NATURAL ENEMY THRESHOLDS FOR GREENBUG, *SCHIZAPHIS GRAMINUM* (RONDANI), ON WINTER WHEAT

By

DOUGLAS B. JONES

Bachelor of Science

Henderson State University

Arkadelphia, Arkansas

1983

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 2001

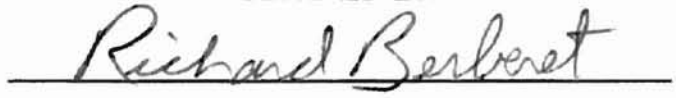
NATURAL ENEMY THRESHOLDS FOR

GREENBUG, *SCHIZAPHIS GRAMINUM*

(RONDANI), ON WINTER WHEAT

Thesis Approved:

Thesis Advisor



Dean of the Graduate College

PREFACE

Chapter I of this thesis is an introduction and chapter II is a literature review focusing on the history and biology of *Schizaphis graminum* (Rondani), *Hippodamia convergens* Guërin-Mëinville, *Lysiphlebus testaceipes* Cresson and *Aphidius colemani* Viereck. Also included is a detailed description of functional responses, natural enemy thresholds and integrated pest management. Chapters III and IV are formal manuscripts of the research I conducted during my M. S. program and are written in compliance with the publication policies and guidelines for manuscript preparation with the Entomological Society of America.

Pursuing and completing this degree would not have been possible without the loving support of my wife Gina, who put up with my long hours and late nights in addition to working long hours herself to support our family during my time at Oklahoma State University. I would like to sincerely thank my major professor Dr. Kristopher Giles for all his assistance and advice throughout my project. Additionally I want to thank Drs. Norman Elliott, Richard Berberet, Tom Royer, and Larry Claypool for their valuable advice and assistance. Special thanks is extended to Tim Johnson, Dr. Wade French, Dr. Roger Fuentes, Les Magee, Jessica Mayes, Kwanza Stewart, and Melissa Riley for helping collect data for this thesis. I also want to thank my sons Nathaniel, Zachary, Phillip and John for pitching in and helping whenever I needed extra help. Above all I want to thank my parents John and Madeline Jones for their faith in me.

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

LIST OF FIGURES

Figure Page

CHAPTER 1: GENERAL INTRODUCTION

Six to seven million acres of winter wheat, *Triticum aestivum* L., are planted in Oklahoma annually (Krenzer et al. 1999). Wheat is grown in Oklahoma for forage production, grain production or a combination of the two (Thompson 1990). Whether for forage or grain production, Oklahoma wheat is attacked by a number of insect herbivores including the greenbug *Schizaphis graminum* (Rondani).

The greenbug was first reported in the United States as an agronomic pest of wheat in 1882 (Hunter and Glenn 1909, Webster and Phillips 1912). Greenbugs can reach tremendous population levels in a short period of time (Starks and Burton 1977). Outbreaks occur in Oklahoma almost every year, and statewide infestations are reported about every 5-10 years (Starks and Burton, 1977). When population levels surpass economic injury levels (EIL's), greenbug feeding reduces yield and crop quality (Burton et al. 1985, Pike and Schaffner 1985, Kieckhefer and Kantack 1988, Massey 1993, Elliott et al. 1994a, Noetzel 1994). In Oklahoma, losses range from $0.5 to $135 million annually, though much of the losses are due to the expense of insecticide use (Starks and Burton 1977, Webster 1995).

Farmers frequently apply insecticides to control aphids without sufficient cost/benefit justification (Wratten et al. 1990). Many fields are treated when aphid populations are too low to cause yield loss greater than application costs, or they are treated so late in the growing season that yield losses have already occurred (Wratten et al. 1990). Unnecessary pesticide use reduces farmers profits, exterminates beneficial insects, contributes to development of insecticide resistance, and negatively affects wildlife (Peters et al. 1975, Klass 1982, Grue et al. 1988, Flickinger et al. 1991, Sloderbeck et al. 1991).

Greenbugs are attacked by a number of predators and parasites, including lady beetles, parasitic wasps, spiders, damsel bugs, lacewing larvae and syrphid fly larvae (Royer et al. 1998a). The most important examples of these natural enemies in the Southern Great Plains are Coccinellidae predators such as the convergent lady beetle *Hippodamia convergens* Guërin-Mëinville., and the parasitic hymenopteran *Lysiphlebus testaceipes* Cresson (Ruth et al. 1975, Kring and Gilstrap 1983, 1984, Kring et al. 1985).

A natural enemy threshold is a critical ratio of a natural enemy (*L. testaceipes; H. convergens*) and pest species (*S. graminum*) required to prevent that pest species from reaching populations that exceed economic injury levels (Nyrop and van der Werf 1994). Patrick and Boring (1990) state that when one or two lady beetles per foot of row are present or when 15 to 20 percent of greenbugs are parasitized, chemical control measures should be delayed until it can be determined if the greenbug population is continuing to increase. Additionally, they state that warm weather is required for beneficial insects to have be effective. In Oklahoma, its recommended that 20 to 30 percent of greenbugs must be parasitized and daytime temperatures exceed 13.3 °C, or there should be one or two lady beetles per 0.3m of crop furrow before natural control can be successful (Royer et al. 1998b). A problem with these published natural enemy thresholds is that they are not based on any published experimental data (Elliott et al. 1994a).

Incorporating predictable efficacy of greenbug predators and parasitoids in controlling infestations will help wheat farmers reduce unnecessary pesticide applications. This would increase producer profits, reduce the incidence of greenbug resistance to insecticides and reduce negative impacts of pesticides on the environment.

The overall goals of this research were to investigate reproductive potential of two

greenbug parasitoids (one indigenous and the other a potential candidate for release) over a range of temperatures and to experimentally determine natural enemy thresholds for effective greenbug population regulation during the spring growing season on hard red winter wheat. In the first study I examined functional responses, 24 hour egg production totals and superparasitism of greenbugs by a introduced aphid parasitoid, *Aphidius colemani* Viereck and by the indigenous parasitoid, *Lysiphlebus testaceipes* Cresson.

Functional response curves describe the attack rate by a species of natural enemy to changing host density per unit of time (Jervis and Kidd 1996). Functional responses can be used as indicators to determine the relative effectiveness of natural enemies in different situations. Twenty-four hour egg production is the total number of eggs laid by each wasp during the 24 hours that each wasp was exposed to greenbug hosts in the experiment. Superparasitism refers to those occasions where the wasp oviposits more than one time in a host. Both parasitoids were evaluated at four temperatures (14°, 18°, 22° and 26° C) representing common spring temperatures in Oklahoma. These individual measures provide insights on the effectiveness of *L. testaceipes* and *A. colemani* for regulating greenbug populations in the Southern Great Plains during spring growth of hard red winter wheat.

For the second study, I evaluated parasitism by *L. testaceipes*, and predation by *H. convergens* on greenbug and bird oat-cherry aphids, *Rhopalosiphum padi* L., populations on field-caged hard red winter wheat. These interactions were evaluated concurrently on wheat cultivars having resistance (cv TAM 110) and susceptibility (cv TAM 107) to greenbugs in order to determine natural enemy to greenbug ratios (natural enemy thresholds) necessary to provide effective greenbug regulation. Additionally, I sampled

wheat fields across Oklahoma for aphids, parasitoids and Coccinellid predators to validate natural enemy thresholds from the caged study.

Objectives

I. Examine functional responses and ovipositional rates of *A. colemani* and *L. testaceipes* on winter wheat infested with greenbugs at temperatures commonly present in Oklahoma during March.

II. Determine natural enemy thresholds of *L. testaceipes* and *H. convergens* required for aphid control on greenbug susceptible and resistant winter wheat during early spring.

III. Validate newly developed natural enemy thresholds on field populations of greenbugs infesting winter wheat during the early spring.

Explanation of Thesis Format

This general introduction is followed by a literature review (chapter II), then chapters III and IV, devoted to individual papers to be published, a general summary (chapter V), and appendices. Lists of references are provided for citations in the literature review and papers to be published. In paper I (chapter III) functional responses of the parasitoids *Lysiphlebus testaceipes* and *Aphidius colemani* on *Schizaphis graminum* at four temperatures are examined. The second paper (chapter IV) examines natural enemy (*L. testaceipes* and *H. convergens*) thresholds for aphid control on greenbug susceptible (cv TAM 107) and greenbug resistant (cv TAM 110) winter wheat. These papers follow the general guidelines of the Entomological Society of America for submission to scientific journals.

CHAPTER II: LITERATURE REVIEW

Oklahoma Wheat Production

Winter wheat (*Triticum aestivum* L.) is grown in the southern Great Plains of the United States for grain production, forage and grain production, or for forage production only (Krenzer et al. 1999). In 1998, 6.4 million acres of winter wheat were planted in Oklahoma, of which about 4.3 million acres were harvested for grain with an average yield of 34 bushels per acre (Krenzer et al. 1999). Overall, 50 to 55% of planted wheat is grazed (Thompson 1990, Carver et al. 1991).

In Oklahoma, wheat fields are prepared, generally by plowing, beginning in late summer. Planting dates depend on location and intended purpose for the wheat. In the southern region, wheat for forage and grain production is generally planted from 15 September to 10 October, while grain only wheat is generally planted from 10 October to 30 October. Planting dates are earlier as the location is changed further north and west. Soon after planting, wheat germinates and emerges from the soil as a seedling. Tillering begins when the first tiller appears and continues until stem elongation (jointing) starts. Later, in January to March, warming weather helps initiate jointing, this is characterized by the stem of the tiller becoming hollow and extending upward. Livestock can graze wheat without reducing grain production, from mid-November until the first hollow stem appears. Generally in February when temperatures begin to warm, plants resume growth and tillers extend strongly upward by "jointing". This is characterized by a strong stem that is hollow. Heading begins as the flower spike emerges from the flag leaf sheath and continues until flowering is complete. The seed head matures and is generally harvested in late May or early June (Royer and Krenzer 2000).

There are numerous cultivars of wheat available for planting. Cultivar selection is

governed mostly by grain production potential because of a lack of consistent significant differences in forage production among cultivars (Worrall & Gilmore 1985).

Insect Pests of Wheat

Winter wheat is infested by a number of aphid pests including greenbug (*S. graminum*), Russian wheat aphid (*Diuraphis noxia* Mordviko), bird cherry-oat aphid (*Rhopalosiphum padi* L.), English grain aphid (*Sitobion avenae* Fabricius), and corn leaf aphid (*Rhopalosiphum maidis* Fitch). Other pests include armyworms (*Pseudaletia unipuncta* (Hayworth) and *Spodoptera frugiperda* J. E. Smith), cutworms (*Euxoa auxilaris* Grote and *Agrotis orthogonis* ????), false wireworms in the family Tenebrionidae, Hessian fly (*Mayetolia destructor* Say), mites (*Petrobia latens* ????, *Aceria tosichella* Keifer, and *Pentalius major* ????) and white grubs (*Cyclocephala spp.* and *Phytophaga spp.*)(Royer et al. 1998a).

Aphids are of particular interest because they have been observed to damage wheat from plant emergence to heading. Aphids reproduce rapidly, and are often not detected by farmers until their populations reach deleterious levels (Royer et al. 1998a). Aphid outbreaks occur somewhere in Oklahoma almost every year with widespread outbreaks reported every 5-10 years (Starks and Burton, 1977).

Greenbug

The greenbug, *S. graminum* (Rondani) (Homoptera: Aphididae) was first described in Italy in 1847 (Rondani 1847). The greenbug was later found in Virginia in 1882 (Hunter and Glenn 1909, Pfadt 1962), and has been a serious pest of small grain crops in North America ever since (Porter et al. 1997). Greenbugs infest a wide variety of crops and wild hosts throughout the central United States, feeding on over 70

graminaceous species many of which serve as secondary hosts when winter wheat and other grain crops are not present (Michels 1986).

Wadley (1931) divided plants into four major categories based on the ability of greenbugs to reproduce and cause injury. The first class are preferred host plants such as wheat and oats. The second division of host plants includes rye (*Secale cereale* L.), barley (*Hordeum vulgare* L.), and bluegrass (*Poa pratensis* L.). Growth and reproduction is limited somewhat on these hosts causing greenbug colonies to be short-lived and rarely causing serious injury. The third class, temporary hosts, includes corn (*Zea mays* L.), and at one time sorghum (*Sorghum bicolor* (L.) Moench) where greenbug sometimes feed, but reproduction is rare. The fourth class consists of plants that are quickly abandoned when greenbugs are placed on them. Over the past century greenbugs have expanded their range of preferred hosts to include barley and sorghum and have apparently further developed into several different biotypes capable of flourishing on many host plants previously resistant to their feeding (Beregovoy et al. 1988, Porter et al. 1997).

Except for biotype D, all greenbugs have been characterized by their "virulence profile"; *i.e.* their unique pattern of virulence against a selected array of host plants (Porter et al. 1997). Recently the assumption that greenbug biotypes arose because of selection pressure from host plant resistance, has come into question. Porter et al. (1997) found no correspondence between the introduction of resistant wheat cultivars and the emergence of virulent biotypes. They proposed that the greenbug species may be a complex of host-adapted races that evolved on non-cultivated hosts.

Greenbug Biology

Greenbugs are small light green aphids with a darker green dorsal line (Wadley

1931). Greenbugs have black eyes, and the cornicles, legs, and antennae are black tipped. Greenbugs develop through four nymphal stages, collectively taking about one week to complete under favorable conditions (Metcalf and Metcalf 1993). Greenbugs reproduce mainly by apomictic parthenogenesis when temperatures are above their developmental threshold of about 5° C (Wadley 1931, Walgenbach et al. 1988). Winged alate females reproduce 24 to 48 hours after the last molt, and wingless females are capable of reproduction almost immediately following the final molt (Wadley 1931). Paedogenesis, reproduction by nymphs, occurs in approximately 2% of alate immature greenbugs (Wood and Starks (1975). Reproductive rates of 3.5 nymphs per day by parthenogenic females and ca. one egg per day by oviparous females were described by Wadley (1931). Webster and Starks (1987) recorded a mean of six nymphs produced per day by biotype E greenbugs on TAM 105 wheat at 26-28° C. There are up to 33 greenbug generations per year, though the mean number of generations per year is 21 (Webster and Phillips 1918).

In the autumn, alate males and apterous non-parthenogenetic females (sexuales) may be produced in response to increased scotophase (Mittler and Gordner 1991). After mating oviparous females deposits eggs that overwinter from which apterous parthenogenetic females known as fundatrices hatch in the spring (Dixon 1985, Miyazaki 1987). In the Southern Great Plains, however, the greenbug is thought to overwinter primarily as parthenogenetic females (Webster and Phillips 1918, Wadley 1931).

Greenbugs feed by inserting stylets formed by mandibles and maxillae into the plant tissue to feed on phloem sap, which results in chlorosis, and in many cases eventual death of the plant (Burton 1986). Damage is visible soon after feeding begins due to chlorophyll reduction (Gerloff and Ortman 1971, Niassy et al. 1987, Peters et al 1988).

The two leaf stage, or growth stage 13 (Zadocks et al. 1974), is the most susceptible to greenbug feeding injury (Pike and Schaffner 1985), resulting in both root and shoot biomass reductions that persist throughout the entire growing season and may cause yield reductions (Burton 1986).

Economic Status of Greenbug

In Oklahoma, losses attributable to greenbug damage, range from $0.5 to $135 million annually, though much of the expense of greenbug infestation results from insecticide use (Starks and Burton 1977, Wratten et al. 1990, and Webster 1995). For example, in 1993, greenbugs infested approximately 8 million acres of dryland and 1.2 million acres of irrigated wheat in twelve western states. These figures amount to 41 percent and 93 percent, respectively, of the overall wheat acerage that year. The total cost of infestations were $2.6 million in yield and an additional $1.2 million spent on chemical control (Webster 1995). Specifically in Oklahoma, 13.6% of dryland winter wheat and 89% of irrigated winter wheat were infested, resulting in an estimated $387,000 in losses for Oklahoma farmers (Webster 1995). A more severe outbreak in 1976 resulted in costs of over $80 million to Oklahoma farmers from insecticide applications and yield losses (Starks and Burton 1976).

There have been relatively few studies that have attempted to quantify the relationship between greenbug population density and economic loss in winter wheat. Kieckhefer and Gellner (1992) estimated the economic threshold at 15 greenbugs per plant feeding for 30 days (450 aphid feeding days). Aphid feeding days are calculated by multiplying the number of greenbugs per plant by the number of days that they feed on that plant. Burton and Burd (1993) described a significant dry root weight loss after only

14 days of feeding by 10 greenbugs on TAM 101 wheat. Kieckhefer et al. (1994) estimated reduced grain production at 41 kg of grain per hectare per 100 aphid feeding days.

In Oklahoma, greenbug infestations are measured by one of three general methods. The mean number of aphids per wheat tiller is estimated by selecting three tillers at each of 25 random locations in the field, and calculating the average number of greenbugs present. The second involves determination of the mean number of aphids per 0.3m of crop furrow from counts taken at several random locations throughout the field (Royer et al. 1998b). More recently, a third method utilizing a binomial sequential sampling scheme has been developed (Giles et al. 2000). This method involves looking at 100 randomly selected tillers and noting the presence or absence of greenbugs on each tiller. The number of tillers that were positive for greenbugs is then compared to a chart indicating the probable greenbug population density.

Natural Enemies of Greenbug

Greenbugs are attacked by a number of predators and parasites, including lady beetles, parasitic wasps, spiders, damsel bugs, lacewing larvae and syrphid fly larvae (Royer et al. 1998a). The most important examples of theses natural enemies in the Southern Great Plains are Coccinellidae predators, and the parasitic Hymenoptera (Ruth et al. 1975, Kring and Gilstrap 1983, 1984, Kring et al. 1985).

Coccinellidae

Hodek (1970) proposed that of all aphidophagous insects, Coccinellidae are the most important in regulating aphid populations. Coccinellidae are highly mobile as adults, traveling in some instances many hundreds of kilometers to and from overwintering sites,

such as ones in California and central Mexico (Hodek 1973). There are at least 64 genera and 453 species of aphidophagous Coccinellidae in America north of Mexico (Gordon 1985). Some of the commonly encountered Coccinellidae species in Oklahoma wheat fields are *Hippodamia convergens* Guërin-Mëinville, *Hippodamia sinuata* Muslant, *Coccinella septempunctata* L. and *Coleomegilla maculata* Timberlake (Teetes et al. 1973, Michels et al. 1997, Obrycki and Kring 1998). One of the more frequently encountered is the convergent lady beetle, *H. convergens*, a species of which adults and larvae contribute greatly to greenbug control (Teetes et al. 1973).

Hippodamia convergens are orange, usually with 12 black spots on the elytra. Multi-voltine, *H. convergens* lay orange-yellow eggs in clusters, usually on the undersides of leaves. Larvae are black with abdomen tapered posteriorly and orange stripes across the dorsum. They are voracious predators; adults eat about 30-60 greenbugs per day while the larvae consume about 20-100 per day depending on instar (Hunter and Glenn 1909, Clausen 1916, Fenton and Dahms 1951, Daniels 1965, Chedester 1979). However, *H. convergens* does not necessarily prefer greenbugs over other aphid species (Kring and Gilstrap 1986). Presence of other, possibly less damaging aphid species, such as *R. padi*, *R. maidis*, and *S. avenae,* can be important for greenbug control by attracting Coccinellidae and discouraging their dispersal (Hodek 1973). Of the five aphid species commonly present in Oklahoma wheat fields, all are suitable prey for *H. convergens* (Royer et al. 1998b).

Feeding behavior by *H. convergens* in Oklahoma during the winter wheat growing season has not been well documented. Experiments by Kring et al. (1985) demonstrated that *H. convergens* and other Coccinellidae were not very effective for greenbug control in

early season grain sorghum, but were important later in the growing season when temperatures increased. *Hippodamia convergens* require temperatures above 15°C to complete development beyond the second instar, though the developmental threshold is between 6°C and 12°C (Gutierrez et al. 1981, Obrycki and Tauber 1982, Michels and Behle 1991). Complete development of *H. convergens* requires approximately 350 degree-days when fed greenbugs in the laboratory (Michels and Behle 1991). According to Michels and Behle (1991), *H. convergens* required fewer greenbugs to reach maturity as ambient temperature increased (517 greenbugs at 20°C vs. 230 greenbugs at 30°C)

Hymenopteran Parasitoids

Hymenopteran parasitoids of the greenbug in Oklahoma include *Aphelinus nigritus* (Howard), *Aphelinus varipes* (Foerster), *Diaeretiella rapae* (McIntosh) and *Lysiphlebus testaceipes* (Cresson), which are all primary parasitoids. Of these *L. testaceipes* is the most important (Jackson et al 1970, Walker et al. 1973, Archer et al. 1974, Summy et al. 1979). A complex of hyperparasitoids, including *Aphidencyrtus aphidivorus* (Mayr), *Pachyneuron siphonophorae* (Ashmead), *Charips sp.* and *Asaphes lucens* (Provancher) have also been identified.

Lysiphlebus testaceipes

Lysiphlebus testaceipes is a common parasitoid of cereal aphids found throughout temperate regions of North and South America (Krombein et al. 1979). Female *L. testaceipes* oviposit in all life stages of the greenbug (Webster and Phillips 1912). About 2 days after a greenbug is parasitized, the egg hatches into a larvae that develops first by consuming hemolymph and later all internal organs of the host. After developing through four instars, the immature parasitoid begins to twist and turn inside the host. The

movement expands the host exoskeleton to form a swollen tan colored mummy within which pupation occurs. Before pupating, the larva chews an opening in the host exoskeleton ventrally and fastens it to the leaf surface with silk. Once attached, the parasitoid larva pupates. Upon emergence, the adult chews a circular opening dorsally in the aphid pupal case to emerge and begin another generation (Hardee et al. 1990, Knutson et al. 1993).

When parasitized as adults, greenbugs stop reproducing about three days after being parasitized by *L. testaceipes* (Spencer 1926). Greenbugs parasitized when they are less than three days old will not reproduce at all (Eikenbary and Rogers 1974). *Lysiphlebus testaceipes* has great potential for destroying large numbers of greenbugs without regard for their biotype (Pergande 1902, Sekhar 1957, Wood and Chada 1969, Salto et al. 1983), however its impact has not been extensively studied (Ruth et al. 1975, Kring and Gilstrap 1983, Rice and Wilde 1988, Patrick and Boring 1990).

Lysiphlebus testaceipes has a developmental threshold of 6.6°C and takes 9.3 days to develop from egg to adult at 26°C, in contrast to requiring over 49 days at 10°C (Elliott et al. 1994b). There are conflicting observations on the lower temperature limits for *L. testaceipes* oviposition. Sekhar (1960) reported total ovipositional inactivity at 14°C, while Hunter and Glenn (1909) reported successful oviposition attempts at 3.33°C and feeble attempts at 1.67°C. Hunter and Glenn (1909) also reported that *L. testaceipes* has the ability to survive temperatures below 0°C and oviposit later when temperatures were warmer. Though Oklahoma often experiences temperatures during the winter and spring below developmental requirements as measured in the laboratory, adult parasitoids have been observed during cold weather (<10°C, on a sunny day) when greenbug

population levels are low (D.B. Jones unpublished data).

***Aphidius colemani* Viereck**

Another parasitoid of interest for greenbug control in Oklahoma, is *Aphidius colemani* Viereck. *Aphidius colemani* is a oligophagous parasitoid that is probably probably indigenous to India, but is now found in many other parts of the world (Ramakrishna Ayyar 1934, Starý 1975). It is reported to parasitize several economically important cereal aphids (Elliott et al. 1999). *Aphidius colemani* has been released in the Southern Plains wheat growing region, but has not been reported being established (Prokrym et al. 1998). In areas where the parasitoid is established, *A. colemani* alone, has not been able to keep greenbug populations below economic thresholds (Aalbersberg 1988, Gerding et al. 1989, Prinsloo 1990). However, if established, *A. colemani,* along with *L. testaceipes* and other aphidophagous insects, could contribute to successful biological control of greenbugs (Elliott et al. 1999).

Integrated Pest Management of Greenbug

Nearly half a century ago Painter (1951) stated that the use of insect resistant cultivars of wheat alone will not provide adequate pest control, nor will the continued indiscriminate use of pesticides. Each must be used as components of a much broader management strategy. The principals of Integrated Pest Management (IPM), are to utilize known methods of insect control, both biological and chemical, together in a concerted fashion to keep insect pests under control. Biological control methods include introduced exotic agents, or naturally occurring agents. Chemical control should be used as necessary, in a manner which is least disruptive to biological control (Stern et al. 1959).

Cultural Controls

Conservation tillage provides increased crop residue on the soil surface and has been shown to reduce immigration of greenbugs into wheat fields (Burton and Krenzer 1985); presumably crop residue reduces the attractiveness of fields to greenbugs in comparison to bare soil. Reductions in greenbug populations resulting from conservation tillage are proportional to the amount of residue left on the soil surface, with no-till fields having the largest amount of crop residue. Nitrogen fertilization at recommended rates invigorates wheat allowing it to better tolerate greenbug injury. Under proper fertilization, the rate of greenbug population growth is slow relative to the growth rate of wheat plants, which allows plants to escape some injury (Daniels 1975). Grazing cattle on wheat during winter, a common practice in much of the Southern Great Plains, also reduces greenbug populations (Daniels 1975, Arnold 1981). Grazing after the onset of jointing reduces wheat yields, so cattle are typically removed from fields in late-winter (Redmon et al. 1996). None of these tactics have been used for the sole purpose of controlling greenbugs, but could be included in a comprehensive IPM program.

Host Plant Resistance

Host plant resistance is defined as an intrinsic defense against herbivores (Painter 1951). The three types of intrinsic plant defenses, commonly referred to as "Painter's resistance triangle," are antibiosis (host plant toxins or other compounds that have a deleterious effect on its herbivores), antixenosis (non-preference) and tolerance (ability by the host plant to endure injury by a larger herbivore population than a non-tolerant host plant without an economic loss).

Plant resistance to greenbug feeding is poorly understood. Schuster and Starks

(1973) attempted to define mechanisms of greenbug resistance in sorghum. Some cultivars were highly non-preferred, others were deemed to have an antibiotic effect (determined by reduced greenbug reproduction when compared to susceptible plants), and others still were described as tolerant (measured by differences in plant height for infested and non-infested plants). Other studies have attempted to describe and measure the mechanisms of greenbug feeding and host-plant resistance (Dorschner et al. 1987, Puterka et al. 1988, Tonet and Pires da Silva 1995, Cruz and Vendramim 1998), however none have conclusively identified mechanisms in wheat with resistance to greenbugs.

Resistance of wheat cultivars to greenbug feeding was first described by Wood (1961). Greenbug resistant wheat was first released in 1955 (DS 28A), however, three years later stands of DS 28A were being severely damaged by greenbugs. Subsequently a new greenbug biotype, "B" was proposed to identify this virulence. In 1968, greenbugs damaged sorghum, which was previously considered not to be a preferred host for greenbugs. A new biotype for which sorghum is a preferred host was designated "C". Resistant wheat has been released in the forms of Amigo (1978), Largo (1980), CI 17959 (1982), CI 17882 (1985) and most recently GRS 1201 in 1991. Greenbug biotypes continue to be discovered that are able to overcome new sources of host plant resistance. It has been proposed that greenbugs develop new biotypes in response to development of new sources of resistance (Beregovoy et al. 1988). However there is evidence that new biotypes are simply the expression of genes already present from the greenbug's gene-pool (Porter et al 1997, Anstead 2000).

Host plant resistance has long been considered to be easily integrated with biological control (Van Emden 1995). Integration may not be as simple as once thought,

however, as Hare (1992) has cited 16 studies where interactions between resistant crop varieties and parasitoids were studied. The influence of host plant resistance can be positive, have no apparent effect, or even have a negative effect on parasitoid success . Studies have shown that partial plant resistance or environmental variables can not only influence aphid size and fecundity, but may affect weight and fecundity of female parasitoids of the third trophic level as well (Van Emden 1991, 1995).

Insecticides

When greenbug infestations increase to economic thresholds (ET), most farmers resort to treating with an appropriate insecticide, or risk losing the investment in the crop. Chlorpyrifos, dimethoate, disulfoton, imadacloprid, malathion, methyl parathion, parathion, and parathion with methyl parathion are registered for greenbug control in Oklahoma (Royer et al 1998b).

Widespread use of pesticides for greenbug control has likely contributed to pesticide resistance (Shotkoski et al. 1990, Sloderbeck et al. 1991, Sloderbeck 1992, Peckman and Wilde 1993). Teetes et al. (1975) described greenbug resistance to organophosphate compounds in sorghum, designating the insecticide resistant greenbug as biotype "D". Peters (1975) reported greenbugs collected from sorghum in Oklahoma, South Dakota, and Texas as being resistant to organophosphates. Two types of insecticide resistance have been identified in greenbugs: pattern-1 resistance (target-site resistance) due to altered acetylcholinesterase, and pattern-2 resistance (metabolic resistance) caused by amplified esterases (Shufran et al. 1993). Pattern-2 resistant greenbugs are the most abundant in the Great Plains (Shufran et al. 1997).

Wratten et al. (1990) found that most pesticide applications for greenbug control

in winter wheat are made when greenbug populations are below the economic threshold. Also, others were treated too late in the growing season after most yield loss had already occurred (Wratten et al. 1990). In 1996 over 90% of the acreage in Blaine and Kiowa counties was treated for greenbugs at least once, and often more than once (Carlin Lawrence, Personal communication). Many of these applications were economically unnecessary, or were applied during poor weather conditions making them less effective (S. D. Kindler, Personal communication).

Biological Control

Natural enemies are the fundamental resources of biological control (van Driesche and Bellows 1996). In order to incorporate natural enemies into IPM decisions, natural enemies that are present must be identified and the biology of these species must be described to determine whether they can be relied on to achieve successful control.

Results of past research are not consistent about the roles of natural enemies in greenbug population regulation. Some authors place great emphasis on predators such as the Coccinellidae (Cartwright et al. 1977, Kring and Gilstrap 1984, Kring et al. 1985). Others argue that parasitoids such as *L. testaceipes* are more effective regulators of greenbug populations (Pergrande 1902, Sekhar 1957, Jackson et al 1970, Kring and Gilstrap 1983, Rice and Wilde 1988, Patrick and Boring 1990). It is most probable that greenbug population levels at any particular time are the result of a complex web of many factors including both parasitism and predation, along with other factors such as weather, disease and host-plant resistance.

Functional response curves describe the attack rate shown by a natural enemy to changing host density per unit of time (Solomon 1949, Jervis and Kidd 1996). Functional

response can be used as an indicator to help determine the relative effectiveness of natural enemies in different situations. Holling (1959) proposed four possible functional response types; Type 1 is a constant rise in prey consumed, or hosts parasitized for parasitioids, as prey density rises until the natural enemy is satiated or the parasitoid's egg supply is exhausted (Fig. 1). For Type 2, the response rises at a constantly decreasing rate until a maximum value is reached, time requirements for subduing, killing, eating and digesting the prey, are responsible for the rate change. Type 3 resembles a type 2 functional response except that at lower prey densities the functional response accelerates creating a sigmoidal curve; the acceleration is a representation of ever shorter searching time at moderate prey densities. Type 4 resembles a type 2 response but at high prey densities, the attack rate decreases due to prey species being able to interfere with and slow the natural enemy (Holling 1959, van Alphen and Jervis 1996).

Greenbug control by parasitoids may be explained by examining functional responses. One mechanism may be that as parasitoids encounter higher host densities, attacks may increase as a result of learning (Type 3 functional response), providing a potential mechanism for greenbug control (Murdoch and Oaten 1975). At low host densities control may be achieved by an attack rate that matches host fecundity and stabilizes host-parasitoid populations (Berryman 1999).

Natural enemy thresholds are critical ratios of a pest species to natural enemies required to prevent that pest species from reaching populations that exceed economic injury levels (Nyrop and van der Werf 1994, Wilson 1994). They have been successfully utilized in agricultural production systems by allowing farmers to evaluate whether pest populations will be biologically controlled, or if additional measures are needed (Croft and

Nelson 1972, Wilson et al. 1984, Wilson 1985, Nyrop 1988, Patrick and Boring 1990, Beers et al. 1994). Understanding natural enemy thresholds for *H. convergens* and *L. testaceipes* can help farmers make informed decisions about success or failure of greenbug control. Patrick and Boring (1990) state that when there are one or two lady beetles per 0.3m of furrow or 15 to 20 percent of greenbugs are parasitized, control measures should be delayed until it can be determined if the greenbug population will continue to increase and exceed the economic threshold. Additionally, warm weather is required for beneficial insects to have an impact. In Oklahoma, its recommended that 20 to 30 percent of greenbugs must be parasitized and temperatures are above 13.3 °C, or there should be one or two lady beetles per 0.3m of crop furrow before natural control can be successful (Royer et al. 1998b). A problem with these recommendations is that they are not based on any published experimental data (Elliott et al. 1994a)! Identification of more accurate natural enemy thresholds for greenbug control based on experimental data and a detailed description of the capabilities of natural enemies are needed to improve IPM recommendations and make sound, profitable decisions.

References Cited

Aalbersberg, Y. K., M. C. vander Westhuizen and P. H. Hewitt. 1988. Natural enemies and their impact on *Diuraphis noxia* (Mordvilko)(Hemiptera: Aphididae) populations. Bull. Entomol. Res. 78:111-120.

Anstead, J. 2000. Genetic and biotypic diversity of greenbug, *Schizaphis graminum* (Rondani) populations on non-cultivated hosts. M. Sc. Thesis. Oklahoma State University Stillwater, OK.

Archer, T. L., R. H. Cate, R. D. Eikenbary and K. J. Starks. 1974. Parasitoids collected from greenbugs and corn leaf aphids in Oklahoma in 1972. Ann. Entomol. Soc. Am. 67:11-14.

Arnold, D. C. 1981. Effects of cold temperatures and grazing on greenbug populations in wheat in Noble County, Oklahoma 1975-76. J. Kansas. Entomol. Soc. 54:571-577.

Beers, E. H., L. A. Hull and V. P. Jones. 1994. Sampling pest and beneficial arthropods of apple. In: L. Pedigo and D. G. Buntin (eds.), Handbook of Sampling Methods for Arthropod Pests in Agriculture. CRC Press, Boca Raton, Florida.

Beregovoy, V. H., K. J. Starks and K. G. Janardan. 1988. Fecundity characteristics of the greenbug biotypes C and E cultured on different host plants. Environ. Entomol. 17: 59-62.

Berryman, A. A. 1999. The theoretical foundations of biological control. *In* B. A. Hawkins and H. V. Cornell [eds], Theoretical Approaches to Biological Control. Cambridge University Press. Cambridge UK. pp 3-21.

Burton, R. L. and E. G. Krenzer, Jr. 1985. Reduction of greenbug (Homoptera: Aphididae) populations by surface residues in wheat tillage studies. J. Econ. Entomol.

78:390-394.)

Burton, R. L., D. D. Simon, K. J. Starks and R. D. Morrison. 1985. Seasonal Damage by Greenbugs (Homoptera: aphididae) to a Resistant and a Susceptible Variety of Wheat. J. Econ. Entomol. 78: 395-401.

Burton, R. L. 1986. Effect of greenbug (Homoptera: Aphididae) damage on root and shoot biomass of wheat seedlings. J. Econ. Entomol. 79:633-636.

Burton, R. L. and J. D. Burd. 1993. Relationship between number of greenbugs and damage to wheat seedlings. Southwestern Entomol. 18:263-267.

Cartwright, B. O., R. D. Eikenbary, J. W. Johnson, T. N. Farris and R. D. Morrison. 1977. Field release and dispersal of *Menochilus sexmaculatus*, an imported predator of the greenbug, *Schizaphis graminum*. Environ. Entomol. 6(5): 699-704.

Carver, B. F., E. G. Krenzer Jr. and W. E. Whitmore. 1991. Seasonal forage production and regrowth of hard and soft red winter wheat. Agronomy Journal. 83:533-537

Chedester, L. D. 1979. Feeding habits, reproduction and sexual determination of the convergent lady beetle, *Hippodamia convergens* (Guer.). Texas Agricultural Experiment Station MP-1437.

Clausen, C. P. 1916. Life-history and feeding records of a series of California Coccinellidae. Univ. Calif. Tech. Bull. 1: 251-299.

Croft, B. A. and E. E. Nelson. 1972. An index to predict efficient interaction of *Typhlodromus occidentalis* in control of *Tetranchus mecdanieli* in Southern California apple trees. J. Econ. Entomol. 64: 845.

Cruz, I. and J. D. Vendramim. 1998. Tolerance as a mechanism of resistance of

sorghum to the greenbug, *Schizaphis graminum* (Rond.)(Homoptera: Aphididae). An. Soc. Entomol. Brasil 27:141-148.

Daniels, N. E. 1965. Lady beetles and their consumption of aphids. Texas Agr. Exp. Sta. Annual Report (at Bushland) p. 50-53.

Daniels, N. E. 1975. Factors influencing greenbug infestations in irrigated winter wheat. Texas Agricultural Experiment Station Publication MP-1187, College Station, Texas, USA.

Dixon, A. F. G. 1985. Aphid Ecology. Chapman and Hall, New York. 157 pp.

Dorschner, K. W., J. D. Ryan, R. C. Johnson and R. D. Eikenbary. 1987. Modification of host nitrogen levels by the greenbug (Homoptera: Aphididae): its role in resistance of winter wheat to aphids. Environ. Entomol. 16:1007-1011.

Eikenbary, R. D. and C. E. Rogers. 1974. Importance of alternate hosts in establishment of introduced parasites. Tall timbers conference on ecological animal control by habitat management. Tallahassee, FL pp 119-133.

Elliott, N. C., W. E. Riedell and B. W. Fuller. 1994a. Yield on Spring Wheat in Relation to Level of Infestation by Greenbugs (Homoptera: Aphididae). Can. Ent. 126: 61-66.

Elliott, N. C., J. A. Webster and S. D. Kindler. 1994b. Developmental response of *Lysiphlebus testaceipes* to temperature. Southwestern Entomol. 24:1-4.

Elliott, N. C., B. W. French, J. D. Burd, S. D. Kindler, and D. K. Reed. 1999. Parasitism, adult emergence, sex ratio, and size of *Aphidius colemani* (Hymenoptera: Aphidiidae) on several aphid species. Great Lakes Entomol. 27: 137-142.

Fenton, F. A. and R. G. Dahms. 1951. Attempts at controlling the greenbug by

importation and release of lady beetles in Oklahoma. Proc. Okla. Acad. Sci. 32: 1-2.

Flickinger, E. L., G. Juenger, T. J. Roffe, M. R. Smith and R. J. Irwin. 1991. Poisoning of Canada geese in Texas by parathion sprayed for control of Russian wheat aphid. J. Wildl. Dis. 27: 265-268.

Gerding, M. P., E. S. Zuniga, C. E. Quiroz, H. M. Norambuena and R. M. Vargas. 1989. Relative abundance of parasitoids of *Sitobion avenae* (F) and *Metopolophium dirhodum* (WLK) (Homoptera: Aphididae) in different areas of Chile. Agricult. Technica (Chile) 49:104-114.

Gerloff, E. D. and E. E. Ortman. 1971. Physiological changes in barley induced by greenbug feeding stress. Crop Sci. 11:174-176.

Giles K. L., T. A. Royer, N. C. Elliott and S. D. Kindler. 2000. Development and validation of a binomial sequential sampling plan for the greenbug (Homoptera: Aphididae) infesting winter wheat in the southern plains. J. Econ. Entomol. 93(5): 1522-1530.

Gordon, R. D. 1985. The Coccinellidae (Coleoptera) of America north of Mexico. J. N. Y. Entomol. Soc. 93: 1-912.

Grue, C. E., M. W. Tome, G. A. Swanson, S. M. Borthwick and L. R. DeWeese. 1988. Agricultural chemicals and the quality of prairie-pothole wetlands for adult and juvenile waterfowl - what are the concerns? In. P. J. Stuber (Coord.), Proc. National Symp. Protect. Wetlands from Agricultural Impacts. USDA, Fish and Wildlife Serv. Biol. Rep. 88:55-66.

Gutierrez, A. P., J. U. Baumgaertner and K. S. Hagen. 1981. A conceptual model of growth, development and reproduction in the ladybird beetle, *Hippodamia convergens*

(Coleoptera: Coccinellidae). Can. Entomol. 113:21-33.

Hardee, D. D., P. J. O'Brien, G. W. Elzen and G. L. Snodgrass. 1990. Emergence and survival of the parasitoid *Lysiphlebus testaceipes* from *Aphis gossypii* exposed to aphicides. Southwestern Entomologist 15(2): 211-216.

Hare, D. J. 1992. Effects of plant variation on herbivore-enemy interactions. pp 278-298 *In* Fritz, R. S. & Simms, E. L. (*Eds*) Plant resistance to herbivores and pathogens. University of Chicago Press.

Hodek, I. 1970. Coccinellids and modern pest management. Bioscience 20: 543-552.

Hodek, I. 1973. Biology of Coccinellidae. Academia, Dr. W. Junk, The Hague.

Holling, C. S. 1959. Some characteristics of simple types of predation and parasitism. Can. Ent. 91: 385-398.

Hunter, S. J. and P. A. Glenn. 1909. The greenbug and its enemies. Univ. Kans. Sci. Bull. 9: 221pp.

Jackson, H. B., L. W. Coles, E. A. Woods, Jr. and R. D. Eikenbary. 1970. Parasites reared from the greenbug and corn leaf aphid in Oklahoma in 1968 and 1969. J. Econ. Entomol. 63:733-736.

Kieckhefer, R. W. and B. H. Kantack. 1988. Yield losses in winter grains caused by cereal aphids (Homoptera: Aphididae) in South Dakota. J. Econ. Entomol. 81: 317-321.

Kieckhefer, R. W. and J. L. Gellner. 1992. Yield losses in winter wheat caused by low-density cereal aphid populations. Agron. J. 84:18-183.

Kieckhefer B. W., N. C. Elliot, W. E. Riedell and B. W. Fuller. 1994. Yield of spring wheat in relation to level of infestation by greenbugs (Homoptera: Aphididae). Can. Ent. 126: 61-66.

Klass, E. E. 1982. Effects of pesticides on non-target organisms. In R. B. Dalgren (ed.), Workshop on Midwest Agricultural Interfaces with Fish and Wildlife Resources. U. S. Fish and Wildlife Serv., Agr. Exp. Sta. and Coop. Ext. Serv. Iowa State Univ. Ames, Iowa: 7-9.

Knutson, A., E. P. Boring, III, G. J. Michels, Jr. and F. Gilstrap. 1993. Biological control of insect pests in wheat. TAM Extension Pub. B-5044. 12pp.

Krenzer, G., R. Kochenower, R. Austin and C. Luper. 1999. Grain yield from wheat variety trals 1998-1999. Protection Technology - Crops. 11(18): 21pp. Oklahoma Cooperative Extension Service.

Kring, T. J. and F. E. Gilstrap. 1983. Within-field distribution of greenbug (Homoptera: Aphididae) and it's parasitoids in Texas winter wheat. J. Econ. Entomol. 76: 57-62.

Kring, T. J. and F. E. Gilstrap. 1984. Efficacy of the natural enemies of grain sorghum aphids (Homoptera: Aphididae). J. Kans. Entomol. Soc. 57: 460-467.

Kring, T. J., F. E. Gilstrap and G. J. Michels Jr. 1985. Role of Indigenous Coccinellids in regulating greenbugs (Homoptera: Aphididae) on Texas Grain Sorghum. J. Econ. Entomol. 78: 269-273.

Kring, T. J. and F. E. Gilstrap. 1986. Beneficial role of corn leaf aphid, *Rhopalosiphum madis* (Fitch)(Homoptera: Aphididae), in maintaining *Hippodamia spp.* (Coleoptera: Coccinellidae) in grain sorghum. Crop Protection. 5:125-128.

Krombein, K. V., P. D. Hurd, D. C. Smith and B. D. Burks. (Eds.) 1979. Catalog of Hymenoptera in America North of Mexico. Smithsonian Institute Press, Washington.

Massey, B. 1993. Insects on small grains and their control. Oklahoma Cooperative

Extension Service No. 7176.

Metcalf, M. A. and R. A. Metcalf. 1993. Useful and Destructive Insects: Their Habits and Control. Fifth Edition. McGraw-Hill Inc. New York.

Michels, G. J. 1986. Graminaceous North American host plants of the greenbug with notes on biotypes. Southwestern Entomol. 11:55-66.

Michels, G. J. and R. W. Behle. 1991. A comparison of Coccinella septempunctata and Hippodamia convergens larval development on greenbugs at constant temperatures. Southwestern Entomol. 16:73-80.

Michels, G. J., N. C. Elliott, R. L. Romero and W. B. French. 1997. Estimating Populations of Aphidophagous Coccinellidae (Coleoptera) in Winter Wheat. Environ. Entomol. 26: 4-11.

Mittler, T. E. and N. K. N. Gordner. 1991. Variation between clones of *Schizaphis graminum* (Homoptera: Aphididae) in the photoperiodic induction of sexual morphs. Environ. Entomol. 20(2):433-440.

Miyazaki, M. 1987. Forms and morphs of aphids. In A. K. Minks and P. Harrewijn [eds.], World Crop Pests. Aphids: Their Biology, Natural Enemies and Control. Volume A. Elsevier, New York. 450 pp.

Murdoch, W. W. and A. Oaten. 1975. Predation and population stability. Adv. Ecol. Res. 9: 1-131.

Niassy, A., J. D. Ryan and D. C. Peters. 1987. Variations in feeding behavior, fecundity, and damage of biotypes B and E of *Schizaphis graminum* on three wheat genotypes. Environ. Entomol. 16:1163-1168.

Noetzel, D. 1994. Revised aphid thresholds in small grains. Minnesota Agricultural

Extension Service Publication. 13 pp.

Nyrop, J. P. 1988. Sequential classification of prey-predator ratios with application to European red mite (Acari: Tetranychidae) and *Typhlodromus pyri* (Acari: Phytoseiidae) in New York apple orchards. J. Econ. Entomol. 80:14.

Nyrop, J. P., and W. van der Werf. 1994. Sampling to Predict or Monitor Biological Control. In: L. Pedigo and D. G. Buntin (eds.), Handbook of Sampling Methods for Arthropod Pests in Agriculture. CRC Press, Boca Raton, Florida.

Obrycki, J. J. and M. J. Tauber. 1982. Thermal requirements for development of *Hippodamia convergens* (Coleoptera: Coccinellidae). Ann. Entomol. Soc. Am. 75:678-683.

Obrycki, J. J. and T. J. Kring. 1998. Predaceous coccinellidae in biological control. Annu. Rev. Entomol. 43: 295-321.

Painter, R. H. 1951. Insect resistance in crop plants. University of Kansas Press, Lawrence, Kansas.

Patrick C. D. and E. P Boring, III. 1990. Managing insect and mite pests of Texas small grains. TA&M Extension Publication # B-1251. 11 pp.

Peckman, P. S. and G. E. Wilde. 1993. Sublethal effects of permethrin on fecundity and longevity of *Hippodamia convergens* (Coleoptera: Coccinellidae). J. Kansas Entomol. Soc. 66:361-364.

Pergande, L. 1902. The southern grain louse. USDA Div. Entomol. Bull. 38 New Series: 7-19.

Peters, D. C., E. A. Wood Jr. and K. J. Starks. 1975. Insecticide Resistance in Selections of the Greenbug. J. Econ. Entomol. 68: 339-340.

Peters, D. C., D. Kerns, G. Puterka and R. W. McNew. 1988. Feeding behavior, development, and damage by biotypes B, C and E of *Schizaphis graminum* in wintermalt and post barley. Environ. Entomol. 17:503-507.

Pfadt, R. E.. 1962. Insect pests of small grains. *In*, R. E. Pfadt (ed.), Fundamentals of Applied Entomology, pp. 213-248. Macmillan Co., New York. 668pp.

Pike, K. S. and R. L. Schaffner. 1985. Development of autumn populations of cereal aphids, *Rhopalosiphum padi* (L.) and *Schizaphis graminum* (Rondani) (Homoptera: Aphididae) and their effects on winter wheat in Washington State. J. Econ. Entomol. 78: 676-680.

Porter, D. R., J. D. Burd, K. A. Shufran, J. A. Webster and G. L. Teetes. 1997. Greenbug (Homoptera: Aphididae) biotypes: Selected by resistant cultivars or preadapted opportunists? J. Econ. Entomol. 90:1055-1065.

Prinsloo, G. J. 1990. Some aspects of the biology of the aphidophagous parasitoids *Aphidius colemani* (Viereck) and *Aphelinus asychis* Walker, M.Sc. Thesis, University of Orange Free State, Bloemfontein, Republic of South Africa.

Prokrym, D. R., K. S. Pike, and D. J. Nelson. 1998. Biological control of *Diuraphis noxia* (Homoptera: Aphididae): Implementation and evaluation of natural enemies: S. S. Quisenberry, and F. B. Peairs Eds. Response model for an introduced pest - the Russian wheat aphid. Thomas A. Publications in Entomology. Published by the Entomological Society of America. Landover MD pp 167-183.

Puterka, G. J., D. C. Peters, D. L. Kerns. J. E. Slosser, L. Bush, D. W. Worrall and R. W. McNew. 1988. Designation of two new greenbug (Homoptera: Aphididae) biotypes G and H. J. Econ. Entomol. 81:1754-1759.

Ramakrishna Ayyar, T. V. 1934. Hymenopterous parasites of economic importance in S. India. Madras Agricultural J. 22: 430-443.

Redmon, L. A., E. G. Krenzer, Jr., D. J. Bernado and G. W. Horn. 1996. Effect of wheat morphological stage at grazing termination on economic return. Agron. J. 88: 94-97.

Rice M. E. and G. E. Wilde. 1988. Experimental evaluation of predators and parasitoids in suppressing greenbugs (Homoptera: aphididae) in sorghum and wheat. Environ. Entomol. 17: 836-841.

Rondani, C. 1847. Nuov. Ann. Sci. Bologna Ser. 2 vols 8,9.

Royer, T. A., K. L. Giles and N. C. Elliott. 1998a. Insects and mites on small grains. Oklahoma State University Extension Facts F-7176.

Royer, T. A., K. L. Giles and N. C. Elliott. 1998b. Small grain aphids in Oklahoma. Oklahoma State University Extension Facts F-7183.

Royer, T. A., and E. G. Krenzer. 2000. Wheat Management in Oklahoma: A Handbook for Oklahoma's Wheat Industry. Oklahoma State University Extension Publication E-831.

Ruth, W. E., R. W. McNew, D. W. Caves and R. D. Eikenbary. 1975. Greenbugs (Homoptera: Aphididae) forced from host plants by *Lysiphlebus testaceipes* (Hymenoptera: Braconidae). Entomophaga. 20: 65-71.

Salto, C. E., R. D. Eikenbary and K. J. Starks. 1983. Compatability of *Lysiphlebus testaceipes* (Hymenoptera: Braconidae) with greenbug (Homoptera: Aphididae) biotypes "C" and "E" reared on susceptible and resistant oat varieties. Environ. Entomol. 12:603-604.

Schuster, D. J. and K. J. Starks. 1973. Greenbugs: components of host-plant resistance in sorghum. J. Econ. Entomol. 66:1131-1134.

Sekhar, P. S. 1957. Mating, oviposition and discrimination of hosts by *Aphidius testaceipes* (Cres.) and *Praon aguite* Smithi primary parasites of aphids. Ibid. 50: 370-375.

Sekhar, P. S. 1960. Host relationships of *Aphidius testaceipes* (Cresson) and *Praon aguti* (Smith), primary parasites of aphids. Can. J. Zoo. 38: 593-603.

Shotkoski, F. A., Z. B. Mayo and L. L. Peters. 1990. Induced Disulfoton resistance in greenbugs (Homoptera: Aphididae). J. Econ. Entomol. 83: 2147-2152.

Shufran, R. A., G. E. Wilde and P. E. Sloderbeck. 1993. Current distribution of pesticide resistant greenbugs based on gel-electrophoresis, pp 10-12. In Proceedings, Greenbug Workshop and Symposium, Alberquerque, NM, February 9-10. Cooperative Extension Service, New Mexico State University, Las Cruces.

Shufran, R. A., G. E. Wilde and P. E. Sloderbeck. 1997. Life history study of insecticide resistant and susceptible greenbug (Homoptera: Aphididae) strains. J. Econ. Entomol. 90:1577-1583.

Sloderbeck, P. E., M. A Chowdhury, L. J. DePew and L. L. Buschman. 1991. Greenbug (Homoptera: Aphididae) resistance to Parathion and Chlorpyrifos-Methyl. J. Kans. Entomol. Soc. 64: 1-4.

Sloderbeck, P. E. 1992. Discovery of pesticide resistant greenbugs in Kansas. In P. E. Sloderbeck (Comp.), Proceedings of the Greenbug Workshop, 4 February, Garden City, Kansas.

Solomon, M. E. 1949. The natural control of animal populations. J. Animal Ecol. 18:

1-45.

Spencer, H. 1926. Biology of the parasite and hyper parasites of aphids. Ibid 19: 119-157.

Starks, K. J. and R. L. Burton. 1977. Preventing Greenbug [*Schizaphis Graminum*] Outbreaks [Small Grains and Sorghum]. Leafl U S Dep Agric 309, Rev., 11 pp.

Starý, P. 1975. *Aphidius colemani* Viereck: its taxonomy, distribution, and host range. Acta Entomol. Bohemoslov. 72: 156-163.

Stern, V. M., R. F. Smith, R. van den Bosch and K. S. Hagen. 1959. The integration of chemical and biological control of the spotted alfalfa aphid: the integrated control concept. Hilgardia. 29(2): 81-102.

Summy, K. R., F. E. Gilstrap and S. M. Corcoran. 1979. Parasitization of greenbugs and corn leaf aphids in west Texas. Southwestern Entomol. 4:176-180.

Thompson, J. D. 1990. Varietal and clipping effects on seasonal forage distribution, grain yield and economic returns for winter wheat utilized as a dual purpose crop. M.S. Thesis, Oklahoma State University, Stillwater, OK.

Teetes, G. L., E. G. Lopez and C. A. Schaefer. 1973. Population density relationships between the greenbug and associated predators and parasites "Grain sorghum insect and mite research on the High Plains of Texas." A report to the Texas Grain Sorghum Producers Board.

Teetes, G. L., C. A. Schaefer, J. R. Gipson, R. C. McIntyre and E. E. Latham. 1975. Greenbug resistance to organophophorus insecticides on the Texas high plains. J. Econ. Entomol. 68:214-216.

Tonet, G. L. and R. F. Pires da Silva. 1995. Resistance of wheat genotypes to the

greenbug *Schizaphis graminum* (Rondani, 1852)(Hom., Aphididae). Pesq. Agropec. Bras. 30:299-306.

van Alphen, J. J. M. and M. A. Jervis. 1996. Foraging behaviour. In: M. Jervis and N. Kidd (eds.), Insect Natural Enemies: Practical approaches to their study and evaluation. Chapman and Hall, New York, New York.

van Driesche, R. G. and T. S. Bellows. 1996. Biological Control. Chapman and Hall. New York.

van Emden, H. F. 1991. The role of host plant resistance in insect pest mis-management. Bull. Entomol. Res. 81:123-126.

van Emden, H. F. 1995. Host plant-Aphidophaga interactions. Agriculture, Ecosystems and Environment. 52:3-11.

Wadley, F. M. 1931. Ecology of *Toxoptera graminum*, especially as to factors affecting importance in the northern United States. Ann., Entomol. Soc. Am. 24: 325-395.

Walker, A. L., D. G. Bottrell and J. R. Cate, Jr. 1973. Hymenopterous parasites of biotype C greenbug in the high plains of Texas. Ann. Entomol. Soc. Am. 66:173-176.

Webster, F. M. and W. J. Phillips. 1912. The spring grain-aphid or "greenbug," USDA Bur. Entomol. Bull 110. 153 p.

Webster, F. M. and W. J. Phillips. 1918. Contribution to the knowledge of *Toxoptera graminum* in the South. J. Agric. Res. 14(2):97-111.

Webster, J. A. and K. J. Starks. 1987. Fecundity of *Schizaphis Graminum* and *Diuraphis Noxia* (Homoptera: Aphididae) at Three Temperature Regimes. J. Kans. Entomol. Soc. 60: 580-582.

Webster, J. A. 1995. Economic impact of the greenbug in the western United States:

1992-1993. Volume Publication No. 155. Great Plains Agricultural Council, Stillwater, Oklahoma.

Wilson, L. T., M. A. Hoy, F. G. Zalom and J. M. Smilanick. 1984. Sampling mites in almonds. I. Within-tree distribution and clumping pattern of mites with comments on predator-prey interaction. Hilgardia. 52:1.

Wilson, L. T. 1985. Estimating abundance and impact of arthropod natural enemies in IPM systems. In: M. A. Hoy and D. C. Herzog (eds.), Biological control in agricultural IPM systems. Academic Press, New York.

Wilson, L. T. 1994. Estimating abundance, impact, and interactions among arthropod natural enemies in cotton agroecosystems. In: L. Pedigo and D. G. Buntin (eds.), Handbook of Sampling Methods for Arthropod Pests in Agriculture. CRC Press, Boca Raton, Florida.

Wood, E. A. 1961. Biological studies of a new greenbug biotype. J. Econ Entomol. 54:1171-1173.

Wood, E. A. Jr. and H. L. Chada. 1969. Chemical control of the sorghum "greenbug," Guymon, Oklahoma 1968. Okla. Agr. Exp. Sta. Prog. Rep. P-616. 4p.

Wood, E. A. and K. J. Starks. 1975. Incidence of paedogenesis in the greenbug. Environ. Entomol. 4(6):1001-1002.

Worrall, W. D. and E. C. Gilmore. 1985. Forage production of small grains for the rolling plains. Research Report MP-1584, Texas Agricultural Experiment Station. College Station, Texas.

Wratten, S. D., A. D. Watt, N. Carter, and J. C. Entwhistle. 1990. Economic consequences of pesticide use for grain aphid control on winter wheat in 1984 in England.

Crop Prot. 9: 73-78.

Zadocks, J. C., T. T. Chang, and C. F. Konzak. 1974. A decimal code for the growth stages of cereals. Weed Res. 14:415-421.

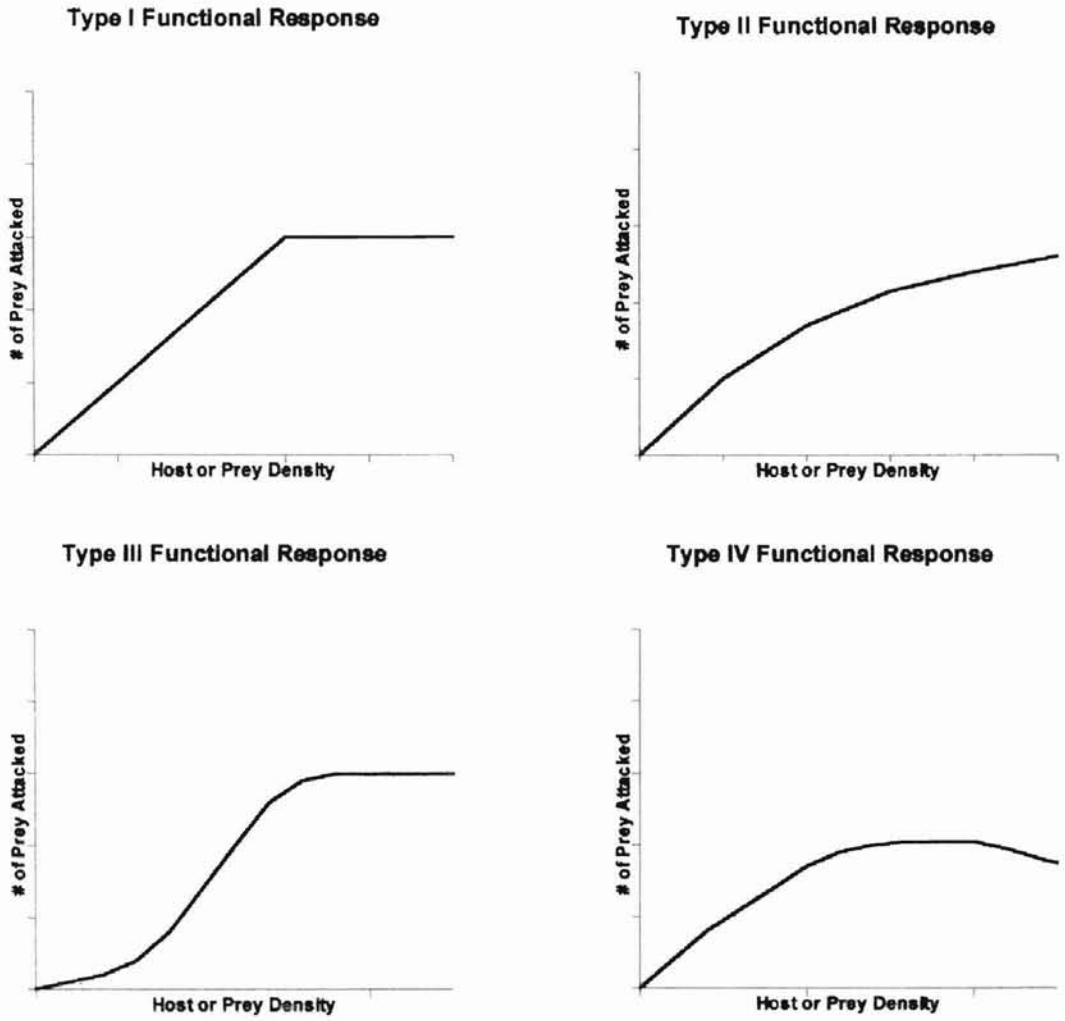


Figure 1. The four types of functional response observed in predators and parasitoids. Y-axis label refers to number of hosts parasitized or consumed. (Jervis and Kidd 1996)

CHAPTER III: FUNCTIONAL RESPONSES OF AN INTRODUCED PARASITOID AND AN INDIGENOUS PARASITOID OF THE GREENBUG, *SCHIZAPHIS GRAMINUM* (RONDANI)

Abstract

Functional responses and superparasitism by the indigenous parasitoid wasp *Lysiphlebus testaceipes* Cresson (Hymenoptera: Aphidiidae) and the introduced parasitoid *Aphidius colemani* Viereck (Hymenoptera: Aphidiidae) on greenbug, *Schizaphis graminum* (Rondani) (Homoptera: Aphididae) were measured at four temperatures (14°, 18°, 22°, and 26° C) during a 24-hour period (12:12 L:D). At each temperature, from 5 to 75 greenbugs were exposed to individual wasp mating pairs for 24 hours. Ultimately, 176 *A. colemani* females and 204 *L. testaceipes* females were evaluated. At all experimental temperatures, attack rates for both wasps most closely fit the Type III functional response model. *Aphidius colemani* functional response, oviposition rate, and prevalence of superparasitism were not temperature-dependent. However, these parameters were temperature-dependent for *L. testaceipes*. *Aphidius colemani* achieved higher parasitism rates than *L. testaceipes* at lower temperatures suggesting that *A. colemani* may be an effective addition to the parasitoid guild for biological control of greenbug during cooler periods in the Southern Great Plains when greenbug populations approach economic thresholds.

Introduction

Aphidius colemani Viereck and *Lysiphlebus testaceipes* Cresson are oligophagous parasitoids in the family Aphidiidae. Although it is believed to be indigenous to India, *Aphidius colemani* has been released and established in many other regions of the world (Ramakrishna Ayyar 1934, Starý 1975). This parasitoid is currently produced commercially for biological control of *Myzus persicae* Sulzer and *Aphis gossypii* Glover in contained cropping systems such as greenhouses (Grasswitz 1998). *Aphidius colemani* is reported to parasitize several economically important cereal aphids, including the greenbug, *Schizaphis graminum* (Rondani) (Starý 1975), and has been observed parasitizing a number of aphid hosts other than small grain aphids (Elliott et al. 1994). This parasitoid has been released in the Southern Plains region of the U. S., but has not been recovered (Prokrym et al. 1998). If established, *A. colemani* could potentially contribute to integrated control of greenbugs (Elliott et al. 1994).

The nearctic parasitoid *L. testaceipes* utilizes greenbug and other cereal aphids as hosts throughout the central and western wheat growing regions of the U. S. and is considered to be one of the most important biological control agents of greenbug in the Southern Plains (Jackson et al. 1970). *Lysiphlebus testaceipes* has been observed to regulate greenbug populations below economic thresholds in both wheat and sorghum (Kring et al. 1985, Rice and Wilde 1988, Patrick and Boring 1990). Greenbug populations are reduced directly through mortality and indirectly by reduced reproductive potential (Hight et al. 1972). When parasitized, the reproductive span of a greenbug is 0 to 5 days versus 25 to 30 for non-parasitized greenbugs. Those parasitized as first and second instar nymphs often do not reproduce at all (Hight et al. 1972).

Efficacy of parasitoids appears to be closely tied to ambient temperature. In Oklahoma, it is recommended that 20 to 30 percent of greenbugs must be parasitized and daytime temperatures remain consistently above 13.3°C before biological control can be successful (Royer et al. 1998). These recommendations are based on the fact that the developmental thresholds for greenbug is considerably lower than that for *L. testaceipes* (Hight et al. 1972, Walgenbach et al. 1988, Patrick and Boring 1990, Elliott et al. 1999). Because of the difference of developmental thresholds it is assumed that at low temperatures, populations of parasitoids increase much more slowly than greenbug populations and fail to regulate greenbugs. However, these recommendations do not incorporate the effects of temperature on the functional response of parasitoids (attack rate as a function of host density per unit of time, Solomon 1949).

Knowledge of parasitoid biology is important for implementing a integrated management program for greenbug control. Information for each species such as, functional response, egg laying capacity, prevalence of superparasitism and the effects of temperature on extent of parasitism provide insights about chances for successful biological control by *L. testaceipes* and *A. colemani* in the Southern Plains. Functional response analyses are commonly used to help predict the potential for parasitoids to regulate prey populations (Oaten and Murdoch 1975). Initially, functional response research was conducted by Holling (1959a, b) who developed mathematical models to describe parasitoid responses to changing host density. Type I responses are exemplified by a parasitoid with a constant attack rate over all host densities and a random search pattern. The number of hosts parasitized per female in a Type I system is directly proportional to host density and represented by a linear response until satiation is reached

(Hassell 1978). Type II responses incorporate handling time, which refers to the act of subduing the host, determining host acceptance, oviposition, and then perhaps cleaning and resting before moving on to search for more hosts. With some exceptions, most arthropods possess a Type II response (Holling 1961, Sandness and McMurtry 1970, Tostowaryk 1972, Hassell et al. 1977). Type III functional response model, is depicted by a sigmoidal curve with an accelerating attack rate as the host density increases. The rate then decreases as handling time increases when approaching satiation.

The objective of this study was to evaluate functional responses of *A. colemani* and *L. testaceipes* (numbers of greenbugs parasitized) on greenbugs infesting winter wheat at 4 different temperatures (representing a common range of daytime temperatures in central Oklahoma in March). The numbers of parasitoid larvae present within parasitized greenbugs were counted by dissections to estimate ovipositional and superparasitism rates.

Materials and Methods

Greenbug and Parasitoid Colonies. Biotype "E" greenbugs were obtained from colonies maintained at the USDA-ARS Plant Science and Water Conservation Research Laboratory at Stillwater, OK and established on wheat (cv 2137) grown in a fritted clay and sphagnum moss medium. Colonies and all wheat plants were kept inside fine mesh cages within a greenhouse to prevent infestation by feral greenbugs and parasitoids. Fresh plants were supplied to cages housing colonies as needed.

Cages with two layers of fine mesh spaced 2.5 cm apart for sides were constructed to house parasitoid colonies which were maintained in growth chambers set at 22°C and a photo-period of 12:12 (L:D). The double-layered cages prevented feral parasitoids from ovipositing into greenbugs through the mesh and permitted ample airflow. *Aphidius*

colemani females were obtained from a colony at Plant Science and Water Conservation Research Laboratory at Stillwater, OK. *Lysiphlebus testaceipes* was isolated from specimens collected at Perkins, OK in the fall of 1999. Pots of wheat infested by greenbugs were in the colonies every 3-4 days to maintain a steady supply of parasitoids.

Functional Response Studies. Functional response experiments were conducted with *Aphidius colemani* in 1999 and *L. testaceipes* in 2000. A 4 x 4 factorial randomized complete block design was used for the studies with *A. colemani* involving four temperatures (14°, 18°, 22° and 26° C) and targeted greenbug densities of 10, 30, 50, and 70 greenbugs per parasitoid female. Temperatures recorded at Oklahoma City Rogers Airport during March for the years from 1988 to 1998 were used to determine the approximate range of experimental temperatures. This is an important time period when greenbug populations can exceed the economic injury level (EIL) and natural enemies can be important for their control (Kring and Gilstrap 1983). *Lysiphlebus testaceipes* was evaluated at the same temperatures, but the targeted greenbug densities were expanded to include 5, 15, 25, 35, 45, 55, 65, and 75 greenbugs per conetainer.

In both experiments, greenbug densities were targeted, but were not exact, because of handling mortality and paedogenesis that caused tested densities to vary slightly. Greenbugs were placed on individual plants within conetainers and caged for these studies. Wheat seed (cv 2137) was planted in 5 cm diameter by 20 cm tall conetainers. When wheat plants reached approximately 30 cm tall (about 3-4 weeks old), they were thinned to 2 tillers and threaded through a 0.6 cm diameter hole in a 5 cm by 0.6 cm thick plexiglass disk. The disk was fit to the conetainer at soil level, and cotton filled up the remaining area of the hole to maintain a sealed experimental arena. A 5 cm

diameter by 30 cm tall clear acetate tube was then fitted around the top of the conetainer. Two 5 cm holes in the side of each acetate tube were covered with fine mesh polyester netting to allow ventilation. The top of each tube was also covered with netting, and held in place by a rubber band. Greenbugs were established by placing second and third instar greenbugs on wheat tillers in each conetainer with a fine brush. By only using similar age greenbugs, complicating factors such as prey age preference by the wasps were avoided. Greenbugs were allowed to settle for 4 hours before introducing wasps.

Each experimental block for the *A. colemani* studies consisted of 16 conetainers divided into four groups (one group per temperature), each group consisted of one conetainer of each targeted density. The *L. testaceipes* experiment was similar except that there were four additional targeted densities (32 conetainers for each experimental block). The experiment was replicated 11 times for *A. colemani* and 10 times for *L. testaceipes*.

During the night cycle preceding experimental setup, parasitoid colonies were purged of all adult wasps. This ensured that all wasps tested were of similar age, having emerged within 12 hours. Newly emerged wasps were aspirated individually into vials and sexed. Single females and males were then placed into each conetainer infested with greenbugs at the beginning of the night cycle.

Conetainers were placed into growth chambers set at the range of temperatures previously stated. After 24 h, both wasps in each conetainer were removed and survival was recorded. If a female did not survive, data were not recorded from that conetainer. During the 2h hour period that the parasitoids were exposed to greenbugs, both species of wasps were only active during the 12 hour light period and were quiescent when lights were off. The conetainers were then placed in a 26°C chamber for 2.5 days to allow

parasitoids to develop into larvae before dissections were attempted. Eggs are quite difficult to detect, thus waiting until larvae were present greatly improved the accuracy of data gained from dissections. Greenbugs were held at 5°C to arrest parasitoid development, until they were dissected into a solution of 2% saline and 1% detergent.

Dissections were completed by grasping the greenbug head with a pair of fine forceps and "pricking" the anal region with a second pair of fine forceps to open the body cavity. The second pair of forceps were used to gently squeeze the contents from the greenbug into the dissecting solution. The contents were then examined for the presence of parasitoid larvae. Numbers of larvae present in each greenbug and the total numbers of greenbugs dissected were recorded. The total number of parasitoid larvae present were assumed to be approximately equal to the total number of eggs each wasp can lay in 24 hours (this includes daytime active periods and night time inactivity by these parasitoids), though there may be some small differences due to egg mortality (Hofsvang and Hågvar 1978, van Steenis 1993).

Statistical Analysis. The effects of temperature and greenbug density on the linear relationships for functional responses, 24 hour oviposition rates, and superparasitism rates, were tested using PROC GLM at $P = 0.05$ level of significance ($N_{A.\ colemani} = 176$, $N_{L.\ testaceipes} = 204$). A dummy variable procedure was used to compare regression lines at each temperature for the regression equation:

$$y = \beta_0 + \beta_1 D + \beta_2 X + \beta_3 DX + \beta_4 DX^2 + \beta_5 DX^2 + \beta_6 DX^3 + \beta_7 DX^3$$

In this equation, **y** = the number of hosts parasitized, the number of eggs laid in 24 hours,

or the number of greenbugs superparasitized depending on which analysis is being performed, β_{0-7} are estimates generated by the PROC GLM, D is the dummy variable represented by 0 or 1, and X is the greenbug density. Coefficients of determination (r^2 values) were calculated by PROC NLIN (SAS Institute 1996) to determine which nonlinear model, Types I, II, or III, best described the functional responses. The models are:

Type I: $N_A = aTN$,

Type II: $N_A = aTN/(1+aT_hN)$,

Type III: $N_A = N[1-\exp(-a(T - T_hN_A))]$

In these models, N_A is the number of hosts parasitized, N is the initial host density, T is the time available for searching during the experiment, a is the instantaneous rate of discovery, and T_h is the amount of time the parasitoid spent handling the host. Though a and T_h can be measured by observation (Mills and Gutierrez 1999), it was not practical to do so in this experiment, therefore parameters a and T_h from the functional response models were estimated using PROC NLIN (Royama 1971, SAS Institute 1996).

Results

Functional Responses. At all temperatures, *A. colemani* and *L. testaceipes* attack rates most closely followed type III functional response curves (Table 3.1). Temperature did not significantly influence the functional response of *A. colemani,* (Table 3.2; Fig. 3.1A). Parasitism by *A. colemani* reached a maximum of 55 greenbugs parasitized out of 70 total greenbugs at 18°C, however this maximum was not significantly different from maximums for the other temperatures. Temperature significantly influenced functional response of *L. testaceipes* for which parasitism ranged from means of 30 parasitized

greenbugs per 70 total greenbugs at 14°C to about 50 parasitized greenbugs per 70 total greenbugs at 26°C (Fig. 3.1B). Though not statistically compared with *A. colemani*, fewer greenbugs were parasitized by *L. testaceipes* at the same density for experimental temperatures of 14°C, 18°C and 22°C. Functional responses appeared similar for the two species at 26°C (Fig. 3.1)

Parasitoid 24-Hour Oviposition. Ovipositional rates for each species were slightly higher than attack rates indicating the occurrence of superparasitism (Table 3.3; Fig 3.2). For example, 60 *L. testaceipes* larvae were dissected from a group of 75 greenbugs at 26°C while only 50 of those 75 greenbugs were parasitized. Ovipositional rates for *A. colemani* were similar at all temperatures (Fig. 3.2A). *Lysiphlebus testaceipes* ovipositional rate was significantly reduced at 14°C and 18°C as compared to 22°C and 26°C (Fig. 3.2B). This was especially evident at low host densities. Larvae produced by *L. testaceipes* at 22°C and 26°C were similar (Fig. 3.B). Compared with *L. testaceipes*, *Aphidius colemani* produced higher numbers of larvae at all temperatures and densities except at 26°C where they both produced nearly the same number of larvae at high greenbug densities (Fig. 3.2).

Superparasitism. The prevalence of superparasitism by *A. colemani* was not consistently affected by temperature (Table 3.4; Fig. 3.3A). However, the extent of superparasitism by *L. testaceipes* was temperature dependent. The lowest superparasitism by *L. testaceipes* occurred at 14°C, with rates becoming higher as temperatures increased (Fig. 3.3). Comparisons of superparasitism at 14°C, 18°C and 22°C reveals a higher prevalence of supernumerary larvae for *A. colemani* than *L. testaceipes* at all greenbug densities. However, as greenbug density approached 60 greenbugs per conetainer at

26°C, super-parasitism by *L. testaceipes* exceeded *A. colemani's* (Fig. 3.3B). Maximum numbers of larvae found in one greenbug were 10 and 13 for *A. colemani* and *L. testaceipes,* respectively.

Discussion

Lysiphlebus testaceipes has been observed to suppress greenbug populations in winter wheat, but cold temperatures may limit its effectiveness (Ramaseshiah et al. 1968, Tyler and Jones 1974, Elliott et al. 1999). My results show that *L. testaceipes* attack rate declines somewhat at 18°C and dramatically at 14°C (Fig. 3.1B). The change in functional response with temperature changes is not isolated to *L. testaceipes*; other parasitoid species including *Cephalonomia waterstoni* (Gahan)(Hymenoptera: Bethylidae), and *Cardiochiles phillippinensis* Ashmead (Hymenoptera: Braconidae) have been observed to have significantly reduced functional responses in relation to temperature (Flinn 1991, Runjie et al. 1996). These results indicate that when temperature is below 14°C, parasitism by *L. testaceipes* cannot be expected to increase proportionally with increasing greenbug populations, because greenbugs can reproduce even when temperatures dip to 5.86°C (Walgenbach et al. 1988).

There are conflicting observations on the lower temperature limits for *L. testaceipes* oviposition. Sekhar (1960) reported no ovipositional activity at 14°C, while Hunter and Glenn (1909) reported successful oviposition at 3.3°C and feeble attempts at 1.67°C. Hunter and Glenn (1909) also reported that *L. testaceipes* was able to survive temperatures below 0°C, and oviposits later when temperatures were warmer. I have personally observed active adult *L. testaceipes* during cold weather (<10°C, on a sunny day).

The attack rate of *A. colemani* was not affected by the four temperatures tested. This suggests that *A. colemani* could parasitize greenbugs at lower temperatures than *L. testaceipes*. Considering this attribute alone, *A. colemani* appears to be a good candidate parasitoid for control of greenbug in late fall and early spring in the Southern Plains, when temperatures are frequently below 14 °C.

Both species laid more eggs than the number of greenbugs parasitized. This may simply reflect the tendencies of both wasps to superparasitize in the closed environment of the conetainer cage. Studies by Messenger (1968) also recorded similar superparasitism rates that were independent of host density for an aphid parasitoid *Praon exsoletum* (Nees).

Incorporation of *A. colemani* and *L. testaceipes* into a comprehensive integrated control program for greenbug regulation, requires a thorough understanding of their biology and effectiveness over a range of plant growth stages and environmental conditions. The results of this study provide insights as to how these parasitoids behave at different temperatures and also gives insights about greenbug control mechanisms.

Acknowledgments

I wish to thank Mr. Les Magee, Mr. Tim Johnson, Ms. Leila Johnson, Dr. Larry Claypool and Dr. Roger Fuentes for their contributions toward this research project.

References Cited

Elliott, N. C., B. W. French, J. D. Burd, S. D. Kindler and D. K. Reed. 1994. Parasitism, adult emergence, sex ratio, and size of *Aphidius colemani* (Hymenoptera: Aphidiidae) on several aphid species. Great Lakes Entomol. 27: 137-142.

Elliott, N. C., J. A. Webster and S. D. Kindler. 1999. Developmental response of *Lysiphlebus testaceipes* to temperature. Southwest Entomol. 24: 1-12.

Flinn, P. W. 1991. Temperature-dependent functional response of the parasitoid *Cephalonomis waterstoni* (Gahan)(Hymenoptera: Bethylidae) attacking rusty grain beetle larvae (Coleoptera: Cucujidae). Environ. Entomol. 20(3): 872-876.

Grasswitz, T. R. 1998. Effect of adult experience on the host-location behavior of the aphid parasitoid *Aphidius colemani* Viereck (Hymenoptera: Aphidiidae). Biological Control 12:177-181.

Hassell, M. P., J. H. Lawton and J. R. Beddington. 1977. Signoid functional responses by invertebrate predators and parasitoids. J. Anim. Ecol. 46:249-262.

Hassell, M. P. 1978. The dynamics of arthropod predator-prey systems. Princeton University Press, Princeton.

Hight, S. C., R. D. Eikenbary, R. J. Miller and K. J. Starks. 1972. The greenbug and *Lysiphlebus testaceipes*. Environ. Entomol. 1:205-209.

Hofsvang, T., and E. B. Hågvar. 1978. Larval morphology and development of *Aphidius colemani* Viereck and *Ephedrus cerasicola* Starý (Hym., Aphidiidae). Norw. J. Ent. 25: 1-8.

Holling, C. S. 1959a. The components of predation as revealed by a study of small-mammal predation of the European pine sawfly. Can. Entomol. 91: 293-320.

Holling, C. S. 1959b. Some characteristics of simple types of predation and parasitism. Can. Ent. 91: 385-398.

Holling, C. S. 1961. Principles of insect predation. Ann. Rev. Entomol. 6: 163-182.

Hunter, S. J. and P. A. Glenn. 1909. The greenbug and its enemies. Univ. Kans. Sci. Bull. 9: 221pp.

Jackson, H. B., L. W. Coles, E. A. Wood and R. D. Eikenbary. 1970. Parasites Reared from the Greenbug and Corn Leaf Aphid in Oklahoma in 1968 and 1969. [*Schizaphis Graminum, Rhopalosiphum Maidis*]. J. Econ. Entomol. 63:733-736.

Kring, T. J. and F. E. Gilstrap. 1983. Within-field distribution of greenbug (Homoptera: Aphididae) and it's parasitoids in Texas winter wheat. J. Econ. Entomol. 76: 57-62.

Kring, T. J., F. E. Gilstrap and G. J. Michels Jr. 1985. Role of Indigenous Coccinellids in regulating greenbugs (Homoptera: Aphididae) on Texas Grain Sorghum. J. Econ. Entomol. 78: 269-273.

Messenger, P. S. 1968. Bioclimatic studies of the aphid parasite Praon exsoletum. 1. Effects of temperature on the functional response of females to varying host densities. Can. Ent. 100: 728-741.

Mills, N. J. and A. P. Gutierrez. 1999. Biological control of insect pests: a tritrophic perspective. *In* B. A. Hawkins and H. V. Cornell [eds], Theoretical Approaches to Biological Control. Cambridge University Press. Cambridge UK. pp 89-102.

Oaten, A. and W. W. Murdoch. 1975. Functional response and stability in predator-prey systems. Amen. Nat. 109: 289-298.

Patrick C. D. and E. P Boring, III. 1990. Managing insect and mite pests of Texas

small grains. TA&M Extension Publication # B-1251. 11 pp.

Prokrym, D. R., K. S. Pike, and D. J. Nelson. 1998. Biological control of *Diuraphis noxia* (Homoptera: Aphididae): Implementation and evaluation of natural enemies: S. S. Quisenberry, and F. B. Peairs Eds. Response model for an introduced pest - the Russian wheat aphid. Thomas A. Publications in Entomology. Published by the Entomological Society of America. Landover MD pp 167-183.

Ramakrishna Ayyar, T. V. 1934. Hymenopterous parasites of economic importance in S. India. Madras Agricultural J. 22: 430-443.

Ramaseshiah, G., K. V. Bhat and P. R. Dharmadhikari. 1968. Influence of host aphid, host plant and temperature on the laboratory breeding of *Lysiphlebus testaceipes*. Indian J. Ent. 30:281-285.

Rice M. E. and G. E. Wilde. 1988. Experimental evaluation of predators and parasitoids in suppressing greenbugs (Homoptera: aphididae) in sorghum and wheat. Environ. Entomol. 17: 836-841.

Royama, T. 1971. A comparative study of model for predation and parasitism. Res. Pop. Ecol. Suppl. 1: 1-91.

Royer, T. A., K. L. Giles and N. C. Elliott. 1998. Small grain aphids in Oklahoma. Oklahoma State University Extension Facts F-7183.

Runjie, Z., K. L. Heong and L. T. Domingo. 1996. Relationship between temperature and functional response in *Cardiochiles phillippinensis* (Hymenoptera: Braconidae), a larval parasitoid of *Cnaphalocrocis medinalis* (Lepidoptera: Pyralidae). Environ. Entomol. 25(6): 1321-1324.

Sandness, J. N. and J. A. McMurtry. 1970. Functional response of three species of

Phytoseiidae (Acarina) to prey density. Can. Entomol. 102: 692-704.

SAS Institute. 1996. SAS user's guide: statistics, version 6.12. ed. SAS Institute, Cary NC.

Sekhar, P. S. 1960. Host relationships of *Aphidius testaceipes* (Cresson) and *Praon aguti* (Smith), primary parasites of aphids. Can. J. Zoo. 38: 593-603.

Solomon, M. E. 1949. The natural control of animal populations. J. Animal Ecol. 18: 1-45.

Starý, P. 1975. *Aphidius colemani* Viereck: its taxonomy, distribution, and host range. Acta Entomol. Bohemoslov. 72: 156-163.

Tostowaryk, W. 1972. The effect of prey defense on the functional response of *Podisus modestus* (Hemiptera: Pentatomidae) to densities of the sawflies *Neodiprions swainei* and *N. pratto banksianae* (Hymenoptera: Neodiprionidae). Can. Entomol. 104: 61-69.

Tyler, B. M. J. and P. A. Jones. 1974. Influence of Low Temperature on Development and Successful Emergence of *Lysiphlebus testaceipes*, a Parasite of the Greenbug. [Schizaphis Graminum]. Environ. Entomol. 3:377-379.

van Steenis, M. J. 1993. Intrinsic rate of increase of *Aphidius colemani* Vier. (Hym., Braconidae), a parasitoid of *Aphis gossypii* Glov. (Hom., Aphididae), at different temperatures. J. Appl. Ent. 116:192-198.

Walgenbach, D. D., N. C. Elliot and R. W. Kieckhefer. 1988. Constant and fluctuating temperature effects on developmental rates and life table statistics of the greenbug. J. Econ. Entomol. 81:501-507.

Table 3.1. Goodness of fit for functional response prediction regressions for *Lysiphlebus testaceipes* and *Aphidius colemani* at 14°, 18°, 22° and 26°C (24 hours 12:12 L:D) on greenbugs.

Parasitoid Species[a]	Temperature	Type I r^2	Type II r^2	Type III r^2
L. testaceipes	14°	0.660	0.606	**0.925**
	18°	0.734	0.773	**0.941**
	22°	0.699	0.750	**0.946**
	26°	0.659	0.924	**0.947**
A. colemani	14°	0.891	0.907	**0.953**
	18°	0.927	0.935	**0.966**
	22°	0.864	0.870	**0.912**
	26°	0.871	0.887	**0.926**

[a] *Lysiphlebus testaceipes* host densities ranged from 5 to 75 greenbugs per container and *A. colemani* host densities were 10 to 70 greenbugs per conetainer. Type I, II and III functional response curves were evaluated using SAS PROC NLIN to generate r^2 values indicating best fit (bold).

Table 3.2. Statistical results for PROC GLM dummy variable analysis of functional response for *Aphidius colemani* and *Lysiphlebus testaceipes* at 14°, 18°, 22° and 26°C (24 hours 12:12 L:D) on greenbugs.

	Aphidius colemani		
Temp.	18°	22°	26°
14°	F=1.41; df=4,80; *P*=0.2381	F=0.43; df=4,80; *P*=0.7870	F=0.55; df=4,79; *P*=0.7020
18°		F=0.61; df=4,80; *P*=0.6567	F=0.73; df=4,79; *P*=0.5757
22°			F=1.07; df=4,79; *P*=0.3779
	Lysiphlebus testaceipes		
	18°	22°	26°
14°	F=5.55; df=4,101; *P*=0.0005	F=7.35; df=4,97; *P*<0.0001	F=13.93; df=4,94; *P*<0.0001
18°		F=0.66; df=4,94; *P*=0.6233	F=1.26; df=4,91; *P*=0.2932
22°			F=0.69; df=4,87; *P*=0.6001

Table 3.3. Statistical results for PROC GLM dummy variable analysis of parasitoid 24-hour oviposition for *Aphidius colemani* and *Lysiphlebus testaceipes* at 14°, 18°, 22° and 26°C (24 hours 12:12 L:D) on greenbugs.

	Aphidius colemani		
Temp.	18°	22°	26°
14°	F=1.82; df=4,80; *P*=0.1330	F=1.40; df=4,80; *P*=0.2415	F=1.36; df=4,79; *P*=0.2760
18°		F=0.35; df=4,80; *P*=0.8400	F=0.79; df=4,79; *P*=0.5373
22°			F=1.86; df=4,79; *P*=0.4902
	Lysiphlebus testaceipes		
	18°	22°	26°
14°	F=4.58; df=4,101; *P*=0.0019	F=8.76; df=4,97; *P*<0.0001	F=13.03; df=4,94; *P*<0.0001
18°		F=1.55; df=4,94; *P*=0.1928	F=2.25; df=4,91; *P*=0.0696
22°			F=0.63; df=4,87; *P*=0.6422

Table 3.4. Statistical results for PROC GLM dummy variable analysis of the incidence of superparasitism by *Aphidius colemani* and *Lysiphlebus testaceipes* at 14°, 18°, 22° and 26°C (24 hours 12:12 L:D) on greenbugs.

	Aphidius colemani		
Temp.	18°	22°	26°
14°	F=1.47; df=4,80; *P*=0.2184	F=2.77; df=4,80; *P*=0.0328	F=1.90; df=4,79; *P*=0.1193
18°		F=0.46; df=4,80; *P*=0.7651	F=0.43 df=4,79; *P*=0.7834
22°			F=0.85; df=4,79; *P*=0.4950
	Lysiphlebus testaceipes		
	18°	22°	26°
14°	F=1.50; df=4,101; *P*=0.2091	F=9.17; df=4,97; *P*<0.0001	F=7.42; df=4,94; *P*<0.0001
18°		F=3.92; df=4,94; *P*=0.0055	F=2.78; df=4,91; *P*=0.0314
22°			F=1.04; df=4,87; *P*=0.3926

Fig. 3.1 A & B. Functional responses of *Aphidius colemani* and *Lysiphlebus testaceipes* respectively at 14°, 18°, 22° and 26°C (12:12 L:D) on greenbugs. Regressions with the same letter listed in the legend have slopes that are not significantly different at P=0.05, SAS PROC GLM.

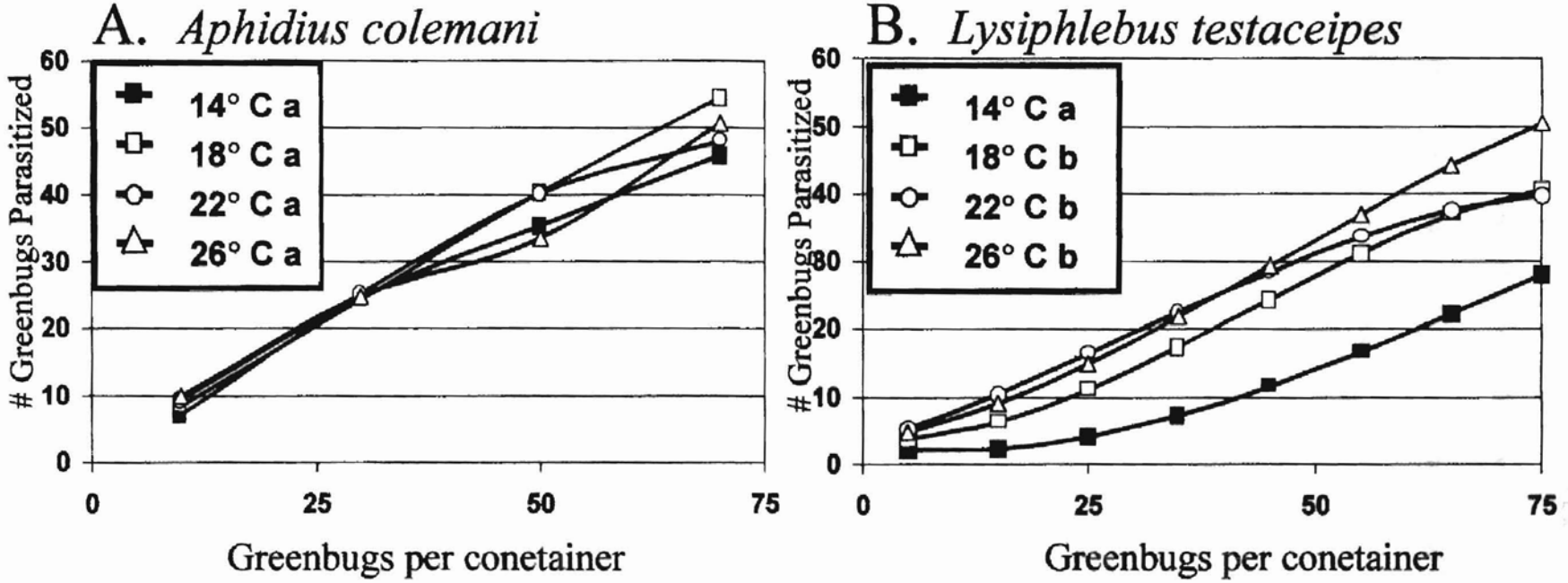
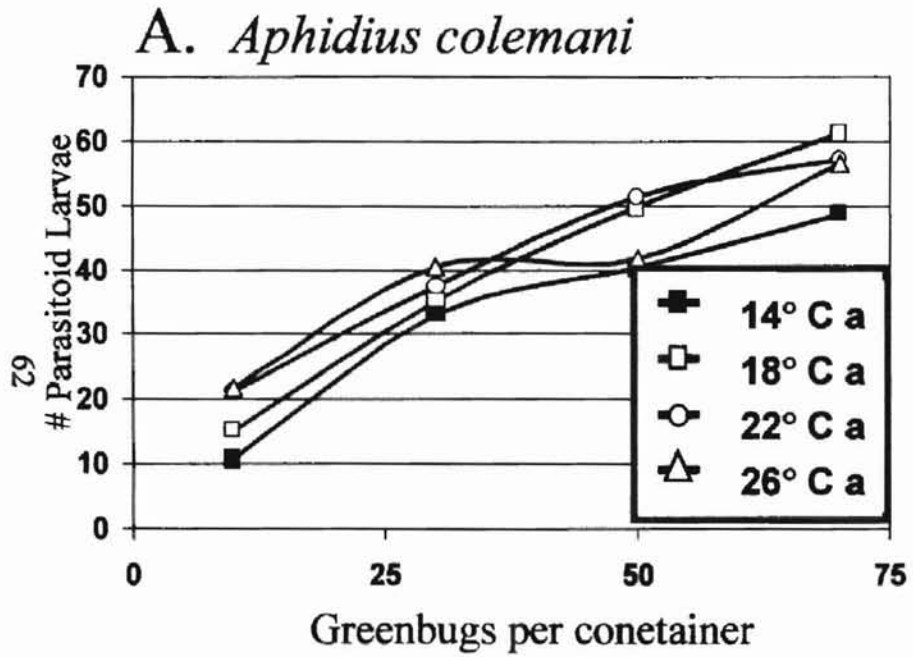


Fig. 3.2 A & B. Total number of parasitoid larva found by dissection of greenbugs parasitized by *Aphidius colemani* and *Lysiphlebus testaceipes* at 14°, 18°, 22° and 26°C (12:12 L:D). Regressions with the same letter listed in the legend have slopes that are not significantly different at P=0.05, SAS PROC GLM.


A. Aphidius colemani
Parasitoid Larvae
0
10
20
30
40
50
60
70
0
25
50
75
Greenbugs per conetainer
14° C a
18° C a
22° C a
26° C a

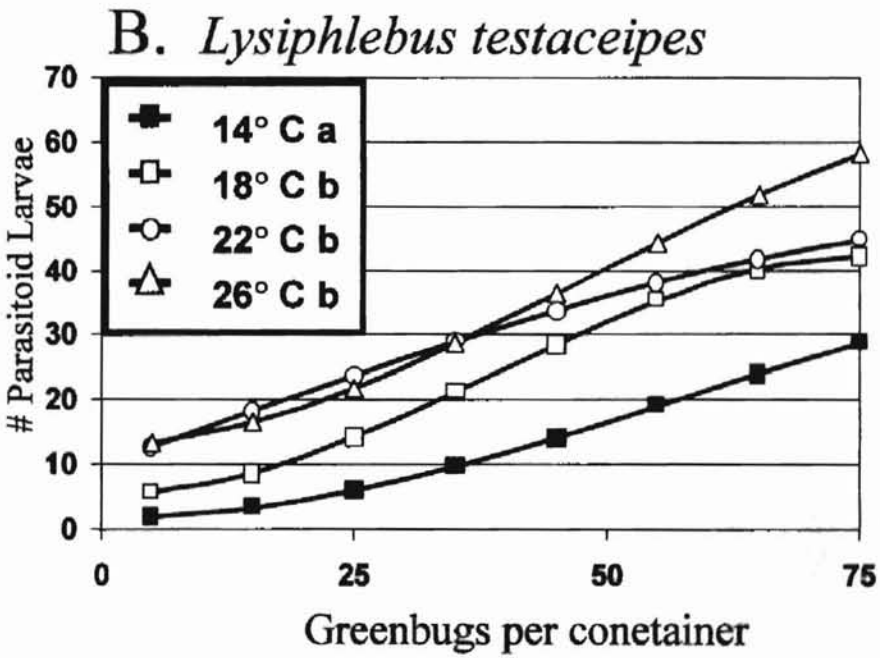
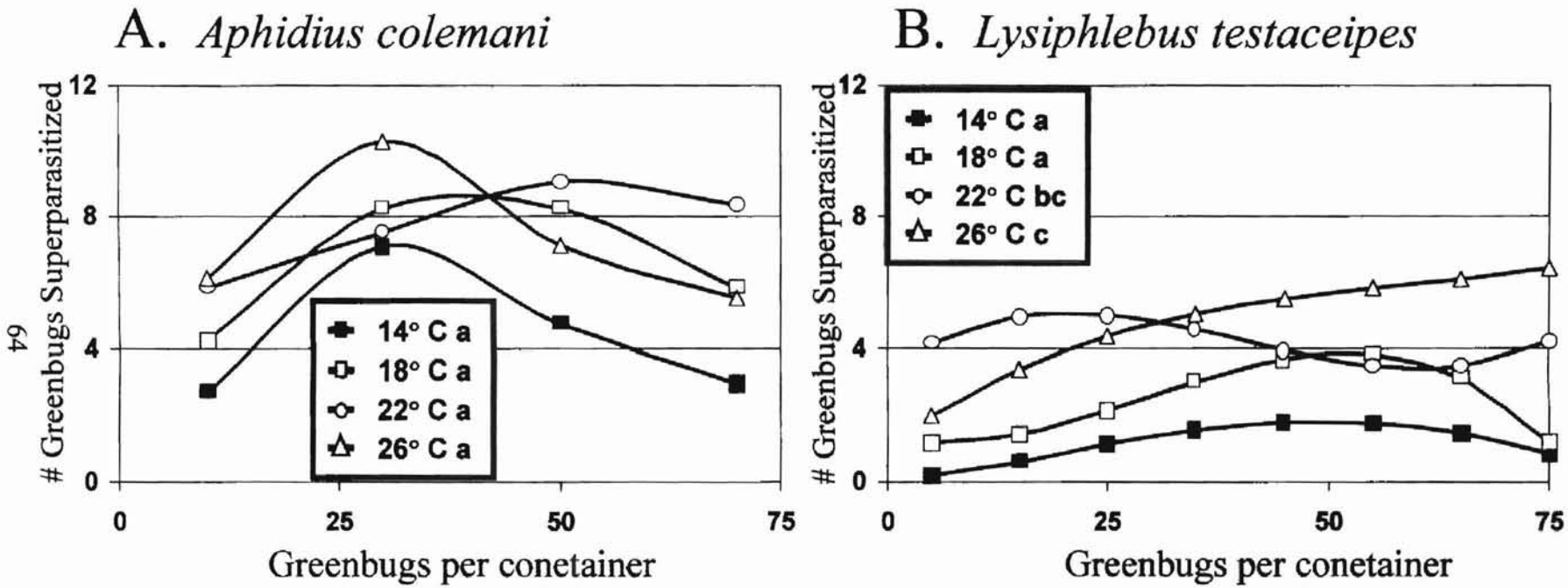

B. Lysiphlebus testaceipes
Parasitoid Larvae
0
10
20
30
40
50
60
70
0
25
50
75
Greenbugs per conetainer
14° C a
18° C b
22° C b
26° C b

Fig. 3.3 A & B. Superparasitism of greenbugs by *Aphidius colemani* and *Lysiphlebus testaceipes* respectively at 14°, 18°, 22° and 26°C (12:12 L:D). Regressions with the same letter listed in the legend have slopes that are not significantly different at P=0.05, SAS PROC GLM.


A. Aphidius colemani
Greenbugs Superparasitized
12
8
4
0
0
25
50
75
Greenbugs per conetainer
14° C a
18° C a
22° C a
26° C a
B. Lysiphlebus testaceipes
Greenbugs Superparasitized
12
8
4
0
0
25
50
75
Greenbugs per conetainer
14° C a
18° C a
22° C bc
26° C c

CHAPTER IV: SUPPRESSION OF APHIDS BY THE PARASITOID *LYSIPHLEBUS TESTACEIPES* CRESSON AND THE PREDATOR *HIPPODAMIA CONVERGENS* GUËRIN-MËINVILLE ON WINTER WHEAT: EVALUATION OF NATURAL ENEMY THRESHOLDS

Abstract

Field-caged colonies of greenbug, *Schizaphis graminum* (Rondani), were established on both susceptible (TAM 107) and greenbug resistant (TAM 110) winter wheat during spring of 1999 and 2000 in Grady County, Oklahoma. Initial infestation levels ranging from 0.1 to 10 aphids per tiller of wheat were established within cages by releasing greenbugs in February of each year. Infestations of *Rhopalosiphum padi* (L.) aphids were also present in cages during 1999. After establishing greenbugs, newly emerged *Lysiphlebus testaceipes* Cresson mating pairs were released in cages (40 cages in 1999 and 56 cages in 2000) establishing aphid : released adult parasitoid ratios from 2:1 to >200:1. *Hippodamia convergens* Guërin-Mëinville adults were released in 32 other cages (3 and 6 mating pairs), establishing aphid : beetle ratios from 1:1 to >100:1. Twenty-four other cages served as controls. Cages with aphid to released parasitoid ratios less than 67:1 (>1.5% parasitism rate), kept aphid intensities below economic thresholds on both wheat cultivars. *Hippodamia convergens* were able to maintain aphids below economic thresholds only when initial aphid to beetle ratios were <10:1.

Introduction

The greenbug, *Schizaphis graminum* (Rondani), is a serious pest on wheat, *Triticum aestivum* L, throughout North America. When greenbug infestations surpass economic injury levels (EIL's), grain yield and quality are reduced (Burton et al. 1985, Pike and Schaffner 1985, Kieckhefer and Kantack 1988, Kieckhefer et al. 1994, Noetzel 1994). Damaging infestations of greenbugs occur in Oklahoma almost every year with widespread outbreaks recorded every 5-10 years (Starks and Burton, 1977). In Oklahoma, losses due to greenbugs range from $0.5 to $135 million annually, though much of the losses are due to the expense of insecticide applications (Starks and Burton 1977, Webster 1995).

Without viable alternative management options, many Oklahoma wheat farmers rely solely on insecticide applications to suppress damaging greenbug populations (Massey 1993). During severe greenbug outbreaks, millions of acres are sprayed with insecticides (Shotkoski et al. 1990). These widespread insecticide applications are sometimes economically justifiable. However during most years in Oklahoma, high greenbug populations are usually localized, remaining near or below economic injury levels (EIL) in most parts of the state making widespread insecticide applications questionable (K. L. Giles unpublished data).

Greenbugs are attacked by a number of predators and parasites (Royer et al. 1998). Important examples of theses natural enemies in the Southern Great Plains are Coccinellidae predators such as the convergent lady beetle *Hippodamia convergens* Guërin-Mëinville, and the Nearctic parasitoid *Lysiphlebus testaceipes* Cresson (Ruth et al. 1975, Kring and Gilstrap 1984, Kring et al. 1985, Rice and Wilde 1988). While *H.*

convergens is not the only predator that attacks greenbugs, it is often the most abundant predator found in wheat fields (Rice and Wilde 1988, K. L. Giles unpublished data). *Lysiphlebus testaceipes* parasitizes greenbug and other cereal aphids throughout the wheat growing regions of the United States and is considered to be one of the most important biological control agents of greenbugs in the Southern Plains (Jackson et al. 1970). *Lysiphlebus testaceipes* has been observed to suppress greenbug populations below economic thresholds in both wheat and sorghum (Rice and Wilde 1988, Patrick and Boring 1990).

A natural enemy threshold is the critical ratio of natural enemy vs. pest species population densities required to prevent a pest species from exceeding economic injury levels (Nyrop and van der Werf 1994). For parasitoids, this ratio is generally expressed as a percentage, based on the ratio of apparent parasitized aphids to total aphids. That is, aphid mummies are counted along with aphids present on samples taken from the field. The number of mummies present only represents the number of parasitoids that have completed development to the point that a mummy is apparent. This ignores the aphids that are parasitized, but have not yet formed a mummy. Raising aphids collected for a few days until each parasitoid completes development will provide a more accurate estimate of the "true parasitism rate."

In Oklahoma, Royer et al. (1998) recommends "if one or two predators, such as Coccinellidae, larvae of Chrysopidae and/or larvae of Syrphidae are present per 0.3m of winter wheat furrow, or 20 to 30 percent of greenbugs are parasitized and temperatures are above 13°C, there is a good possibility that aphid populations will be suppressed without insecticidal control." Patrick and Boring (1990) state when there are one or two

lady beetles per 0.3m of furrow or when 15 to 20 percent of greenbugs are parasitized in winter wheat, insecticide application should be delayed until it can be determined if the greenbug population is continuing to increase. Additionally, they state that "warm" weather is required for beneficial insects to have an impact. Though widely publicized, these recommendations are not based on any published experimental data (Elliott et al. 1994). The statement "requiring warm weather for beneficial insects to have an impact" is also vague, making management decisions less reliable. Identification of more reliable natural enemy thresholds based on experimental data and detailed descriptions of natural enemy capabilities are needed to improve management recommendations for greenbug.

My objective was to determine parasitism and predation ratios for greenbug control (population maintenance below economic injury levels) by *L. testaceipes* and *H. convergens* on spring growth of winter wheat in Oklahoma. Because cultivars with host resistance to greenbug may alter natural enemy effectiveness as shown by Starks et al. (1972), Rice and Wilde (1989), Campbell et al. (1992), I evaluated natural enemy to greenbug ratios on susceptible and resistant wheat cultivars. Additionally, I evaluated effectiveness of the newly determined natural enemy threshold ratios by monitoring natural enemy and greenbug populations in wheat fields throughout Oklahoma.

Materials and Methods

I evaluated natural enemy thresholds on greenbug biotype 'E' susceptible wheat (cv. TAM 107) and on resistant wheat (cv. TAM 110). These winter wheat cultivars (0.4 ha each) were established in Grady County, Oklahoma in late September 1998 and 1999. Seeds were drill planted at a rate of 57kg/ha in rows spaced 20 cm apart. Planting was done in fallow soil or soil that had not grown wheat during the previous growing season

to reduce the emergence of "volunteer" wheat. Fields were fertilized with urea (57kg/ha) in 1999. In 2000, fertilizer was not applied.

Experiments were conducted in March and April, 1999 and 2000. This is an important time period when greenbug populations often exceed EILs and natural enemies can be important for their control (Kring and Gilstrap 1983). Population levels for aphids and natural enemies were chosen to match a wide variety of natural enemy-prey ratios encompassing the current natural enemy recommendations in Oklahoma (Royer et al. 1998). Cages with *H. convergens* were established having both 1 and 2 beetles per 0.3m of furrow over a range of greenbug population levels (Table 4.1). Cages with *L. testaceipes* were established with initial aphid to parasitoid ratios from 2:1 to >200:1 (50 to <0.5% parasitism rates). These parasitism ratios were created by considering the released parasitoids to represent mummies. This is to simulate the apparent parasitism rate that a farmer would be more likely to sample for.

Insect Cages. Cages were constructed from 100% polyester "no-see-um" netting (Seattle Fabrics Inc., Seattle, WA) and a lightweight nylon reinforced tarp material was used for the base of each cage. Cage frames consisted of a pair of loops made from ½ inch lightweight metal electrical conduit, their legs were set into the ground such that the apex of each loop was perpendicular to the other. When the fabric cage was pulled over the frame, it created a cage 61 cm wide by 61 cm long by 61 cm tall (Fig 4.1). On one side of the fabric, a pair of cloth tubes (sleeves) were attached for sampling access (Fig. 4.1). The sleeves were then tied tightly together sealing the tubes. The nylon tarp base of the cage was buried into the soil. The top of the fabric was gathered together and tied with a short nylon rope to allow initial access to the cage and to simplify fitting the fabric

to each of the hoops in the field.

Ninety-six insect cages were installed in January 1999 and 112 in January 2000. Half of the cages were placed over TAM 110 wheat and the other half over TAM 107. Cages were placed about 10 m apart in a square grid pattern. A few days after cages were established in the field (>1 month prior to releasing greenbugs), each cage was sprayed with permethrin (300 ml per hectare) and a week later with Malathion (1200 ml per hectare) in an attempt to kill any aphids and natural enemies that were present in the cages before the experiment.

Insect colonies. Biotype "E" greenbugs were obtained from USDA-ARS, Stillwater, OK and established on hard red winter wheat variety 2137, grown in a fritted clay and sphagnum moss medium. Insect colonies and all wheat plants were isolated inside double-layered, fine mesh cages in a greenhouse. Pots of wheat were supplied every few days as needed. These greenbugs were used to maintain parasitoid colonies and to provide greenbugs for infesting field cages.

Lysiphlebus testaceipes were isolated from specimens collected at Perkins, OK and raised in growth chambers at 22°C and a photo period of 12:12 (L:D). Cages with 2 layers of fine mesh spaced 2.5 cm apart were constructed to house parasitoid colonies. Double layering the cage sides prevented feral parasitoids from ovipositing into greenbugs through the cage while permitting ample airflow through the cage. A fresh pot (15 cm diameter) of infested wheat, was placed in each parasitoid colony every 3-4 days to maintain a steady supply of parasitoids. During the night cycle before release of wasps into field cages, wasp colonies were purged of all adults leaving only mummies. This was done to ensure that all wasps used for subsequent releases were of similar age (within 12

hours).

Hippodamia convergens lady beetles were purchased in January of each year from the Beneficial Insect Company, Fort Mill, SC. Beetles were placed into a large cage and maintained on a daily diet of greenbugs and a wheat-yeast-honey mixture. Beetles were sexed and paired up the day before release into field cages.

1999 Experiment. For each cultivar, twelve combinations of greenbug/natural enemy were randomly assigned to 48 field cages (Table 4.1). Each combination was replicated in 4 times. Greenbug levels were targeted at light, moderate and heavy infestations (about 0.1, 1.0 and 10.0 greenbugs per tiller). These greenbug infestation levels represented infestations well below and within the economic threshold range of 8 to 20 greenbugs per tiller in Oklahoma (Royer et al. 1998). Over the course of the experiment, in the absence of natural enemies, light infestations were expected to slowly increase and possibly exceed economic thresholds. Moderate infestations were expected to exceed economic thresholds and heavy infestations were expected to rapidly exceed economic thresholds.

Greenbugs were released on 22 February by clipping and placing infested tillers from greenhouse colonies into assigned cages. Lightly infested cages received about 1/4 pot of clippings, moderately infested cages received a full pot of clipped tillers and heavily infested cages received 4 pots of clippings (N. C. Elliott unpublished data). The following week, field cages were inspected to confirm greenbug densities were near targeted levels. This initial sample revealed that insecticide applications failed to eliminate *Rhopalosiphum padi* (L.) from the cages. I collected and cultured *R. padi* from all field cages, verifying that no parasitoids survived with the aphids. Because populations of greenbugs were

below targeted levels, additional greenbugs were added on 4 March to cages as needed in an effort to establish targeted greenbug densities.

Twelve cages were assigned as untreated checks, 4 light, 4 moderate and 4 heavy greenbug infestations (Table 4.1). Another 12 cages were assigned to receive *H. convergens* (6 female and 6 male), 4 with light greenbug infestations, 4 with moderate and 4 with heavy infestations. Four additional cages with moderate greenbug infestations received 3 male and 3 female *H. convergens*. Releases of 12 predators per cage represented a predator density of two *H. convergens* per 0.3m of crop furrow. Twelve more cages received 4 male and 4 female *L. testaceipes* wasps; 4 with light, 4 with medium and 4 with heavy greenbug infestations. The final 8 cages had medium greenbug infestations with half getting 28 wasps (14 male:14 female) and the other half getting 48 wasps (24 male: 24 female).

On 9 March, after sampling cages to determine the baseline greenbug infestations, all *H. convergens* and half of the *L. testaceipes* (equal numbers of males and females) were placed into cages. Half of the wasps were released a week (15 March) after the first were released to better reproduce multiple life stages typically present in wheat fields.

Field cages were sampled using the arm sleeves after carefully untying them to prevent entry or escape of insects. Using a small pair of scissors, 10 arbitrarily selected tillers were clipped at ground level and placed in a labeled plastic bag that was sealed before removing it from the cage. All field samples were placed in an ice chest and returned to the laboratory where they were examined for greenbugs, other aphids, mummies, and natural enemies. A sub-sample (up to 20 aphids) of the aphids collected from each cage were placed on wheat in caged conetainers and reared for 7 days. This

sub-sample along with the mummy count provided estimates of actual parasitism levels in each cage on each sampling date.

Sampling continued every week (Julian dates 68-96) until 6 April, 1999, when wheat reached boot stage. All remaining wheat in the cage was then inspected to determine the exact number of tillers present at the end of the experiment. Adding 10 tillers for each sample taken, would then give an estimate of the number of wheat tillers present in each cage at the onset of the experiment.

2000 Experiment. Experimental setup for 2000 was similar to 1999 except for the addition of 16 cages (Treatments 7 and 8, Table 4.1) to provide additional wasp/aphid ratios. All 16 additional cages were assigned medium greenbug infestations. Half received 12 wasps (6 male: 6 female) and the other half received 20 wasps (10 male: 10 female).

Greenbugs were released into cages on 2 February 2000. Sampling on 9 February revealed that no greenbugs survived in cages following the initial release. On 18 February, after two weeks of wet weather, greenbugs were released again. Sampling on 25 February, identified cages that were below targeted greenbug infestation levels; additional greenbugs were added later as needed. On 4 March, *H. convergens* and half of the *L. testaceipes* were released into assigned cages. The rest of the parasitoids were released on 9 March. Field cages were sampled every week until wheat reached boot stage on 6 April 2000, when a final sample was taken and the total number of tillers in each cage was counted.

Evaluation of Natural Enemy Thresholds. Ten 0.4 ha wheat fields were provided by cooperators throughout Central Oklahoma in late autumn 1999. Fields were

located close to Ponca City, Billings, Perry, Enid (2), Kingfisher, El Reno (2), Minco, and Chickasha, Oklahoma. Each cooperator pledged to not treat these fields with insecticides during the 1999-2000 wheat growing season. Fields were sampled by clipping 120 arbitrarily selected wheat tillers and inspecting them for living aphids and parasitoid mummies. Mean aphid numbers per tiller (intensity) was determined by dividing the total aphids collected by the number of tillers sampled. Aphids were returned to the laboratory, placed on wheat grown inside conetainers and examined several times over the following 7 days for development of mummies. Additionally 12 arbitrarily selected locations (0.3m of furrow) were examined for lady beetles (both adults and larvae) to determine the mean number of lady beetles per 0.3m of furrow. The ratio of aphids to lady beetles was determined by multiplying the mean aphid intensity by 40 (estimated mean number of tillers per 0.3m of furrow) and dividing this product by the mean number of lady beetles per 0.3m of furrow, giving an estimate of the number of aphids per lady beetle present in the field.

Sampling was initiated on 18 January, 2000 and continued until wheat reached boot stage in early April 2000. Using results from my 1999 experiment, observed parasitism and predation rates were used to predict success or failure of natural enemies for control of greenbugs on these 10 wheat fields. Success of aphid control was based on whether aphid intensities exceeded the economic threshold of 8-20 greenbugs per tiller (Royer et al. 1998).

Statistical Analysis. Because *R. padi* were present in all cages in 1999, data collected were analyzed twice, first using combined aphid numbers (*S. graminum* and *R. padi*) and again using only greenbug population data. Data collected in 2000 were

analyzed using greenbug intensity data (only greenbugs were present in cages).

Since initial aphid levels were only targeted, not exact, it was necessary to calculate initial ratios of aphids per parasitoid, greenbugs per parasitoid, aphids per beetle and greenbugs per beetle for each cage, along with initial greenbugs and initial total aphids per tiller. I calculated the total population of aphids/greenbugs present in each cage using equation [1];

$$T_{pop} = (A/10) \times (S + 10 \text{ x } N) \qquad [1]$$

where T_{pop} is total initial number of aphids/greenbugs in the cage, A is the number of aphids/greenbugs in the last sample taken just before natural enemies were introduced, 10 is the number of tillers clipped in each sample, S is the number of tillers in the cage at the end of the experiment and N is the number of samples taken from each cage after the baseline sample.

Initial parasitism ratios (expressed as percent parasitism were calculated by equation [2];

$$P_{rate} = [P / (T_{pop} + P)] \text{ x } 100 \qquad [2]$$

where P_{rate} is the parasitism ratio expressed as a percentage, P is the total number of parasites released, and T_{pop} is the initial number of aphids/greenbugs in the cage.

Initial aphids per *H. convergens* beetle were calculated with equation [3];

$$PRED_{rate} = T_{pop} / B \qquad [3]$$

where $PRED_{rate}$ is the initial number of aphids/greenbugs per beetle, T_{pop} is the initial number of aphids/greenbugs in the cage and B is the total number of beetles released in the cage.

Data from each cage were analyzed using PROC GLM (SAS Institute 1996). Based on these analyses I was able to statistically group ($P < 0.05$) regressions with similar initial natural enemy to aphid ratios for each wheat cultivar (Table 4.2). Data from cages without natural enemies were used to construct control data groupings that were paired up with data from cages with natural enemies with similar initial aphid per tiller intensities (Table 4.2). Aphid/greenbug population growth for natural enemy data groupings were compared to their corresponding controls using PROC MIXED (SAS Institute 1996), again using $P < 0.05$ level of significance.

Results

All Aphids (1999). Statistical values are reported in Table 4.3. Compared to untreated controls, cages containing *L. testaceipes* had slower aphid population growth over sampling dates (Fig. 4.2 A-D). Cages with initial aphid to *L. testaceipes* ratios greater than 67:1 (<1.5 percent initial parasitism) on TAM 107 wheat eventually approached the upper economic threshold of 20 aphids per tiller (Royer et al. 1998).. However, such a low parasitism rate still significantly limited aphid intensities to less than half of corresponding aphid intensities in control cages (Fig. 4.2A). Cages with initial aphid to *L. testaceipes* ratios greater than 67:1 (<1.5 percent initial parasitism) on TAM 110 wheat also displayed significantly slower population growth such that aphid intensities

on 6 April were less than half of the corresponding control (Fig 4.2B). In cages with initial aphid to *L. testaceipes* ratios less than 67:1 (>1.5 percent initial parasitism), mean aphid intensities never reached the lower economic threshold of 8 aphids per tiller on each wheat cultivar (Fig. 4.2 C&D). However, mean aphid intensities on TAM 110 wheat were not significantly different from controls over all sampling dates (Fig. 4.2D).

Aphid intensities in TAM 107 cages treated with *H. convergens* with mean initial aphid to beetle ratios under 10 to 1 stayed below 5 aphids per tiller throughout the experiment. However, mean aphid intensities on 6 April (Julian date 96) were not significantly different from the corresponding control even though it was approaching 20 aphids per tiller(Fig 4.3A). Aphid intensities in cages with mean initial aphid to beetle ratios that exceeded 25 aphids per *H. convergens*, were not significantly different from corresponding control cages for all sampling dates (Fig 4.3C).

Success of *H. convergens* in regulating aphid numbers on TAM 110 wheat were similar to that observed on TAM 107 wheat. Mean aphid intensities in cages with initial aphid to *H. convergens* ratios less than 10 to 1 stayed low, never exceeding 5 aphids per tiller, however they were not significantly different from the corresponding control cages for all sampling dates (Fig. 4.3B). Mean aphid intensities in cages with more than 25 aphids per *H. convergens* beetle initially, continued to grow, paralleling the untreated control cages (Fig 4.3D).

Greenbugs Only (1999). Statistical values are reported in Table 4.3. Mean greenbug intensities in TAM 107 Cages with initial greenbug to *L. testaceipes* ratios greater than 25:1 (<4.0% initial parasitism) stayed below 10 greenbugs per tiller and were significantly different from mean greenbug intensities in corresponding control cages

which exceeded 20 greenbugs per tiller (Fig. 4.4A). Mean greenbug intensities in TAM 110 cages with initial greenbug to *L. testaceipes* ratios greater than 25:1 (<4.0% initial parasitism) also stayed below 10 greenbugs per tiller for all sampling dates, but were not significantly different from mean greenbug intensities in corresponding control cages (Fig. 4.4B). Mean greenbug intensities in cages with initial greenbug to *L. testaceipes* ratios less than 25:1 (>4.0% initial parasitism), stayed below 2 greenbugs per tiller on both TAM 107 and TAM 110 wheat, but were not significantly different from greenbug intensities in corresponding control cages (Fig. 4.4C&D).

Greenbug intensities in cages with initial greenbug to *H. convergens* ratios less than 50 to 1 stayed well below treatment thresholds on TAM 107 wheat, but were not significantly different from corresponding check cages. However, when initial greenbug to *H. convergens* ratios were greater than 50 to 1, mean greenbug intensities stayed just under 10 greenbugs per tiller, but were significantly less than control cages where mean greenbug intensities reached 23 greenbugs per tiller (Fig. 4.5C). On TAM 110 wheat, greenbug intensity means stayed less than 5 greenbugs per tiller for all initial greenbug to *H. convergens* ratios over all sampling dates and were not significantly different from controls (Fig. 4.5B&D).

2000 Experiment. Statistical values are reported in Table 4.4. For all cages in the 2000 experiment, greenbug populations did not increase regardless of treatment, including controls. On TAM 107 wheat, greenbug intensities stayed constant or decreased to near 0 greenbugs per tiller by the end of the experiment (Figs 4.6A-D and 4.7A-D). In all cases, greenbug intensities did not exceed 8 greenbugs per tiller with most cages never exceeding 4 greenbugs per tiller (Figs 4.6A-D and 4.7A-D). Cages with *L. testaceipes*

exhibited slight greenbug population drops, but they were not significantly different from the controls (Figs 4.6A-D). Greenbug intensities dropped quickly to near 0 greenbugs per tiller for all cages on TAM 110 wheat (Figs. 4.6B&D and 4.7B&D).

Evaluation of Natural Enemy Thresholds. In all 10 fields, aphid intensities including greenbugs never exceeded an average of 1.5 aphids per tiller (Table 4.5); well below the lower 8 greenbugs per tiller economic treatment threshold (Royer et al. 1998). Apparent parasitism rates were well above 1.5% for most of the sampling dates and reached peaks of over 50%. Aphids that were returned to the laboratory and reared, revealed actual parasitism rates as high as 100% (Table 4.5). Aphids per lady beetle were measured, but generated results that fluctuated widely (from lows of 24 aphids per beetle to 2460 aphids per beetle). Frequently, no lady beetles were present at all.

Discussion

The presence of *R. padi* in cages during the 1999 season made data analysis more difficult. However, results of this experiment are still useful since greenbugs are not always the only aphid feeding on winter wheat in Central Oklahoma (Royer et al. 1998). Economic thresholds for greenbug are the most stringent of winter wheat pest aphids (Royer et al. 1998). By examining all aphids, Coccinellidae and parasitoids present in the wheat field, decisions can be made based on ratios of natural enemies to all aphids present and be extended to predict greenbug control.

Berryman (1992) suggested that natural enemy to prey ratios solve many of the predator-prey model paradoxes. Use of natural enemy threshold ratios would make decision making models for greenbug and other pest aphids more accurate. *Hippodamia convergens* were able to suppress aphid populations in 1999 only when there were fewer

than 10 aphids per beetle present in the cage (Fig. 4.3). In 2000, greenbug populations decreased over time in cages treated with *H. convergens*. However since greenbug populations did not increase in untreated cages, I cannot state that *H. convergens* was responsible for greenbug control. The observed aphid control in 1999, at low aphid to beetle ratios is similar to results reported by Eigenbrode et al. (1998); *H. convergens* controlled low levels of *Acyrthosiphon pisum* (Harris) on *Pisum sativum* L. in cages, but was not observed to provide control at high aphid to beetle ratios.

Results of this experiment do not support the recommendation that one or two lady beetles per 0.3m of crop furrow will provide greenbug control, except for very low aphid populations (less than 10 aphids per beetle). However, because *H. convergens* is a generalist predator and greenbugs and *R. padi* are only a part of its overall diet (Hodek 1973, Murdoch et al. 1985), a caged study may not adequately define their abilities. My experiment provided little time for progeny to develop and provide control; *H. convergens* require about 30 days to complete development from egg to adult at 20°C, in addition to requiring temperatures above 15°C to complete development beyond the first 2 larval instars (Butler and Dickerson 1972, Gutierrez et al. 1981, Obrycki and Tauber 1982, Michels and Behle 1991). Small larvae (first and second instar) were present in the cages, but there was insufficient time for development into the more voracious third and fourth instars (Chedester 1979, Okrouhla et al. 1983). Given more time with moderate temperatures, *H. convergens* may have been able to reduce larger aphid populations in the cages. Additionally, and perhaps most importantly, cages prevent large aggregative responses by adult beetles, which may be important for aphid suppression by *H. convergens* (Rowlands and Chapin 1978, K. L. Giles unpublished data)

Lysiphlebus testaceipes was able to regulate aphid and greenbug intensities below 8 aphids per tiller when initial aphid to parasitoid ratios were <67:1 (>1.5% initial parasitism rate) on both greenbug resistant and susceptible winter wheat (Figs. 4.2 C&D). This is far below the recommendations of 15 to 20% mummified greenbugs by Patrick and Boring (1990) and 20 to 30% mummified greenbugs by Royer et al (1998). These results may be influenced somewhat by a cage effect, but provide empirical measurements of *L. testaceipes* capabilities for greenbug control , which here to fore have been lacking. Since *L. testaceipes* has been observed to limit greenbug populations in winter wheat (Ramaseshiah et al. 1968, Tyler and Jones 1974), it is probable that *L. testaceipes* is indeed able to provide control of greenbug in most instances when the parasitism of the overall aphid population is between 1.5 and 15%. However, the effective ratio could be heavily influenced by temperature because laboratory studies for *L. testaceipes* on greenbug show that parasitism rates are greatly reduced at temperatures below 18°C (D. B. Jones unpublished data).

For the validation part of this study I tentatively identified natural enemy thresholds for aphids in winter wheat (>1.0% for *L. testaceipes* and <10 aphids per *H. convergens* beetle). My results demonstrated that I did not make any wrong decisions (Type II errors; aphids exceeded economic thresholds when they were expected to be suppressed) about aphid control in spring 2000 when I examined apparent parasitism percentages at all 10 locations (Table 4.5). However when I relied on aphid to lady beetle ratios, I was wrong in every instance (aphid to lady beetle ratios predicted failure of biological control yet aphid populations never exceeded 1.5 aphids per tiller (Table 4.5). These results would indicate that *L. testaceipes* and other parasitoids are primarily

responsible for aphid control in winter wheat production. However, lady beetles, such as *H. convergens* are still important, adding to aphid control when their populations are high enough. Further validation research over several years must be performed to effectively document the probabilities of success.

Acknowledgments

I wish to thank Mr. Les Magee, Mr. Tim Johnson, Ms. Melissa Riley, Ms. Kwanza Stewart, Dr. Larry Claypool and Dr. Roger Fuentes for their contributions toward this research project.

References Cited

Berryman, A. A. 1992. The origins and evolution of predator-prey theory. Ecology. 75(5): 1530-1535.

Burton, R. L., D. D. Simon, K. J. Starks and R. D. Morrison. 1985. Seasonal Damage by Greenbugs (Homoptera: aphididae) to a Resistant and a Susceptible Variety of Wheat. J. Econ. Entomol. 78: 395-401.

Butler, G. D. and W. A. Dickerson. 1972. Life Cycle of the Convergent Lady Beetle in Relation to Temperature. [*Hippodamia convergens*]. J. Econ. Entomol. 65: 1508-1509.

Campbell, R. K., D. K. Reed, J. D. Burd and R. D. Eikenbary. 1992. Russian wheat aphid and drought stresses in wheat: tritrophic interactions with *Diaeretiella rapae* and plant resistance. Insect-Plant Relationships. 8: 297-298.

Chedester, L. D. 1979. Feeding habits, reproduction and sexual determination of the convergent lady beetle, *Hippodamia convergens* (Guer.). Texas Agricultural Experiment Station publication MP-1437. 4p.

Eigenbrode, S. D., C. White, M. Rohde and C. J. Simon. 1998. Behavior and effectiveness of adult *Hippodamia convergens* (Coleoptera: Coccinellidae) as a predator of *Acyrthosiphon pisum* (Homoptera: Aphididae) on a wax mutant of *Pisum sativum*. Environ. Entomol. 27(4): 902-909.

Elliott, N. C., B. W. French, J. D. Burd, S. D. Kindler and D. K. Reed. 1994. Parasitism, adult emergence, sex ratio, and size of *Aphidius colemani* (Hymenoptera: Aphidiidae) on several aphid species. Great Lakes Entomol. 27: 137-142.

Gutierrez, A. P., J. U. Baumgaertner and K. S. Hagen. 1981. A conceptual model of growth, development and reproduction in the ladybird beetle, *Hippodamia convergens*

(Coleoptera: Coccinellidae). Can. Entomol. 113:21-33.

Hodek, I. 1973. Biology of Coccinellidae. Academia, Dr. W. Junk, The Hague.

Jackson, H. B., L. W. Coles, E. A. Wood and R. D. Eikenbary. 1970. Parasites Reared from the Greenbug and Corn Leaf Aphid in Oklahoma in 1968 and 1969. [*Schizaphis Graminum, Rhopalosiphum Maidis*]. J. Econ. Entomol. 63:733-736.

Kieckhefer, R. W. and B. H. Kantack. 1988. Yield losses in winter grains caused by cereal aphids (Homoptera: Aphididae) in South Dakota. J. Econ. Entomol. 81: 317-321.

Kieckhefer B. W., N. C. Elliot, W. E. Riedell and B. W. Fuller. 1994. Yield of spring wheat in relation to level of infestation by greenbugs (Homoptera: Aphididae). Can. Ent. 126: 61-66.

Kring, T. J. and F. E. Gilstrap. 1983. Within-field distribution of greenbug (Homoptera: Aphididae) and it's parasitoids in Texas winter wheat. J. Econ. Entomol. 76: 57-62.

Kring, T. J. and F. E. Gilstrap. 1984. Efficacy of the natural enemies of grain sorghum aphids (Homoptera: Aphididae). J. Kans. Entomol. Soc. 57: 460-467.

Kring, T. J., F. E. Gilstrap and G. J. Michels Jr. 1985. Role of Indigenous Coccinellids in regulating greenbugs (Homoptera: Aphididae) on Texas Grain Sorghum. J. Econ. Entomol. 78: 269-273.

Massey, B. 1993. Insects on small grains and their control. Oklahoma Cooperative Extension Service No. 7176.

Michels, G. J. and R. W. Behle. 1991. A comparison of Coccinella septempunctata and Hippodamia convergens larval development on greenbugs at constant temperatures. Southwestern Entomol. 16:73-80.

Murdoch, W. W., J. Chesson and P. L. Chesson. 1985. Biological control in theory and practice. The American Naturalist. 125:322-366.

Noetzel, D. 1994. Revised aphid thresholds in small grains. Minnesota Agricultural Extension Service Publication. 13 pp.

Nyrop, J. P., and W. van der Werf. 1994. Sampling to Predict or Monitor Biological Control. In: L. Pedigo and D. G. Buntin (eds.), Handbook of Sampling Methods for Arthropod Pests in Agriculture. CRC Press, Boca Raton, Florida.

Obrycki, J. J. and M. J. Tauber. 1982. Thermal requirements for development of Hippodamia convergens (Coleoptera: Coccinellidae). Ann. Entomol. Soc. Am. 75:678-683.

Okrouhla, M., S. Chakrabarti and I. Hodek. 1983. Developmental rate and feeding capacity in *Cheilomenes sulphura* (Coleoptera: Coccinellidae). Vest. Cs. Spolec. Zool. 47: 105-117.

Patrick C. D. and E. P Boring, III. 1990. Managing insect and mite pests of Texas small grains. TA&M Extension Publication # B-1251. 11 pp.

Pike, K. S. and R. L. Schaffner. 1985. Development of autumn populations of cereal aphids, *Rhopalosiphum padi* (L.) and *Schizaphis graminum* (Rondani) (Homoptera: Aphididae) and their effects on winter wheat in Washington State. J. Econ. Entomol. 78: 676-680.

Ramaseshiah, G., K. V. Bhat, and P. R. Dharmadhikari 1968. Influence of host aphid, host plant and temperature on the laboratory breeding of *Lysiphlebus testaceipes*. Indian J. Ent. 30:281-285.

Rice M. E. and G. E. Wilde. 1988. Experimental evaluation of predators and

parasitoids in suppressing greenbugs (Homoptera: aphididae) in sorghum and wheat. Environ. Entomol. 17: 836-841.

Rice M. E. and G. E. Wilde. 1989. Antibiosis effect of sorghum on the convergent lady beetle, a third-trophic level predator of the greenbug. J. Econ. Entomol. 82: 570-573.

Rowlands, M. L. J. and J. W. Chapin. 1978. Prey Searching Behavior in Adults of *Hippodamia Convergens* (Coleoptera: Coccinellidae). J. Ga. Entomol. Soc. 13: 309-315.

Royer, T. A., K. L. Giles and N. C. Elliott. 1998. Small grain aphids in Oklahoma. Oklahoma State University Extension Facts F-7183.

Ruth, W. E., R. W. McNew, D. W. Caves and R. D. Eikenbary. 1975. Greenbugs (Homoptera: Aphididae) forced from host plants by *Lysiphlebus testaceipes* (Hymenoptera: Braconidae). Entomophaga. 20: 65-71.

SAS Institute. 1996. SAS user's guide: statistics, version 6.12. ed. SAS Institute, Cary NC.

Shotkoski, F. A., Z. B. Mayo and L. L. Peters. 1990. Induced disulfoton resistance in greenbugs (Homoptera: Aphididae). J. Econ. Entomol. 83(6): 2147-2152.

Starks, K. J., R. Muniappan and R. D. Eikenbary. 1972. Interaction between plant resistance and parasitism against the greenbug on barley and sorghum. Ann. Entomol. Soc. Am. 65: 650-655.

Starks, K. J. and R. L. Burton. 1977. Preventing Greenbug [*Schizaphis Graminum*] Outbreaks [Small Grains and Sorghum]. Leafl U S Dep Agric 309, Rev., 11 pp.

Tyler, B. M. J. and P. A. Jones. 1974. Influence of Low Temperature on Development and Successful Emergence of *Lysiphlebus testaceipes*, a Parasite of the Greenbug. [Schizaphis Graminum]. Environ. Entomol. 3:377-379.

Webster, J. A. 1995. Economic impact of the greenbug in the western United States: 1992-1993. Volume Publication No. 155. Great Plains Agricultural Council, Stillwater, Oklahoma.

Table 4.1 Targeted greenbug natural enemy treatments for caged natural enemy threshold experiment.

Treatment[a]	*Schizaphis graminum* Targeted intensity (# per tiller)	*Lysiphlebus testaceipes* Females· Males	*Hippodamia convergens* Females· Males
1	0.1	-	-
2	1.0	-	-
3	10.0	-	-
4	0.1	4·4	-
5	1.0	4·4	-
6	10.0	4·4	-
7[b]	1.0	6·6	-
8[b]	1.0	10·10	-
9	1.0	14·14	-
10	1.0	24·24	-
11	0.1	-	6·6
12	1.0	-	6·6
13	10.0	-	6·6
14	0.1	-	3·3

[a] each treatment was replicated four times on winter wheat cultivars TAM 107 (greenbug susceptible) and TAM 110 (greenbug resistant).

[b] additional treatments for 2000 experiment.

Table 4.2. Data groups for 1999 and 2000 using initial predation and parasitism ratios.

	TAM 107 Wheat					
	1999				2000	
Natural Enemy	Ratio[a]	Aphids per tiller[b] (# of cages)	Ratio[a]	Greenbugs per tiller[b] (# of cages)	Ratio[a]	Greenbugs per tiller[b] (# of cages)
L. testaceipes	<1.5%	2.5-5.7 **(8)**	<4.0%	0.9-4.5 **(5)**	<4.0%	0.8-5.2 **(7)**
Control		2.3-5.1 **(6)**		0.8-6.8 **(5)**		1.0-5.5 **(6)**
L. testaceipes	>1.5%	0.1-2.0 **(12)**	>4.0%	0.01-1.7**(15)**	>4.0%	0.02-3.1**(17)**
Control		0.3-2.6 **(6)**		0.01-1.3 **(9)**		0.1-2.8 **(10)**
H. convergens	>25:1	0.7-4.1 **(7)**	>50:1	0.8-4.0 **(3)**	>20:1	0.5-4.1 **(7)**
Control		0.9-5.1 **(8)**		0.8-4.5 **(4)**		1.0-5.5 **(6)**
H. convergens	<10:1	0.1-0.5 **(6)**	<50:1	0.01-1.0**(12)**	<20:1	0.04-1.1 **(7)**
Control		0.9-1.4 **(3)**		0.01-1.3**(10)**		0.1-1.3 **(9)**
	TAM 110 Wheat					
L. testaceipes	<1.5%	3.0-11.4 **(6)**	<4.0%	0.8-9.4 **(6)**	<4.0%	1.3-4.7 **(7)**
Control		2.9-13.1 **(5)**		1.1-10.6 **(6)**		1.3-3.4 **(6)**
L. testaceipes	>1.5%	0.2-2.9 **(8)**	>4.0%	0.04-4.4**(12)**	>4.0%	0.04-1.8**(18)**
Control		0.3-2.9 **(7)**		0.03-3.2 **(9)**		0.02-1.9 **(7)**
H. convergens	>25:1	1.4-7.1**(10)**	>20:1	0.5-2.1 **(8)**	>20:1	0.1-1.1 **(9)**
Control		1.3-9.1 **(7)**		0.6-1.9 **(4)**		0.02-1.3 **(6)**
H. convergens	<10:1	0.4-1.3 **(5)**	<20:1	0.1-0.8 **(8)**	<20:1	1.0-7.6 **(5)**
Control		0.7-1.3 **(5)**		0.03-0.6 **(5)**		1.3-3.4 **(6)**

[a] For *H. convergens*, ratio is the number of aphid/greenbug per beetle. For *L. testaceipes*, the ratio of unparasitized to parasitized aphid/greenbugs is expressed as a percentage.

[b] Based on analysis, PROC GLM (SAS Institute 1996), ($P < 0.05$) linear regressions were combined into major data groups for each wheat cultivar. Data from cages without natural enemies were used to construct control data groups.

Table 4.3. Differences of least squares means for 1999 data groups vs their control groups using initial predation and parasitism ratios generated by PROC MIXED (SAS Institute 1996).

1999 All Aphids on TAM 107 Wheat

Julian Date →		68		75		82		89		96	
Ratio	*df*	*t*	*P>t*	*t*	*P>t*	*t*	*P>t*	*t*	*P>t*	*t*	*P>t*
<1.5% *L. testaceipes*	60	0.08	0.93	0.05	0.96	0.01	0.99	0.66	0.51	2.48	0.02
>1.5% *L. testaceipes*	75	0.17	0.86	0.73	0.47	1.29	0.20	2.18	0.03	4.25	0.00
<10 aphids/beetle	35	0.22	0.83	0.71	0.48	1.20	0.24	2.68	0.01	6.05	0.00
>25 aphids/beetle	65	0.05	0.96	0.08	0.94	0.21	0.83	0.59	0.56	0.45	0.66

1999 All Aphids on TAM 110 Wheat

Julian Date →		68		75		82		89		96	
Ratio	*df*	*t*	*P>t*	*t*	*P>t*	*t*	*P>t*	*t*	*P>t*	*t*	*P>t*
<1.5% *L. testaceipes*	45	0.06	0.95	0.29	0.77	0.53	0.60	0.61	0.54	2.92	0.01
>1.5% *L. testaceipes*	65	0.09	0.93	0.74	0.46	1.39	0.17	1.94	0.06	0.95	0.35
<10 aphids/beetle	40	0.02	0.98	0.31	0.76	0.65	0.52	0.97	0.34	2.68	0.01
>25 aphids/beetle	75	0.16	0.88	0.25	0.80	0.35	0.73	0.14	0.89	0.60	0.55

1999 Greenbugs Only on TAM 107 Wheat

Julian Date →		68		75		82		89		96	
Ratio	*df*	*t*	*P>t*	*t*	*P>t*	*t*	*P>t*	*t*	*P>t*	*t*	*P>t*
<4% *L. testaceipes*	40	0.11	0.91	0.73	0.47	1.35	0.19	1.94	0.06	2.29	0.03
>4% *L. testaceipes*	110	0.14	0.89	1.11	0.27	2.07	0.04	1.79	0.08	3.11	0.00
<50 greenbugs/beetle	90	0.02	0.99	0.23	0.82	0.44	0.66	0.69	0.49	0.80	0.42
>50 greenbugs/beetle	25	0.03	0.98	0.26	0.79	0.56	0.58	1.36	0.19	1.98	0.06

1999 Greenbugs Only on TAM 110 Wheat

Julian Date →		68		75		82		89		96	
Ratio	*df*	*t*	*P>t*	*t*	*P>t*	*t*	*P>t*	*t*	*P>t*	*t*	*P>t*
<4% *L. testaceipes*	50	0.02	0.99	0.33	0.75	0.67	0.51	0.84	0.40	0.79	0.43
>4% *L. testaceipes*	105	0.08	0.94	0.95	0.35	1.82	0.07	2.70	0.01	2.39	0.02
<20 greenbugs/beetle	55	0.16	0.88	0.39	0.70	0.94	0.35	1.26	0.21	0.27	0.79
>20 greenbugs/beetle	70	0.46	0.64	0.58	0.57	0.69	0.49	1.40	0.17	2.56	0.01

Table 4.4. Differences of least squares means for 2000 data groups vs their control groups using initial predation and parasitism ratios generated by PROC MIXED (SAS Institute 1996).

2000 All Aphids on TAM 107 Wheat

Julian Date →		64		69		75		84		90		97	
Ratio	*df*	*t*	*P>t*	*t*	*P>t*	*t*	*P>t*	*t*	*P>t*	*t*	*P>t*	*t*	*P>t*
<4% *L. testaceipes*	24	0.00	0.99	0.10	0.92	0.10	0.92	0.03	0.97	0.07	0.95	0.10	0.92
>4% *L. testaceipes*	162	0.32	0.75	0.39	0.69	0.42	0.68	0.48	0.63	0.28	0.78	0.24	0.81
<20 aphids /beetle	96	0.15	0.88	1.79	0.08	1.17	0.24	2.67	0.01	1.64	0.10	0.56	.058
>20 aphids /beetle	24	0.24	0.81	1.62	0.12	1.89	0.07	0.34	0.74	0.98	0.33	1.39	0.18

1999 All Aphids on TAM 110 Wheat

Julian Date →		64		69		75		84		90		97	
Ratio	*df*	*t*	*P>t*	*t*	*P>t*	*t*	*P>t*	*t*	*P>t*	*t*	*P>t*	*t*	*P>t*
<4 *L. testaceipes*	54	0.29	0.78	1.22	0.23	0.05	0.96	0.58	0.56	0.26	0.80	0.18	0.86
>4% *L. testaceipes*	126	0.89	0.37	0.59	0.56	0.18	0.86	0.04	0.97	0.09	0.93	0.02	0.98
<20 aphids /beetle	60	0.19	0.85	0.54	0.59	0.99	0.33	0.26	0.80	0.06	0.95	0.64	0.53
>20 aphids /beetle	78	1.29	0.20	1.10	0.28	0.54	0.59	0.79	0.43	0.41	0.68	0.03	0.98

Table 4.5. Validation data from each 0.4 ha winter wheat field in various locations in Oklahoma in 2000.

		Julian Date					
Site		18	40	52	66	73	88
1	Parasitism[a] (b)	10% (40)	37% (62)	31% (54)	19% (39)	0% (31)	0% (0)
	aphids/tiller[c] [d]	1.5 [0.7]	0.3 [0.1]	0.3 [0.2]	0.4 [0.3]	0.3 [0.3]	0.5 [0.4]
	aphids/beetle[e]	NA	NA	1034	348	NA	NA
2	Parasitism[a] (b)	13% (30)	18% (26)	20% (32)	29% (49)	25% (40)	19% (27)
	aphids/tiller[c] [d]	1.4 [0.7]	0.7 [.04]	0.6 [0.3]	0.3 [0.2]	0.6 [0.3]	0.6 [0.5]
	aphids/beetle[e]	NA	1212	786	288	NA	1092
3	Parasitism[a] (b)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
	aphids/tiller[c] [d]	0 [0]	0 [0]	0 [0]	0 [0]	0 [0]	0 [0]
	aphids/beetle[e]	NA	NA	NA	NA	NA	NA
4	Parasitism[a] (b)	6% (14)	3% (100)	7% (54)	11% (56)	17% (42)	20% (40)
	aphids/tiller[c] [d]	0.8 [0.6]	1.0 [0.7]	0.5 [0.3]	0.4 [0.2]	0.4 [0.3]	0.4 [0.3]
	aphids/beetle[e]	1176	NA	NA	NA	NA	NA
5	Parasitism[a] (b)	6% (37)	11%(100)	10% (95)	6% (79)	0% (67)	4% (10)
	aphids/tiller[c] [d]	0.1 [0]	0.2 [0.1]	0.1 [0]	0.1 [0]	0.1 [0]	0.4 [0.1]
	aphids/beetle[e]	NA	NA	290	51	24	324
6	Parasitism[a] (b)	0% (0)	0% (0)	4% (33)	5% (44)	5% (46)	4% (37)
	aphids/tiller[c] [d]	0 [0]	0 [0]	0.1 [0]	0.1 [0.1]	0.2 [0.1]	0.2 [0.1]
	aphids/beetle[e]	NA	NA	NA	78	62	60
7	Parasitism[a] (b)	9% (9)	0% (0)	0% (0)	0% (0)	0% (0)	0% (7)
	aphids/tiller[c] [d]	0.1 [0.1]	0 [0]	0 [0]	0.1 [0.1]	0.1 [0.1]	0.4 [0.2]
	aphids/beetle[e]	NA	NA	NA	NA	NA	NA
8	Parasitism[a] (b)	12% (68)	47% (89)	32%(100)	26% (46)	21% (30)	7% (11)
	aphids/tiller[c] [d]	1.0 [0.6]	0.1 [0.1]	0.3 [0.1]	0.3 [0.2]	0.3 [0.2]	0.5 [0.4]
	aphids/beetle[e]	NA	192	342	734	NA	314
9	Parasitism[a] (b)	32% (67)	62% (85)	62% (62)	45% (55)	12% (34)	2% (2)
	aphids/tiller[c] [d]	1.2 [0.5]	0.2 [0.1]	0.2 [0.1]	0.1 [0.1]	0.1 [0.1]	0.4 [0.3]
	aphids/beetle[e]	2460	792	55	73	204	NA
10	Parasitism[a] (b)	17% (86)	43%(100)	37% (63)	53% (66)	31% (75)	12% (23)
	aphids/tiller[c] [d]	1.4 [0.7]	0.6 [0.2]	0.7 [0.2]	0.5 [0.2]	0.3 [0.2]	0.3 [0.2]
	aphids/beetle[e]	NA	231	228	232	612	NA

[a] Apparent percent parasitism determined by the proportion of mummies collected to the number of aphids present.

[b] Percent parasitism determined by the proportion of mummies that develop when a sub-sample (up to 50 aphids) of collected aphids was incubated for 7 days in addition to apparent percent parasitism; ((Mummies + (%mummies from incubated sample X non-mummy aphids collected)) X 100) / (Mummies + aphids collected).

[c] Total aphids per tiller, determined by total aphids collected divided by total wheat tillers sampled.

[d] Greenbugs per tiller, determined by total greenbugs collected divided by total wheat tillers sampled.

[e] mean number of aphids per lady beetle.

Fig. 4.1. Schematic diagram of field cages. Cages were constructed from 100% polyester "no-see-um" netting (Seattle Fabrics Inc., Seattle, WA) and lightweight nylon reinforced tarp material was used for the base that was buried into the soil. Each cage was supported by a pair of hoops made from ½ inch lightweight metal electrical conduit, set into the ground such that the apex of each hoop was perpendicular to the other. Cages were 61cm wide by 61cm deep by 61cm tall. On one side of the netting, a pair of 40 cm long access tubes are sewn into the netting for access.

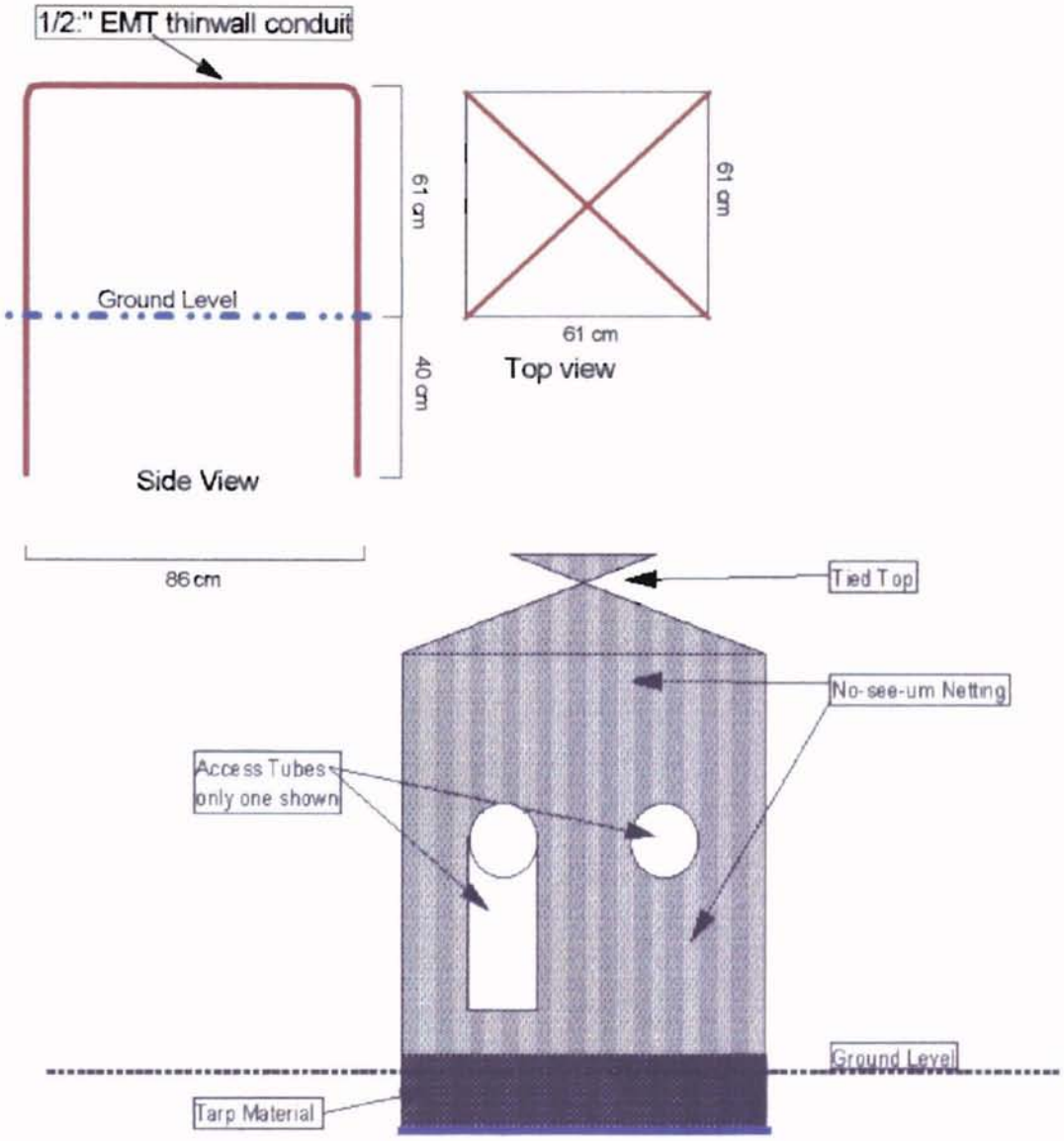
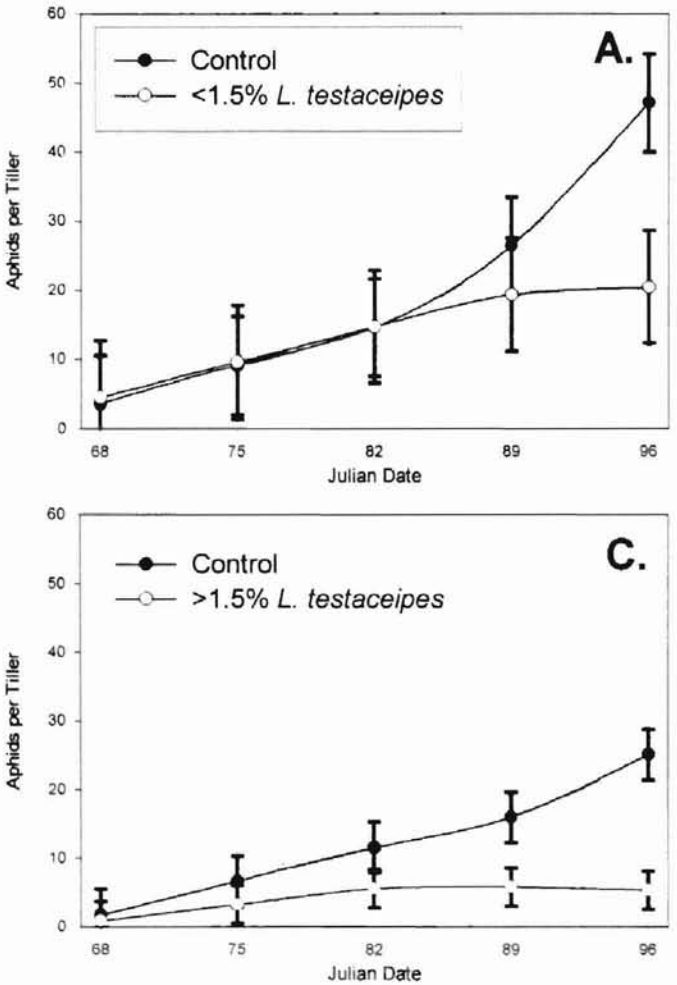

1/2:" EMT thinwall conduit
61 cm
Ground Level
40 cm
Side View
61 cm
61 cm
Top view
86 cm
Tied Top
No-see-um Netting
Access Tubes
only one shown
Ground Level
Tarp Material

Fig. 4.2 A-D. Total aphid intensities (aphids per tiller) on winter wheat over time (Julian dates 68-96 in 1999) in cages located in Grady county, Oklahoma. Pooled data were analyzed by SAS PROC MIXED (SAS Institute 1996), 95% confidence intervals are expressed for each line.

Lysiphlebus testaceipes on TAM 107 Wheat (1999)



Lysiphlebus testaceipes on TAM 110 Wheat (1999)

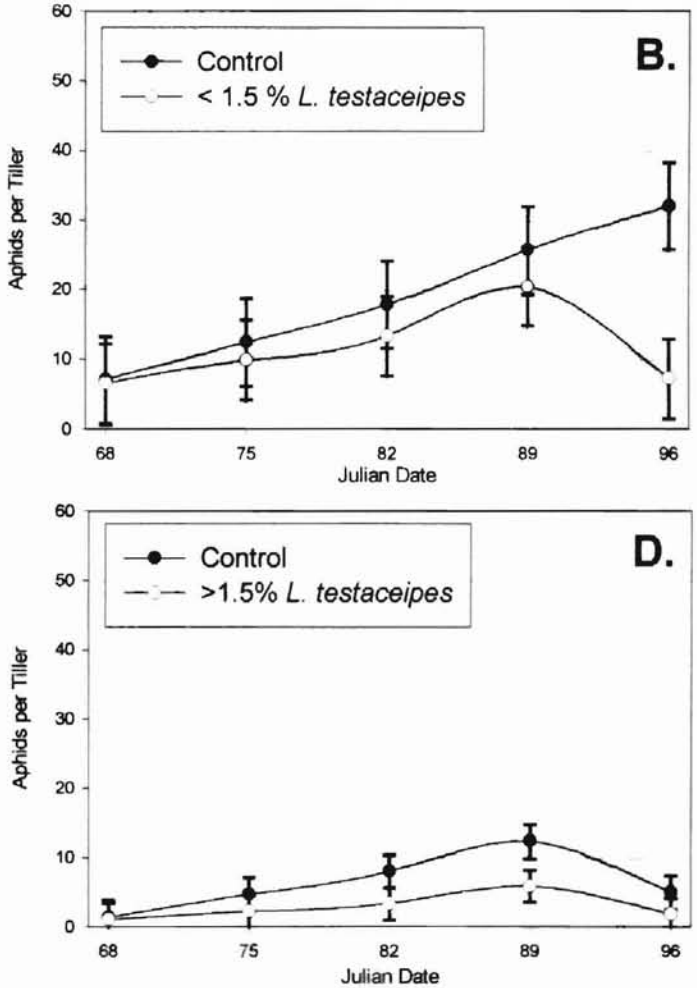
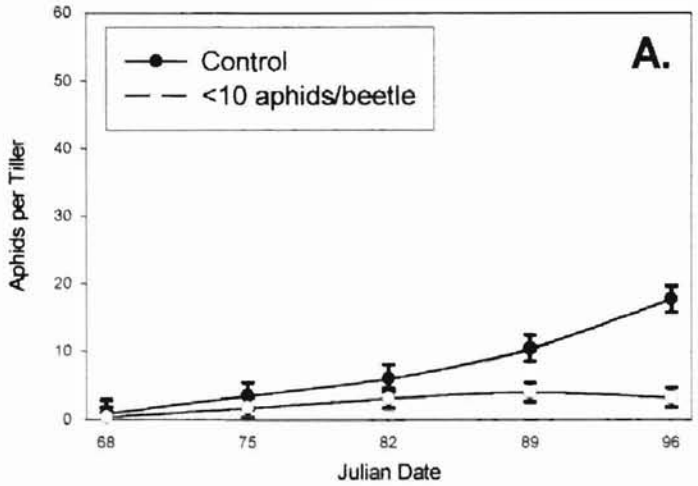
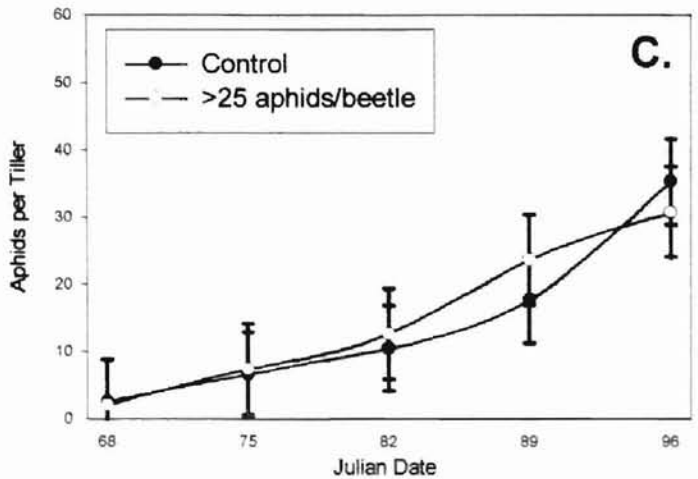


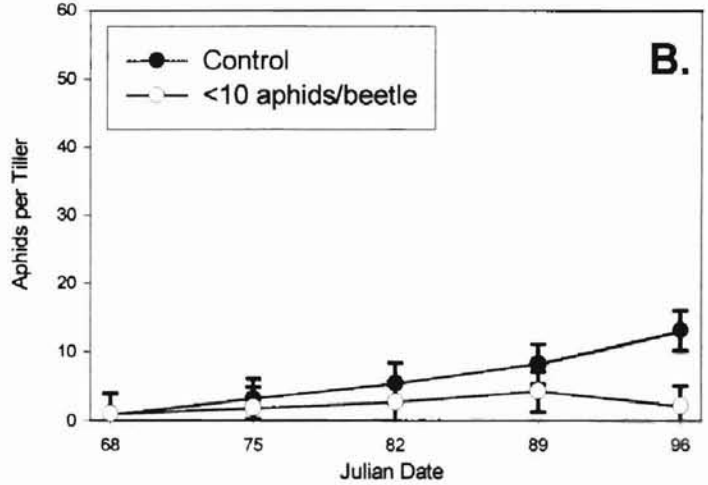
Fig. 4.3 A-D. Total aphid intensities (aphids per tiller) on winter wheat over time (Julian dates 68-96 in 1999) in cages located in Grady county, Oklahoma. Pooled data were analyzed by SAS PROC MIXED (SAS Institute 1996), 95% confidence intervals are expressed for each line.

Hippodamia convergens on TAM 107 Wheat (1999)





Hippodamia convergens on TAM 110 Wheat (1999)



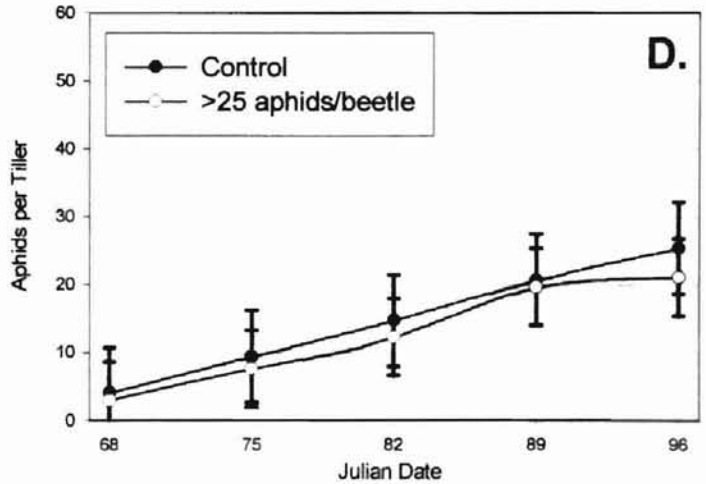
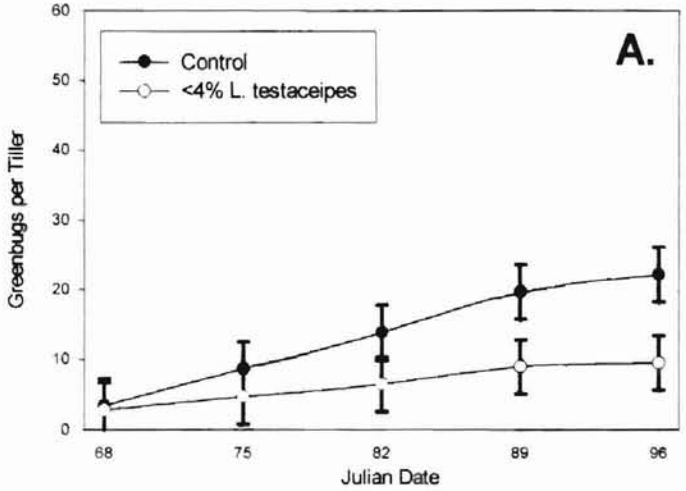
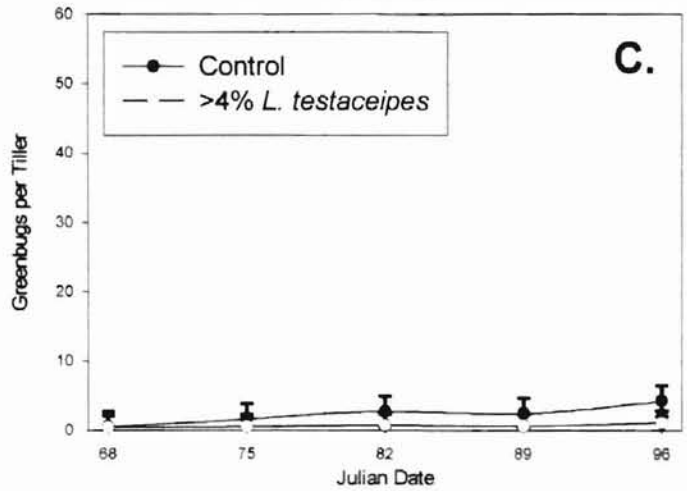


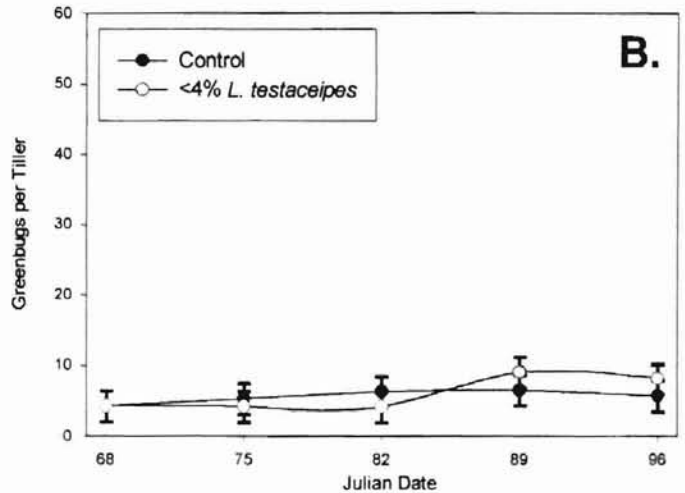
Fig. 4.4 A-D. Greenbug intensities (greenbugs per tiller) on winter wheat over time (Julian dates 68-96 in 1999) in cages located in Grady county, Oklahoma. Pooled data were analyzed by SAS PROC MIXED (SAS Institute 1996), 95% confidence intervals are expressed for each line.

Lysiphlebus testaceipes on TAM 107 Wheat (1999)





Lysiphlebus testaceipes on TAM 110 Wheat (1999)



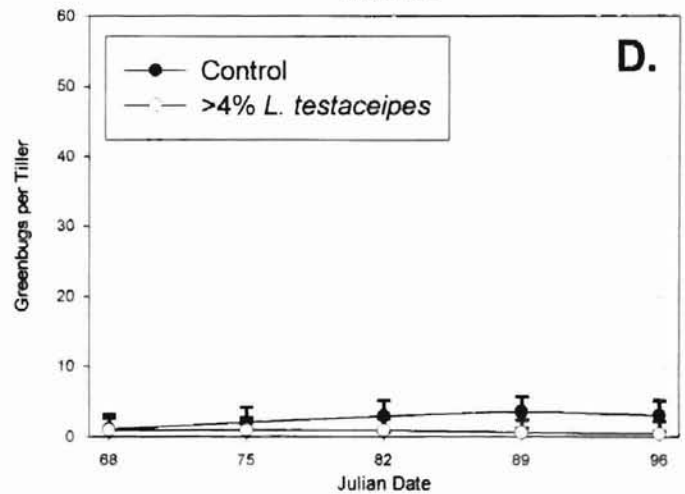
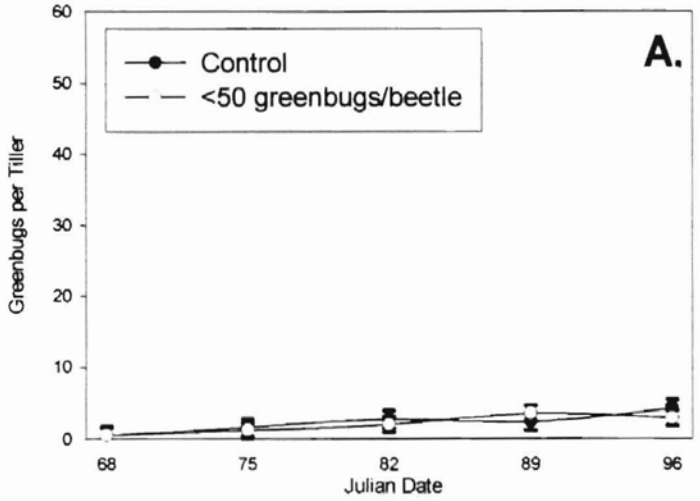
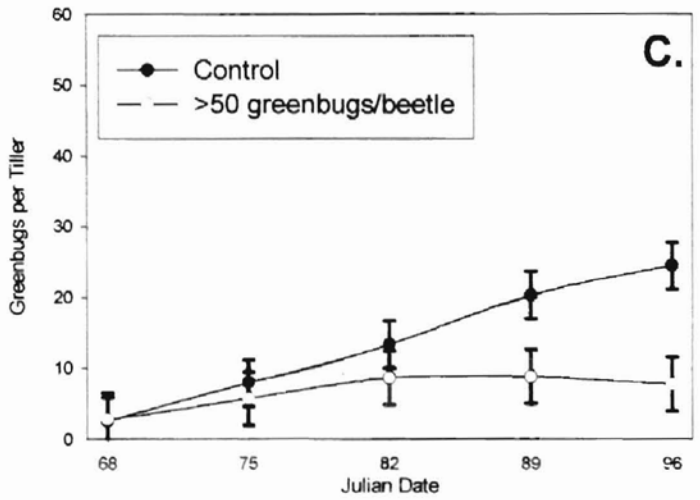


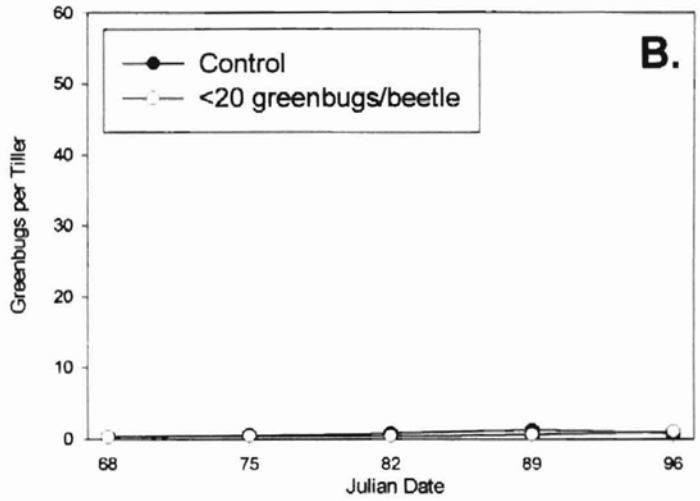
Fig. 4.5 A-D. Greenbug intensities (greenbugs per tiller) on winter wheat over time (Julian dates 68-96 in 1999) in cages located in Grady county, Oklahoma. Pooled data were analyzed by SAS PROC MIXED (SAS Institute 1996), 95% confidence intervals are expressed for each line.

Hippodamia convergens on TAM 107 Wheat (1999)





Hippodamia convergens on TAM 110 Wheat (1999)



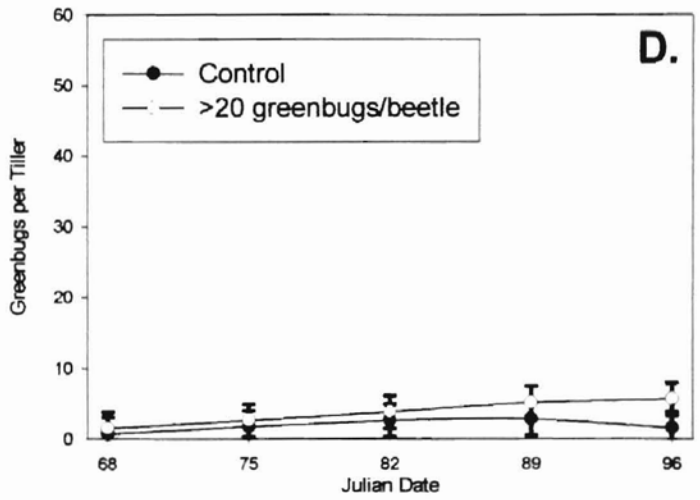
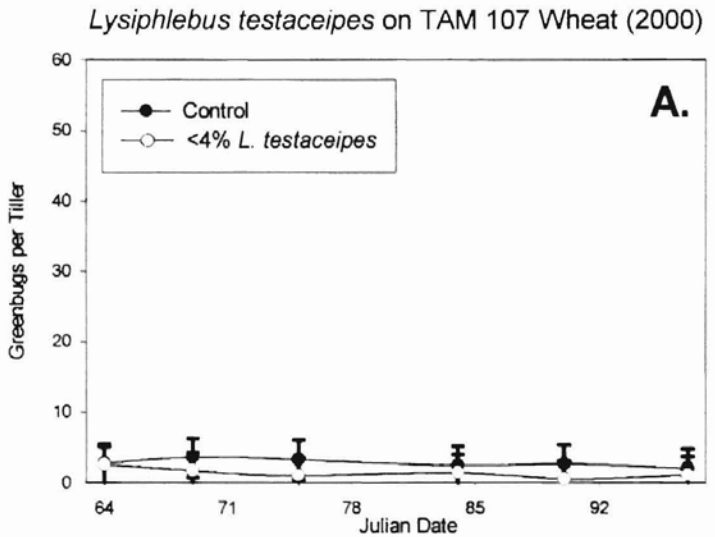
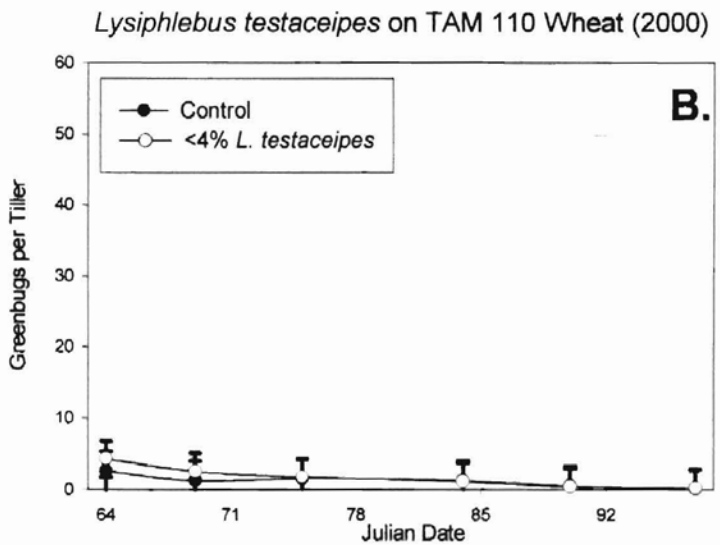
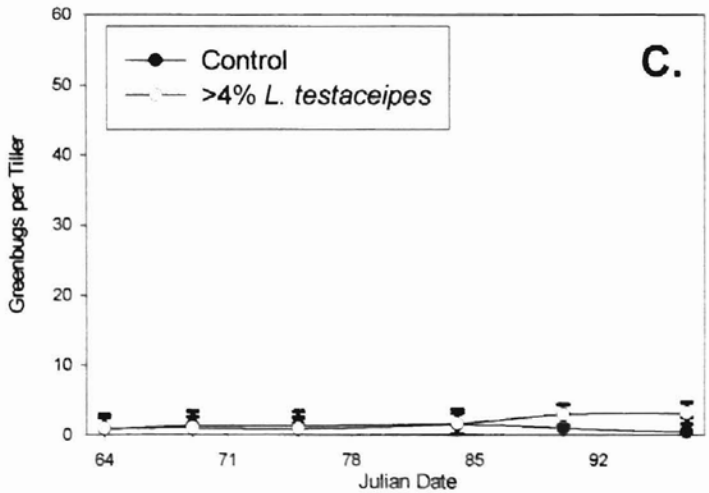


Fig. 4.6 A-D. Greenbug intensities (greenbugs per tiller) on winter wheat over time (Julian dates 64-97 in 2000) in cages located in Grady county, Oklahoma. Pooled data were analyzed by SAS PROC MIXED (SAS Institute 1996), 95% confidence intervals are expressed for each line.


Lysiphlebus testaceipes on TAM 107 Wheat (2000)
A.
Control
<4% *L. testaceipes*
Greenbugs per Tiller
Julian Date
0
10
20
30
40
50
60
64
71
78
85
92


Lysiphlebus testaceipes on TAM 110 Wheat (2000)
B.
Control
<4% *L. testaceipes*
Greenbugs per Tiller
Julian Date
0
10
20
30
40
50
60
64
71
78
85
92


C.
Control
>4% *L. testaceipes*
Greenbugs per Tiller
Julian Date
0
10
20
30
40
50
60
64
71
78
85
92

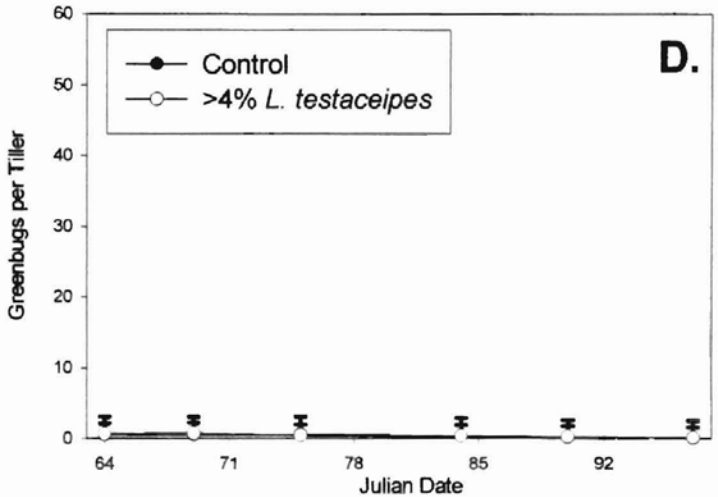
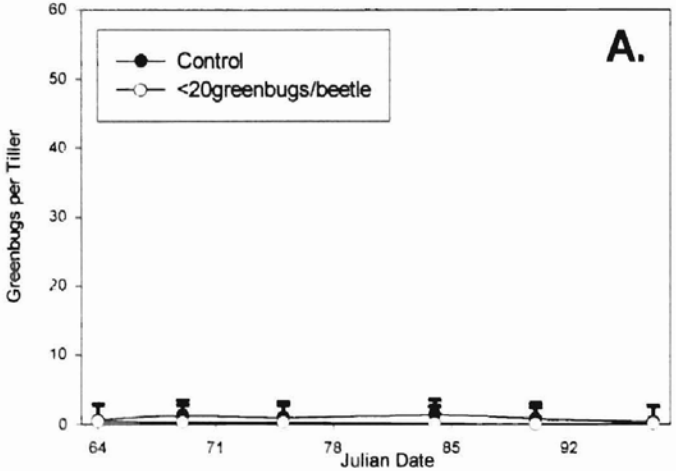
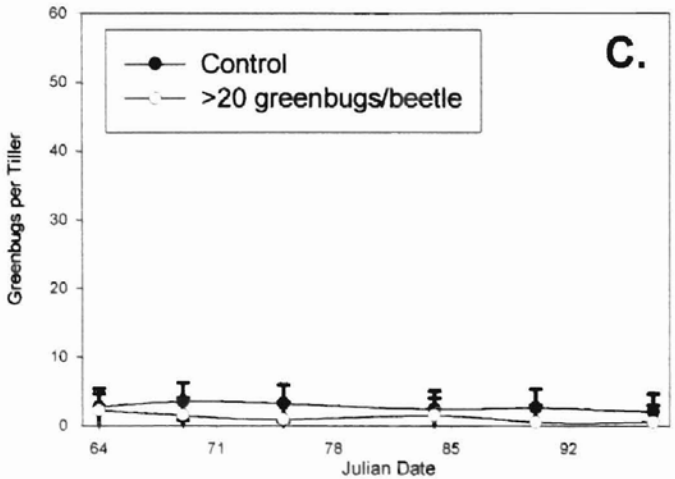

D.
Control
>4% *L. testaceipes*
Greenbugs per Tiller
Julian Date
0
10
20
30
40
50
60
64
71
78
85
92

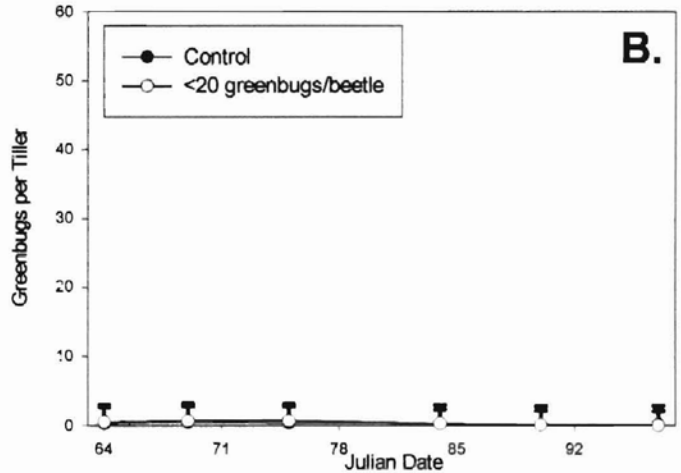
Fig. 4.7 A-D. Greenbug intensities (greenbugs per tiller) on winter wheat over time (Julian dates 64-97 in 2000) in cages located in Grady county, Oklahoma. Pooled data were analyzed by SAS PROC MIXED (SAS Institute 1996), 95% confidence intervals are expressed for each line.

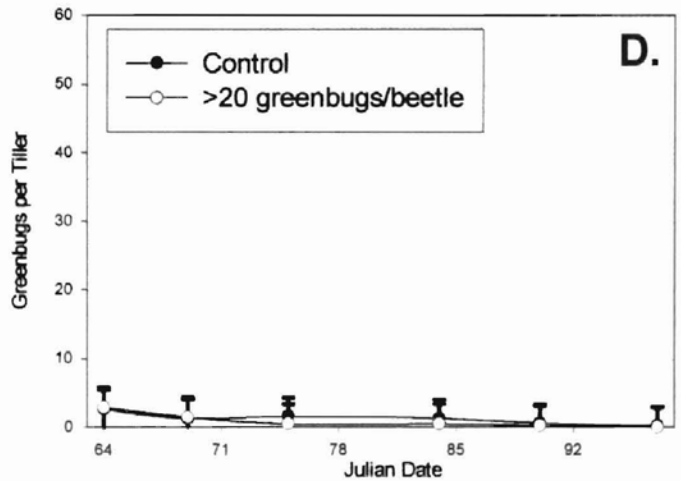
Hippodamia convergens on TAM 107 Wheat (2000)





Hippodamia convergens on TAM 110 Wheat (2000)





CHAPTER V: SUMMARY AND CONCLUSIONS

These studies provide new and more accurate knowledge about natural enemy control of greenbug in Oklahoma winter wheat. Furthermore, my results provide insights about greenbug control outside of Oklahoma. Results from my functional response experiment describes how *L. testaceipes* attack rate declines somewhat at 18°C and dramatically at 14°C. These results suggest that when the temperature does not exceed 14°C, *L. testaceipes* cannot be expected to suppress large, increasing greenbug populations, since greenbugs can reproduce even when temperatures decline to 5.8°C (Walgenbach et al. 1988). The type 3 functional response by *L. testaceipes* on greenbug also provides insights about how this parasitoid may control greenbug at low host densities by having an attack rate that matches host fecundity and stabilizes the host-parasitoid populations (Berryman 1999) and as parasitoids encounter higher host densities, attacks may increase as a result of learning (Murdoch and Oaten 1975).

The functional response of *A. colemani* was not affected by the four temperatures tested, suggesting that *A. colemani* could parasitize aphids at lower temperatures than *L. testaceipes*. Looking at this attribute alone, *A. colemani* appears to be a good candidate for additional late fall and early spring greenbug control in the Southern Great Plains, when temperatures are frequently below 14°C. *Aphidius colemani* has yet to be established in Great Plains (Prokrym et al. 1998). However, results from this experiment should encourage further research about this endeavor because *A. colemani* exhibits a higher attack rate at low temperature than *L. testaceipes*.

Results of my second experiment do not support recommendations that one or two lady beetles per 0.3m of crop furrow will provide effective greenbug control, except possibly for low aphid populations. However, my caged study may not adequately define

lady beetle abilities. This is because *H. convergens* is a generalist predator and greenbugs and *R. padi* are only a part of their overall diet (Hodek 1973, Murdoch et al. 1985). Additionally, only first and second instar *H. convergens* larvae were able to develop in the time frame of the experiment. Given more time with moderate temperatures, *H. convergens* may have been able to reduce larger aphid populations in the cages. Additionally, cages prevented adult beetles from traveling to areas in the field with large aphid populations, which may be more important for aphid suppression by *H. convergens* (Rowlands and Chapin 1978, K. L. Giles unpublished data).

Lysiphlebus testaceipes was able to suppress aphid intensities below 8 aphids per tiller at aphid to parasitoid ratios of <67:1 (>1.5%) on both greenbug resistant and susceptible winter wheat (Figs. 4.2 C&D). This is far below the recommendations of 15 to 20% by Patrick and Boring (1990) and 20 to 30% by Royer et al (1998). These results may be influenced somewhat by a cage effect, but provide the most precise measurements of *L. testaceipes* capabilities for greenbug control to date. Since *L. testaceipes* has been observed to limit greenbug populations in winter wheat (Ramaseshiah et al. 1968, Tyler and Jones 1974), *L. testaceipes* should be able to provide control of greenbug in most instances when the apparent parasitism of the overall aphid population is between 1.5 and 15%. However, the effective ratio could be heavily influenced by temperature because my functional response studies of *L. testaceipes* on greenbug show that parasitism rates are greatly reduced when temperatures are below 14 °C (D. B. Jones unpublished data).

I identified natural enemy thresholds for aphids in winter wheat (>1.0% for *L. testaceipes* and <10 aphids per *H. convergens* beetle), and attempted to validate those thresholds in ten fields throughout Oklahoma in 2000. I can state that I did not make any

type I or II errors, but all my decisions were that biological control would be successful. Further research is necessary to obtain conditions where biological control would fail and test those situations with my natural enemy thresholds. Validation testing these natural enemy thresholds in situations where biological control of greenbug is both successful and not successful, would make reliable predictions of greenbug population suppression in a comprehensive integrated greenbug management program possible.

References Cited

Berryman, A. A. 1999. The theoretical foundations of biological control. *In* B. A. Hawkins and H. V. Cornell [eds], Theoretical Approaches to Biological Control. Cambridge University Press. Cambridge UK. pp 3-21.

Hodek, I. 1973. Biology of Coccinellidae. Academia, Dr. W. Junk, The Hague.

Murdoch, W. W. and A. Oaten. 1975. Predation and population stability. Adv. Ecol. Res. 9: 1-131.

Murdoch, W. W., J. Chesson and P. L. Chesson. 1985. Biological control in theory and practice. The American Naturalist. 125:322-366.

Patrick C. D. and E. P Boring, III. 1990. Managing insect and mite pests of Texas small grains. TA&M Extension Publication # B-1251. 11 pp.

Prokrym, D. R., K. S. Pike, and D. J. Nelson. 1998. Biological control of *Diuraphis noxia* (Homoptera: Aphididae): Implementation and evaluation of natural enemies: S. S. Quisenberry, and F. B. Peairs Eds. Response model for an introduced pest - the Russian wheat aphid. Thomas A. Publications in Entomology. Published by the Entomological Society of America. Landover MD pp 167-183.

Ramaseshiah, G., K. V. Bhat, and P. R. Dharmadhikari 1968. Influence of host aphid, host plant and temperature on the laboratory breeding of *Lysiphlebus testaceipes*. Indian J. Ent. 30:281-285.

Rowlands, M. L. J. and J. W. Chapin. 1978. Prey Searching Behavior in Adults of *Hippodamia Convergens* (Coleoptera: Coccinellidae). J. Ga. Entomol. Soc. 13: 309-315.

Royer, T. A., K. L. Giles and N. C. Elliott. 1998. Small grain aphids in Oklahoma.

Tyler, B. M. J. and P. A. Jones. 1974. Influence of Low Temperature on Development

and Successful Emergence of *Lysiphlebus testaceipes*, a Parasite of the Greenbug. [Schizaphis Graminum]. Environ. Entomol. 3:377-379. Oklahoma State University Extension Facts F-7183.

Walgenbach, D. D., N. C. Elliot and R. W. Kieckhefer. 1988. Constant and fluctuating temperature effects on developmental rates and life table statistics of the greenbug. J. Econ. Entomol. 81:501-507.

VITA 7

Douglas Bradley Jones

Candidate for the Degree of

Master of Science

Thesis: NATURAL ENEMY THRESHOLDS FOR GREENBUG, *SCHIZAPHIS GRAMINUM* (RONDANI), ON WINTER WHEAT

Major Field: Entomology

Biographical:

Education: Graduated from Murfreesboro High School, Murfreesboro, Arkansas in 1979; Attended Arkansas Tech University, Russellville, Arkansas from 1979-1982; Received Bachelor of Science degree in Biology from Henderson State University, Arkadelphia, Arkansas in December 1983; Completed the requirements for the Master of Science degree in Entomology from Oklahoma State University, Stillwater, Oklahoma in May 2001.

Experience: Research Assistant, Integrated Pest Management Lab, Dept. of Entomology and Plant Pathology, Oklahoma State University, Stillwater, Oklahoma, July 1998 to present; Research Specialist, (Red Imported Fire Ant Control Project), School of Forest Resources, University of Arkansas at Monticello, Monticello, Arkansas; Co-Owner of Wolf Creek Nursery, Delight, Arkansas, 1987 to present; Greenhouse Foreman, McMaster's Florist and Greenhouse, Arkadelphia, Arkansas, 1984 to 1987.

Professional and Honor Memberships: Lambda Chi Alpha (Social Fraternity) 1980 to present; Entomological Society of America, 1998 to present; Delight Area Chamber of Commerce, 1988 to 1992; Rocky Mountain Conference of Entomologists, 1999 to present.