

WINTER ECOLOGY OF THE PARASITOID

***LYSIPHLEBUS TESTACEIPES* CRESSON**

By

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***LYSIPHLEBUS TESTACEIPES* CRESSON**

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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), and *Lysiphlebus testaceipes* Cresson. Also included is a detailed description of functional response, cold weather ecology, supercooling ability and greenbug management. Chapters III, IV, V, and VI are formal manuscripts of the research I conducted during my Ph.D. 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, Tom Phillips, and Mark Payton for their valuable advice and assistance. Special thanks are extended to Tim Johnson, Jennifer Chown, Cole O'Neil, and Dennis Kastl 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.

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CHAPTER I

GENERAL INTRODUCTION

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

The greenbug, described by Rondani (1847), was first reported in the United States as an agronomic pest of wheat in 1882 (Hunter and Glenn 1909, Webster and Phillips 1912). Greenbug feeding reduces yield and crop quality when population levels surpass economic injury levels (EIL's) (Burton et al. 1985, Pike and Schaffner 1985, Kieckhefer and Kantack 1988, Massey 1993, Elliott et al. 1994a, Noetzel 1994). Local greenbug outbreaks occur in Oklahoma almost every year, and statewide infestations are reported about every 5-10 years (Starks and Burton, 1977). 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).

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). One of the most important examples of these natural enemies in the Southern Great Plains is the parasitic wasp *Lysiphlebus testaceipes* Cresson (Hymenoptera: Aphidiidae) (Ruth et al. 1975, Kring and Gilstrap 1983, 1984, Kring et al. 1985).

Lysiphlebus testaceipes has been demonstrated to be an effective biological control agent of greenbugs in winter wheat in Oklahoma (Jones 2001, Giles et al. 2003). Field cage studies by Jones (2001) demonstrated that actual parasitism rates above 4% of

the greenbug population were sufficient to prevent greenbug populations from exceeding economic injury levels. While these studies demonstrated that *L. testaceipes* was effective, they did not investigate the mechanisms of how greenbug populations are suppressed during winter wheat growth. Describing greenbug suppression in Oklahoma by *L. testaceipes* requires an understanding of parasitoid ecology during cold winter months.

Greenbug parasitoids such as *L. testaceipes* are frequently exposed in Oklahoma to temperatures below 0°C yet are active on warmer days (Personal Observation). Insects exposed to temperatures below the melting point of their body fluid are in danger of being killed by a lethal freezing. They may be able to tolerate being in a frozen state, or they avoid freezing by keeping their body fluids supercooled (Zachariassen 1985). A review of the literature found virtually no research about cold weather ecology of aphid parasitoids. As such, little is known about the cold weather ecology of *L. testaceipes*. It is not currently known what the lowest temperature is at which *L. testaceipes* can survive. Measurement of its supercooling point (SCP) will answer that question, but many insects perish before their SCP is reached (Leather et al. 1993). Hunter and Glenn (1909) also reported that *L. testaceipes* has the ability to survive temperatures below 0°C and oviposit later when temperatures are warmer. A more complete study of the functional survivability of *L. testaceipes* is needed to determine survivability at temperatures commonly experienced in Oklahoma. Finally there are conflicting reports about ovipositional ability of *L. testaceipes* at cold temperatures. 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. A study of the functional response at low temperatures will provide answers to this question.

The overall goals of this research are to investigate effects of winter temperatures common to Oklahoma on the survivorship and ovipositional behavior of *L. testaceipes*.

Objectives

- I. Examine effects of cold temperatures on survivorship of *Lysiphlebus testaceipes* through experiments to determine the supercooling point of various life stages of *L. testaceipes* and of its host, the greenbug, *S. graminum*
- II. Determine survivorship of adults and mummies at temperatures commonly experienced during Oklahoma winters.
- III. Investigate and define the minimum temperature required for oviposition by *L. testaceipes* and describe attack rates at increasing temperatures.

Explanation of Thesis Format

This general introduction is followed by a literature review (chapter II), then chapters III, IV, and V, devoted to individual papers to be published, a general summary (chapter VI), and appendices. Lists of references are provided for citations in the literature review and papers to be published. In paper I (chapter III) supercooling ability of *L. testaceipes* is used to determine the lowest temperatures that the parasitoid and its host could possibly survive. The second paper (chapter IV) examines functional survivability of *L. testaceipes* at low temperatures by cooling parasitoid specimens for various time periods and then examining whether they are still able to successfully reproduce. The third paper (chapter V) determines the minimum temperature that *L. testaceipes* adults can successfully oviposit, and attack rates for *L. testaceipes* are

reported for varying prey densities (functional response) from the lowest temperature that *L. testaceipes* can successfully oviposit up to 14°C. All chapters 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, over 6 million acres of winter wheat were planted in Oklahoma, of which about 4 million acres were harvested for grain with an average yield of 34 per bushels acre (Krenzer et al. 1999). Overall, about 50 to 55% of planted wheat is grazed (Thompson 1990, Carver et al. 1991).

In Oklahoma, wheat fields are generally prepared in late summer (Royer and Krenzer 2000). Planting dates depend on the intended purpose of the wheat and location of the field. Wheat for forage and grain production is generally planted in Oklahoma's southern region from 15 September to 10 October, while grain only wheat is generally planted from 10 October to 30 October. Planting generally occurs 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 soon after emergence when the first stem appears and continues until elongation (jointing) starts in the spring. Wheat may cease growth and go dormant during the coldest months (usually December through early February) of the winter. Generally in February when temperatures begin to warm, plants resume growth and tillers extend strongly upward by "jointing". This is characterized by a strong upward extended stem that is hollow. Heading begins in March or April, 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.

There are numerous cultivars of wheat available for planting. Cultivar selection is governed mostly by grain production potential, however, recent research has improved

variety selection with increased emphasis on forage production (Worrall & Gilmore 1985, Krenzer et al. 2001, 2002, 2004).

Insect Pests of Wheat

Winter wheat is attacked by numerous insect herbivores including aphids (Homoptera: Aphididae), armyworms (*Pseudaletia unipuncta* (Hayworth) and *Spodoptera frugiperda* J. E. Smith), cutworms (*Euxoa auxiliaris* Grote and *Agrotis spp.*), 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 *Phylophaga 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 pests that infest winter wheat in Oklahoma and throughout much of the Southern Great Plains include greenbug, *S. graminum*, Russian wheat aphid, *Diuraphis noxia* Mordviko, bird cherry-oat aphid, *Rhopalosiphum padi* L., Rice root aphid, *R. rufiabdominalis* (Sasaki), English grain aphid, *Sitobion avenae* Fabricius, and corn leaf aphid, *Rhopalosiphum maidis* Fitch. Arguably the most important of these aphid pests is the greenbug (Jones 2001, Royer et al. 2002a).

Greenbug

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). First reported in the United States as an agronomic pest of wheat in 1882 (Pfadt 1962),

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).

Greenbug Biology. Greenbugs are small light green aphids with a darker green dorsal line, black eyes and black tipped cornicles, legs and antenna (Wadley 1931). 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 5.86°C (Wadley 1931, Walgenbach et al. 1988). Alate females can 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). Wadley (1931) described reproductive rates of 3.5 nymphs per day by parthenogenic females and about one egg per day by oviparous females. 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 about 21 generations per year, however as many as 33 generations have been observed (Webster and Phillips 1918).

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

Greenbugs feed on phloem sap by inserting stylets formed by mandibles and maxillae into the plant tissue to feed, which results in chlorosis, and in many cases eventual death of the plant (Burton 1986). Injury 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 (Zadoks et al. 1974), is the most susceptible to greenbug feeding injury (Pike and Schaffner 1985). Infestation at this stage can cause root and shoot biomass reductions that persist throughout the entire growing season which can result in significant yield reductions (Burton 1986, Kindler et al. 2002).

Economic Status of Greenbug. In Oklahoma, losses attributable to greenbug damage are estimated to 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). A severe outbreak in 1976 resulted in costs of over \$80 million to Oklahoma farmers from insecticide applications and yield losses (Starks and Burton 1977).

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 is 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. Kindler et al. (2003) reported a 14.5 kg/ha loss of yield for each greenbug per tiller

during years with near average precipitation and a loss of 34.3 kg/ha under severe drought conditions.

Integrated Pest Management of Greenbug

Cultural Controls. The use of cultural controls to manage greenbug in winter wheat has been limited to a few tested approaches. 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), however, their results may have simply been lower numbers rather than reduced immigration; 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 invigorate wheat allowing it to better tolerate greenbug injury. Under proper fertilization, the rate of greenbug population growth is reduced 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, Ismail et al. 2003). Grazing after the onset of jointing reduces wheat yields, so cattle are typically removed from fields in late-winter (Redmon et al. 1996, Ismail et al. 2003). None of these tactics have been used for the sole purpose of controlling greenbugs, but could be included in a comprehensive IPM program.

In the Southern Great Plains region of the U. S., winter wheat, is often grown in continuous monocultures in large acreages. However, it has been well documented that

continuous monocultures can over time lead to increased pest pressures (Ahern and Brewer 2002, Brewer and Elliott 2004, Men et al. 2004, Boyles et al. 2004).

Host Plant Resistance. Host plant resistance is an intrinsic plant defense against herbivores (Painter 1951). There are three types of intrinsic plant defenses collectively referred to as “Painter’s resistance triangle.” Antibiosis is resistance conferred by host plant toxins or other compounds that have a deleterious effect on herbivores. Antixenosis is defined as non-preference of the herbivore for the plant. Tolerance is the ability by the host plant to endure injury by herbivores without sustaining an economic loss.

Winter wheat producers in the Southern Plains have limited but important greenbug-resistant cultivars as tools for IPM. According to Porter et al. (1997), TAM-110 (with the Gb3 resistance gene) confers resistance to the most abundant greenbug biotypes C, I, and E (Porter et al. 1997, Lazar et al. 1998). Greenbug populations are designated by their “biotype” which refers to the way a select group of plants respond to feeding (Porter et al. 1997). TAM 110 is recommended for production in dryer climates (High Plains) because it is susceptible to leaf rust. An Oklahoma adapted general-use cultivar (‘OKField’) with Gb3 will be available during the fall of 2005. This cultivar is also designated as “Clearfield” which will allow for more selective weed management. Greenbug resistant wheat with Gb3 is not immune to infestation, and damage can occur when aphid levels are extremely high, however, resistant cultivars can withstand considerably more feeding injury without yield loss than susceptible cultivars (Lazar et al. 1998, Kindler et al. 2002). Wheat cultivars with greenbug resistance genes have been shown to have little to no effect on parasitoids and Coccinellidae predators (Jones 2001, Fuentes-Granados et al. 2001, Giles et al. 2005). These tritrophic evaluations indicate

that the beneficial effects of resistance and biological control could be interactive (Brewer and Elliott 2004).

While host plant resistance can be integrated with biological control (Van Emden 1995), integration may not be as simple as once thought. Hare (1992) has cited 16 studies where interactions between resistant crop varieties and parasitoids were studied. Host plant resistance can be positive, have no apparent effect, or even have a negative effect on parasitoid success. Studies evaluating negative effects suggest that host plant resistance may affect weight and fecundity of female parasitoids of the third trophic level as well (Van Emden 1991, 1995, Fuentes-Granados et al. 2001).

Greenbug Sampling and Decision Making. Because grain yield losses are directly related to greenbug population levels, a population assessment (sampling) is required to estimate the potential for economic losses, and whether insecticides are cost effective to apply (Royer et al. 1998b, 2002, Kindler et al. 2002, 2003, Elliott et al 2003a, 2003b). 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 and refined into a simple management system. Coined “Glance 'n go,” this method involves looking at randomly selected tillers and noting the presence or absence of greenbugs on each tiller (Royer et al. 2002a, 2002b, 2005a 2005b). Because the proportion of tillers that are infested accurately corresponds

with greenbug density (Giles et al. 2000), samplers can quickly classify high or low greenbug populations through sequential sampling. The important goal of Glance 'n go sampling is not to determine the exact greenbug density in the field, but rather to classify the likely density as being above or below a defined economic threshold (ET). The economic threshold is the density at which control measures should be taken to prevent an increasing pest population from surpassing the economic injury level (EIL: costs of control are equal to crop loss values), above which significant losses to the producer occur (Stern et al. 1959). Due to the simplicity, timesavings, and ease of using Glance 'n go, producers could be more likely to sample for greenbugs and make profitable educated decisions about insecticide applications (Elliott et al. 2003c).

Insecticides: When insect herbivore populations increase above economic thresholds (ET), treating with an appropriate insecticide can mitigate crop losses. Chlorpyrifos, dimethoate, disulfoton, imadacloprid, malathion, and methyl parathion, are registered for greenbug control in Oklahoma (Royer et al 1998b, Criswell 2005). 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). Greenbug resistance (greenbug as biotype “D”) to organophosphate compounds in sorghum was described by Teetes et al. (1975) and by Peters (1975) in Oklahoma, South Dakota, and Texas. 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).

Biological Control: 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). One of the most important examples of natural enemies in the Southern Great Plains is the parasitic Hymenoptera (Ruth et al. 1975, Kring and Gilstrap 1983, 1984, Kring et al. 1985, Jones 2001, Jones et al. 2003, Giles et al. 2003). 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 *Aphidencyrus aphidivorus* (Mayr), *Pachyneuron siphonophorae* (Ashmead), *Charips sp.* and *Asaphes lucens* (Provancher) have also been identified.

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, Jones 2001, Jones et al. 2003, Giles et al. 2003, Royer et al. 2005a, 2005b, 2005c). It is 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. In order to incorporate natural enemies into IPM decisions,

natural enemies must be identified and their biology must be described to determine whether they can be relied on to achieve successful control.

Lysiphlebus testaceipes

Lysiphlebus testaceipes Cresson (Hymenoptera: Aphidiidae) is a solitary endoparasitoid whose geographic range is Nearctic, Neotropical, Oceanic, in addition to being Palearctic because of intentional introductions (Mackauer and Starý 1967, Krombein et al. 1979). It has been observed to attack over 100 aphid species, including the greenbug (Mackauer and Starý 1967, Starý et al. 1988, Pike et al. 2000).

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 larva that develops first by consuming hemolymph and later the 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 a 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).

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, Royer et al. 2001). 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* adults have 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) (D.B. Jones unpublished data). Cold weather activity indicates either prolonged adult survival, and/or brief warm periods that allow pupating parasitoids to complete development.

Lysiphlebus testaceipes has been observed to suppress greenbug populations below EIL's in wheat directly through mortality (probing and/or parasitism), and indirectly by sterilizing greenbugs and reducing reproductive potential (Pergande 1902, Spencer 1926, Sekhar 1957, Wood and Chada 1969, Eikenbary and Rogers 1974, Krombein et al. 1979, Salto et al. 1983, Jones 2001, Giles et al. 2003). Additionally, *L. testaceipes* can cause aphids to drop from the plant in an attempt to avoid parasitism. Once on the ground, aphids are highly subject to desiccation and attack by other natural enemies (Losey et al. 1998).

Lysiphlebus testaceipes has great potential for destroying large numbers of greenbugs and has been identified as a reliable natural enemy of greenbug and other small grain aphids (Pergande 1902, Sekhar 1957, Wood and Chada 1969, Salto et al. 1983, Patrick and Boring 1990, Jones 2001, Giles et al. 2003, Jones et al. 2003, Royer et al. 2005a, 2005b). The effect of *L. testaceipes* on greenbug populations can be dramatic. When parasitized as adults, greenbugs stop reproducing about three days after being parasitized by *L. testaceipes* (Spencer 1926, Eikenbary and Rogers 1974). However the

biology of *L. testaceipes* is not well described, especially during the colder winter months when wheat is grown in the Southern Great Plains. Factors such as overwintering, ovipositional ability and survivability during the colder months have yet to be explored.

Functional Response

Functional response can be used as an indicator to help determine the relative effectiveness of natural enemies in different situations. Functional response describes the change in attack rate by a natural enemy to the change in host density (Solomon 1949, van Alphen and Jervis 1996). There are four distinct functional response types described in the literature. Type I is described as a constant rise in prey consumed, or hosts parasitized as prey density rises until the natural enemy is satiated or the parasitoid's egg supply is exhausted (Fig. 2.1). The type II attack rate rises at a decreasing rate until a maximum value is reached; the time requirements for subduing, killing, eating and digesting the prey, are responsible for the rate change. The type III response resembles a type II functional response except that at low prey densities the functional response accelerates creating a sigmoidal curve; this acceleration represents an ever-shorter searching time at moderate prey densities. A type IV response resembles a type II 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).

Previous work by Jones et al. (2003) measured functional responses and superparasitism by the indigenous parasitoid wasp *L. testaceipes* and the introduced parasitoid *Aphidius colemani* Viereck (Hymenoptera: Aphidiidae) on greenbug, at four temperatures (14, 18, 22, and 26°C) during a 24-hour period (12:12 L:D). At all

experimental temperatures, functional responses for both wasps most closely fit the Type III model. Instantaneous attack rates at 14°C, for *L. testaceipes* were significantly lower than estimates at 22 and 26°C when data were fit to a Type II functional response model. However, *L. testaceipes* was able to find, subdue and oviposit in many greenbug hosts at 14°C. This suggests that *L. testaceipes* should be active at even colder temperatures.

Overwintering Biology

Overwintering strategies allow insects to survive environments unfavorable for continuous reproduction and normal metabolic functions. Overwintering insects generally must contend with lower than optimum temperatures and other adversities associated with winter conditions. Overwintering is frequently associated with hibernation, which can be subdivided into three main subclassifications (Mansingh 1971):

1. Quiescence. A response of individual insects to sudden, non-cyclic deviations of normal weather conditions that are usually short lived. Quiescence is probably only seen in early winter to active insects and results in growth retardation.
2. Oligopause. A fixed period of dormancy in response to cyclic and longer term climatic changes. Due to longer retardation in growth than quiescent insects, insects in oligopause require nutritional reserves and may even feed periodically during the dormant phase.
3. Diapause. A long term period of dormancy that enables an insect to overcome extended periods of adverse weather conditions. There is a definite preparatory phase that is usually initiated by a temperature independent factor, such as photoperiod, that initiates metabolic changes. The insect does not feed while in

diapause and return of favorable conditions will not terminate diapause immediately. Termination of diapause usually requires a complex series of events, such as the accumulation of degree-days, or a critical photoperiod that will allow the insect to emerge from its overwintering state.

Overwintering insects are vulnerable in their overwintering state. Evasion of predation can be impaired due to the overwintering insects immobility. The overwintering site can also cause major problems for the insect. If the overwintering site is flooded or desiccated the insect may be unable to remove itself from these detrimental conditions. Because of these and possibly other factors, overwintering insects usually experience a high mortality rate, evidenced by the bird cherry-oat aphid *Rhopalosiphum padi* L., whose eggs may show a 20 percent winter survival rate (Leather 1980).

There can be benefits to overwintering. Species, such as anholocyclic aphid species, enter diapause and use it as an opportunity to reproduce sexually without sacrificing much of the rapid multiplicative phase of their life cycle (Ward et al. 1984). Additionally insects in full diapause have little problem with starvation, which can be a major mortality factor of insects active in winter (Mansingh 1971).

Site selection is extremely important for an overwintering insect. Factors such as proximity of large bodies of water, and mountains, inclination and aspect of slopes, vegetation, soil types, snow cover, and possibly other factors affect local conditions and can influence survival of the overwintering insect (Flohn 1969, Danks 1978, Wellington and Trimble 1984). Temperature inversions during the winter are also common and can result in temperature differences of a few or many degrees over only a few meters from ground level (Henson et al. 1954, Stark 1959). Soil moisture can further aid moderation

of soil temperature extremes and help overwintering insects by reducing desiccation risks (Calkins and Kirk 1969). Snow cover can also be an important factor by providing insulation for overwintering insects, protecting them from temperature extremes (Mackay and Mackay 1974).

Currently it is not known where *L. testaceipes* overwinters in the colder regions of the Great Plains. It has been speculated that they may over winter as mummies while attached to blades of grass at varying distances above ground. While not observed, it would be advantageous for *L. testaceipes* to drop from the plant and lay in loose soil or under snow cover due to observations that at only a few centimeters of soil depth, temperatures are moderated (Mukerji and Braun 1988). If overwintering does indeed take place above ground then site selection would be critical since a parasitoid that was concealed inside a whorl of dried leaves would be better protected than a completely exposed mummy. *Lysiphlebus testaceipes* may also be able to overwinter in the Southern Great Plains as an adult or as eggs, larvae, or pupae inside a host aphid. In Oklahoma *L. testaceipes* adults have been observed to be actively seeking hosts and ovipositing on warmer days throughout the winter (D. Jones Personal Observation), suggesting that adults are able to survive the cold temperatures and/ or pupating parasitoids are being warmed enough to complete development.

Parasitoids such as *L. testaceipes*, have additional concerns related to diapause. To be successful, parasitoid and host life cycles must be synchronized such that when the parasitoid breaks diapause there are hosts available to perpetuate the parasitoid. It is probable that some aspect of host physiology is responsible for initiation, maintenance and termination of diapause and that they also play a role in synchronizing host and

parasitoid life cycles (Leather et al. 1993). Three main modes of diapause induction and regulation have been suggested (Tauber et al. 1983, 1986). Some are highly dependent on the physiological state of their host. Others are regulated by environmental cues similar to those that affect non-parasitoids, and others are regulated by a combination of host physiology and environmental cues. Most species appear to respond to more than one cue (Saunders et al. 1970, Anderson and Kaya 1974, Eskafi and Legner 1974, Parrish and Davis 1978, Brodeur and McNeil 1989). Parasitoids usually have a well defined stage in their development at which they are sensitive to cues that induce diapause (Leather et al. 1993). Parasitoids don't always simply follow the physiological lead of their hosts. Many parasitoids are able to alter the physiology of their hosts (Holdaway and Evans 1930, Leather et al. 1993). Additionally there are examples of the parasitoid being able to regulate the onset of diapause in their host (Moore 1989).

Cold Hardiness. Currently little is known about whether *L. testaceipes* is able to, or needs to diapause to survive winter conditions in the Southern Great Plains. Cold-hardiness refers to the ability of an insect to survive low temperatures. Cold-hardiness of an insect is a relative term. At any point in the life of an insect, its ability to survive cold extremes may vary greatly. A number of indices can be used to measure cold-hardiness, such as supercooling points or by LT_{50s} . $LTime_{50}$ is the time at a constant temperature required to kill 50 percent of the population, whereas $LTemp_{50}$ is the temperature at a fixed period of time required to kill 50 percent of the population. Insects can improve their survivability through changes in their body chemistry commonly referred as cold acclimation, or also known as cold-hardening (Leather et al. 1993).

Supercooling. Scientific interpretations for cold hardiness are largely based on research by Salt (1961), who stated that “insects hibernating in cold regions are generally able to withstand fairly low temperatures for long periods of time. Under natural conditions, the only mortality directly attributable to temperature is from freezing.” This founding hypothesis has given rise to the classification of insects as being freezing tolerant or intolerant (Salt, 1936, 1961). Insects exposed to temperatures below the melting point of their body fluid are in danger of being killed by a lethal freezing. The supercooling point is usually regulated by the presence of various endogenous, non-water ice nucleating agents (Lee 1991, Lee et al. 1995). In general, one of two strategies allows these insects to survive such extreme conditions. They may be able to tolerate being in a frozen state, or they avoid freezing by keeping their body fluids supercooled (Zachariassen 1985). A pure liquid or solution that remains unfrozen at temperatures below its freezing point is said to be supercooled (Angell 1982). When ice-nucleating agents are absent, small volumes of water will readily supercool until random clustering of water molecules spontaneously form an ice embryo, upon which ice can form. The theoretical lowest temperature that water can supercool is about -40° C.

In biological systems, ice nucleation generally occurs at temperatures warmer than -20° C (Vali 1995). It is thought that nucleation occurs by a heterogeneous process in which a non-water substrate initiates ice formation. The specific subzero temperature at which ice nucleation occurs is determined by a process influenced by both volume and the duration of exposure to cold temperatures (Vali 1995). As volume increases, the capacity of a solution to supercool decreases, while increasing duration of exposure to cold temperatures increases the likelihood that ice nucleation will occur.

Classification of being freezing tolerant or intolerant, depends on whether an insect is able to survive formation of extracellular and possibly intracellular ice. The only protection for freezing intolerant species is to avoid being frozen or by being able to super cool their body fluids by various means that also prevent freezing. Freezing tolerant species are able to withstand freezing frequently by having ice-nucleating agents (proteins or peptides), generally only present in winter, that initiate protective extracellular freezing at high sub zero temperatures (Zachariassen and Hammel 1976, Zachariassen 1980, 1982, Duman, 1980) and or by the presence of polyhydroxy alcohols that limit freeze damage (Duman and Horwath 1983).

While supercooling studies have been performed on many insects, there are surprisingly few studies on parasitoid and aphid supercooling. Hofsvang and Hågvar (1977) examined supercooling in mummies of the aphid parasitoids *Ephedrus cerasicola* Stål and *Aphidius colemani* Viereck (Hymenoptera: Aphidiidae). They found that both parasitoids could be supercooled below -25° C. Archer et al. (1973, 1974) examined cold storage abilities of *L. testaceipes* adults and mummies, but did not examine supercooling or cold acclimation. However these works are very brief and interpretation of their results is difficult. Archer found that *L. testaceipes* adults could be stored for up to 21 days at 7.2° C, but less than 17% were able to survive this long. Mummies of *L. testaceipes* were hardier, surviving up to 90 days.

Cold Acclimation: Insects can increase their ability to withstand low temperatures through acclimation (Salt 1961). This is a process whereby various cues can stimulate the insect to undergo physiological changes and/or behavioral changes that enhance their ability to survive (Leather et al. 1993). Mechanisms of cold acclimation

include removal of ice nucleators including simple actions such as emptying the insect's gut. Other mechanisms include water loss, and accumulation of polyols, sugars, amino acids, and various proteins (Leather et al. 1993). There may be other mechanisms to cold acclimation that are as yet to be determined. Cold acclimation can be observed in many freezing intolerant species as a seasonal increase in the ability to supercool. This type of change in supercooling ability can be seen in the adult beech leaf mining weevil; *Rhynchaenus fagi* L. beetles collected in late June had a supercooling point of -15.4°C , while beetles collected in early January had a mean supercooling point of -23.1°C (Bale 1980).

There are factors that can lower an insect's cold hardiness. The insect may not evacuate its gut contents, keeping the potential ice nucleators that can initiate ice formation in the gut. Body surface moisture can reduce supercooling ability greatly by allowing ice formation on the body exterior that initiates ice formation within the body at warmer temperatures. High water content in the hemolymph can significantly raise the supercooling point. Recent feeding by phloem feeders such as aphids can enhance supercooling due to the diet being largely sugars and polyols, but other types of feeding, such as chewed plant material, may introduce ice nucleating agents into the gut (Leather et al. 1993).

Changes in cold hardiness can be induced in the laboratory. This involves cooling the insect to an intermediate temperature for a period of time where physiological changes may occur. These changes enable the insect to withstand colder temperatures than one of its non-acclimated co-horts (Baust 1973, Duman and Horwath 1983). A mix of one or more of the previously listed factors probably controls induction of cold

hardiness. It is not currently known if *L. testaceipes* is able to cold-acclimate and survive lower temperatures.

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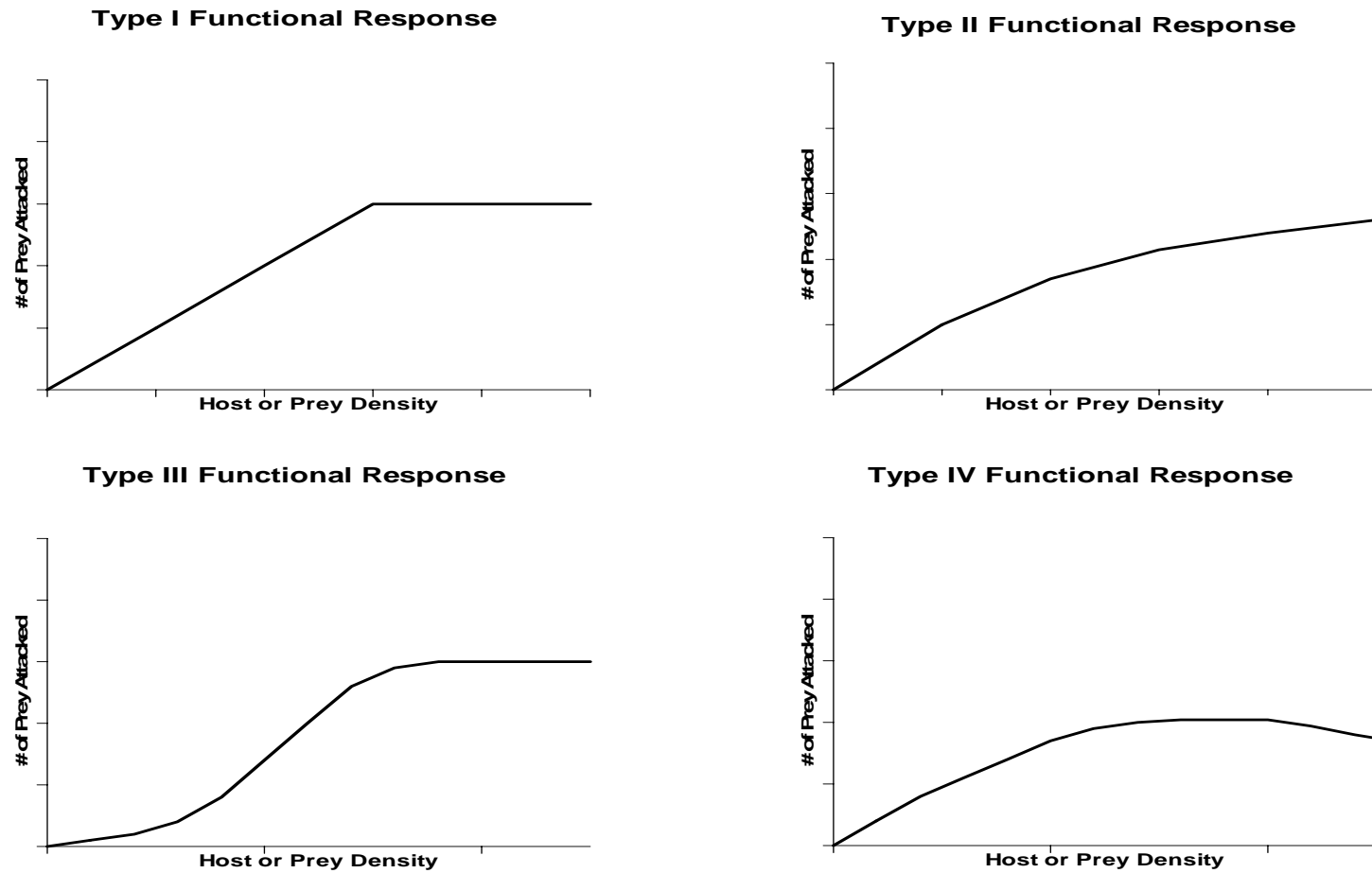


Figure 2.1. The four types of functional response observed in predators and parasitoids. Y-axis label refers to number of hosts parasitized or consumed.

CHAPTER III

SUPERCOOLING POINTS OF *LYSIPHLEBUS TESTACEIPES* AND ITS HOST *SCHIZAPHIS GRAMINUM*

Abstract

Supercooling points (SCP) were measured for various life stages of male and female *Lysiphlebus testaceipes* (Cresson) parasitoids, along with mummies and its aphid host, *Schizaphis graminum* (Rondani). Some parasitoids were acclimated (4 hours at 10° C before cooling down to the SCP) to determine if this could significantly lower the SCP. Acclimation did not improve SCPs for *L. testaceipes*. An inverse relationship between age of the adult parasitoid and its SCP was detected. Non-acclimated male and female parasitoids older than 12h post-emergence spontaneously froze at the warmest mean temperatures ($-20.32^{\circ}\text{C} \pm 1.32\text{ SE}$ and $-22.55^{\circ}\text{C} \pm 0.62\text{ SE}$ respectively). Younger female adult parasitoids (less than 6h post emergence) and mummies had mean SCPs below -26°C . Knowledge of SCPs for *L. testaceipes* and its host *S. graminum* help provide insights about their ability to successfully function throughout the winter in the Southern Great Plains.

Introduction

Lysiphlebus testaceipes (Cresson) (Hymenoptera: Aphidiidae) is a nearctic parasitoid that attacks over 100 aphid species (Mackauer and Starý 1967, Starý et al. 1988, Pike et al. 2000). It is an important parasitoid of cereal aphids in the Southern Great Plains and has been observed to suppress winter wheat (*Triticum Aestivum* L.) pests such as the greenbug, *Schizaphis graminum* (Rondani) (Homoptera: Aphididae) below economic injury levels (Hight et al. 1972, Jones 2001, Giles et al. 2003).

Climatic conditions in Oklahoma are relatively mild during the winter months of wheat growth (Table 3.1). In January, low temperatures average -3° C and highs average 8° C in Oklahoma City (1973-2003; <http://nndc.noaa.gov/>). This transitional area of the Great Plains, however, occasionally experiences dramatic drops in temperature with extremes reaching as low as -22° C (Table 3.1). When temperatures approach colder extremes in the Southern Great Plains, little is known about the relationship between *L. testaceipes* and its greenbug host. Predictability of *L. testaceipes* for regulating greenbug populations is dependent upon its survival during periods of potentially fatal cold winter weather.

Insects exposed to temperatures below the melting point of their body fluid are in danger of being killed by a lethal freezing of that fluid (Salt 1961, Baust 1973, Block 1995). In general, one of two strategies enables insects to survive such extreme conditions. They may be able to tolerate being in a frozen state, or they avoid freezing by various methods including supercooling their body fluids to a point below their actual melting point (Zachariassen 1985). The supercooling point (SCP) of an insect can be lowered by a number of means, including; production of glycerol and other antifreeze

compounds, dehydration of the insect, ingestion of certain substances, and or changes in fatty acids (Sømme 1982). Supercooling points provide a basic indication about the coldest temperature extreme that an insect could survive (Salt 1961, Leather 1993).

The objectives of this study were to (1) determine the supercooling points of various life stages of *L. testaceipes* and its greenbug host, and (2) determine whether conditioning at an intermediate temperature would significantly alter the SCP.

Knowledge of these SCPs would indicate the coldest possible temperature extremes that *L. testaceipes* could survive and provide insights about population interactions with greenbugs during the winter.

Materials and Methods

Insect Preparation. Wheat seed (cultivar '2137') was planted in 5cm diameter by 20cm tall Ray Leach "conetainers™" (Stuewe & Sons Inc., Corvallis, Oregon) and grown for about 3 weeks. A clear acetate tube cage (5cm diameter by 30cm tall) was then fitted around the top of the conetainer™ as previously described (Jones et al 2003). In order to provide adequate ventilation, each cage had two holes in the sides covered with polyester fine mesh netting.

Conetainers™ of wheat were infested with 25-50 3rd instar and older greenbugs from a previously described stock colony of biotype E greenbugs (Jones et al. 2003, 2005). These greenbugs were allowed to settle overnight, after which 5 male/female pairs of *L. testaceipes* from previously described parasitoid colonies reared on wheat (Jones et al. 2003, 2005) were released into each conetainer™ cage. By limiting the number of greenbugs, the fitness of emerging parasitoids was not influenced by plant health (Fuentes-Granados et al. 2001). Parasitized greenbugs were allowed to develop into

mummies, after which they were removed from the conetainer™, and placed individually into 1.5 ml microcentrifuge vials. Vials were kept at 22 ± 1 °C and a photoperiod of 12:12 (L:D). Mummies were allocated such that some were available for mummy SCP analysis, or allowed to develop to provide adult *L. testaceipes* for adult SCP analysis.

Measurement of Supercooling Point. To measure the SCP, four thermocouple appliances were constructed (Fig. 3.1). Each consisted of a 2.5 x 20 cm test tube, with a cork stopper. A 0.3 x 10 cm wooden dowel was placed in the center of the stopper so that it would hang down into the center of the test tube and provide a platform to place the insect specimen on. A 1 m long copper-constantan standard gauge thermocouple wire was extended through the cork stopper and taped to the support dowel. To the distal end of the standard gauge wire, a short length of ultra-fine copper wire was soldered to the end of the copper wire and the same procedure was performed to the constantan lead using constantan ultra-fine wire. The distal ends of the ultra-fine wires were twisted together and secured with a small drop of solder to create the temperature-measuring interface.

Each insect specimen (greenbug, *L. testaceipes* mummy, or *L. testaceipes* adult) was placed singly onto the end of the wooden dowel along with a small drop of petroleum jelly to secure the insect. The ultra-fine thermocouple was then placed in contact with the insect specimen and secured with more petroleum jelly. The specimen was then centered into the test-tube. Each thermocouple was connected to a Sable Systems International TC-1000 thermocouple meter (Sable Systems International, Henderson, NV). The TC-1000 meter self calibrated itself ($< 0.2^{\circ}$ C) against lab-grade internal standards when turned-on and at regular intervals while temperature

measurements were occurring. Temperature measurements were relayed from the meter to a laptop computer via serial cable and were recorded to an ASCII text file. Once connected, each test tube was placed, along with 3 other test tube-thermocouple preparations, inside a wrapping of foam insulation (~ 5 cm thick). This is done to slow the cooling rate to about 1° C per minute. This group of insulated test tubes was then placed into a Styrofoam box and surrounded with crushed dry ice. Temperature measurements were taken every 0.5 seconds until the exotherm associated with the latent heat of fusion is detected (Fig. 3.2). The onset of the exotherm corresponds with the supercooling point for each specimen (Salt 1961).

As insects were available, we determined mean SCPs for the greenbug host, *L. testaceipes* mummies (mummies consisted of greenbugs that had mummified within the previous 24h), freshly emerged adult parasitoids (less than 6h post-emergence males and females), and older parasitoids (greater than 12h post-emergence males and females). Minimums of 20 individual specimens were used to determine each mean SCP.

Acclimation at an intermediate temperature for a few hours can improve the SCP due to changes in the insect's physiology (Sømme 1982, Lee 1991). Most aphid parasitoids including *L. testaceipes* are polyvoltine as are their hosts including the greenbug and, as such, usually respond to day length and temperature as cues for the induction of diapause and diapause associated changes such as increased cold hardiness (Polgár and Hardee 2000). To determine if acclimation significantly changed the supercooling point of *L. testaceipes* males, females, and mummies, these life stages were acclimated at an intermediate temperature of 10°C for four hours before measuring those SCPs. Acclimation at 10°C was chosen because it represents the approximate average

daily temperature during November, prior to the onset of the coldest temperatures during December and January (Table 3.1). Because *L. testaceipes* is a rather short lived parasitoid (personal observation), acclimation time was kept short at four hours. These acclimated adult parasitoids were categorized as being 6 to 10 h post emergence specimens.

Statistical Analysis. An analysis of variance (ANOVA) was performed using PROC MIXED in PC SAS version 8.2 (SAS Institute 1999) to compare SCP's among specimens at a significance level of $P = 0.05$. Student's *t*-test was used to compare differences in mean SCP across treatments.

Voucher Specimens

Voucher specimens of *L. testaceipes* adults, mummies and *S. graminum* adults were deposited in the Department of Entomology and Plant Pathology museum at Oklahoma State University in Stillwater.

Results and Discussion

Mean supercooling points for all treatments ranged from -20.32°C for older male parasitoids to -26.33°C for mummies acclimated at 10°C (Table 3.2). There were significant differences across treatments ($F_{8,218} = 8.72$, $P < 0.0001$). Acclimated and non-acclimated mummies, greenbug hosts and non-acclimated female *L. testaceipes* adults had the lowest mean SCPs, but were not significantly different from one another ($df = 218$, $t < 1.82$, $P > 0.07$). This lack of significant difference in SCPs was not unexpected since parasitoid mummies and their hosts are closely related with respect to their body resources (Brodeur and Boivin 2004). These SCPs for greenbug and *L. testaceipes* are similar to similar species including other cereal aphids such as English grain aphid, *Sitobion avenae* (F.), aphid parasitoids *Aphidius colemani* Viereck, and *Ephedrus cerasicola* Ståfý and the whitefly parasitoid *Eretmocerus eremicus* (Rose & Zolnerowich) (Table 3.3).

A general trend could be discerned that shows as the parasitoid ages, its ability to supercool is reduced (Fig. 3.3). Supercooling points for older parasitoids were at significantly higher temperatures than all other treatments (Table 3.2). Non-acclimated older male parasitoids (older than 12h post-emergence) spontaneously froze at the warmest mean temperature ($-20.32^{\circ}\text{C} \pm 1.32$). Non-acclimated older female parasitoids (>12h post-emergence) had a significantly lower mean SCP of -22.55°C ($df = 218$, $t = 2.68$, $P = 0.008$). Additionally these two age groups had at least a three-fold larger range compared with any of the other age groups (Table 3.2).

For many insects, acclimation for a short period of time at an intermediate temperature can significantly lower the SCP (Sømme 1982, Lee 1991). We determined

that acclimation for four hours at 10° C had no significant effect on mean SCP for *L. testaceipes* mummies (-26.33 vs. -25.94° C; $df = 218$, $t = 0.34$, $P = 0.73$). Additionally, there were no significant differences in mean SCP for acclimated and non-acclimated male *L. testaceipes* (-24.40 vs. -23.95° C; $df = 218$, $t = 0.43$, $P = 0.66$). However, the mean SCP for acclimated female *L. testaceipes* was significantly warmer than for non-acclimated females (-26.13 vs. -23.29° C; $df = 218$, $t = 2.60$, $P = 0.01$).

The observation that acclimation did not significantly lower the SCP for *L. testaceipes* regardless of life stage (Table 3.2) indicated that either (1) no changes were taking place in the parasitoids that could enable them to withstand lower temperatures (Sømme 1982), or (2) our acclimation "treatment" of 10° C for four hours was insufficient to initiate such changes. Additionally, the photoperiod was not changed in this experiment, and perhaps *L. testaceipes* needs a day length cue to alter its SCP. Acclimated adult parasitoids had SCPs that were intermediate to the non-acclimated adults (<6h post-emergence) and the older parasitoids (>12h post-emergence). These acclimated adult parasitoids were on average about 4 hours older than the freshly emerged parasitoids and at least 2 or more hours younger than the older parasitoids. If *L. testaceipes* is indeed able to significantly lower its SCP, other factors may be important, such as the physiological state of its host, or perhaps even the species of the host aphid (Polgár and Hardee 2000). Progeny of *Aphidius matricariae* Haliday would not enter diapause if anholocyclic aphid species such as *Aphis gossypii* Glover and *Myzus ascalonicus* Doncaster were the host aphid. But *A. matricariae* could enter diapause if they developed in apterous virginoparae of the holocyclic aphid species *Myzus persicae* Sulzer (Polgár and Hardee 2000).

Supercooling ability can often be attributed to the accumulation of cryoprotectant chemicals and/or the absence of ice nucleating agents (Lee 1991). Sugars such as glucose, trehalose, and fructose and polyols such as glycerol, mannitol, and sorbitol are known to provide increased supercooling ability and are commonly found in insects (Sømme 1967, 1969, Tanno 1964, Block and Zettel 1980). Perhaps common sugars such as trehalose constitute a high percentage of *L. testaceipes* hemolymph and provide much of their supercooling ability. The depletion of this sugar or some other resource necessary for the parasitoid to live may be responsible for the SCP to be inversely related to the age of the parasitoid. Another possibility was that as the parasitoids aged, they accumulated ice-nucleating agents as a by-product of normal metabolic processes in their hemolymph allowing the parasitoids to freeze at warmer temperatures.

In Oklahoma, parasitoids experience temperatures that range from ideal, to tolerable, to unsuitable, during the winter wheat growing season (Table 3.1). Based on these results, *L. testaceipes* could likely survive even the most extreme temperatures experienced in central Oklahoma (Table 3.1). However SCP is only an indication of how cold an organism can be before it freezes (Bale 1993). The parasitoid may perish at much warmer temperatures or be rendered unable to function in a normal manner much in the same manner that the English grain aphid, a potential host of *L. testaceipes*, has a cold tolerance of -14.6° C, about 12° C above its SCP (Parish and Bale 1991). This situation is likely to occur for *L. testaceipes* in Oklahoma during rapid temperature decreases without protective snow cover.

Lysiphlebus testaceipes is commonly found at latitudes that experience much colder temperatures than Oklahoma (Royer et al. 2001). How these parasitoids survive

the winter at the colder latitudes has yet to be answered. Do these parasitoids survive the winter or do they immigrate in from more temperate regions each spring? As yet we have no clear answers to this question. If these parasitoids were able to overwinter in these colder climates perhaps they were protected by snowfall much in the same manner that snowfall insulates and protects over-wintering Colorado potato beetles (Milner et al. 1992, Hoy 1998). Supercooling ability is only a base value that indicated what temperature *L. testaceipes* might be able to endure, more research is needed to answer the questions about temperatures and winter conditions *L. testaceipes* can survive in the field in Oklahoma.

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Table 3.1. Table of temperatures in Celsius for Oklahoma City, Oklahoma (1973-2003) from the National Virtual Data System, part of the National Oceanic & Atmospheric Administration (NOAA) (<http://nndc.noaa.gov/>).

	September	October	November	December	January	February	March	April
High (normal)	29°C	23°C	16°C	10°C	8°C	12°C	17°C	22°C
Low (normal)	17°C	11°C	3°C	-2°C	-3°C	-1°C	4°C	9°C
Maximum	42°C	36°C	31°C	30°C	27°C	33°C	34°C	38°C
Minimum	2°C	-9°C	-12°C	-22°C	-20°C	-19°C	-16°C	-7°C

Table 3.2. Supercooling points for greenbug, *Lysiphlebus testaceipes* mummies, freshly emerged adults, older adults, and adults conditioned at 10°C.

Species	Acclimation	Life stage	Sex	Mean SCP ± SE (°C)	n	SCP range (°C) (max, min)	Signifi- cance
<i>L. testaceipes</i>	4 h @10°C	Mummy	NA	-26.33 ± 0.20	19	-24.49, -27.79	A
<i>L. testaceipes</i>	None	Adult (< 6 h post emergence)	Fem.	-26.13 ± 0.31	22	-22.25, -27.48	AB
Greenbug	None	Adult	Fem.	-25.98 ± 0.10	22	-25.03, -26.76	ABC
<i>L. testaceipes</i>	None	Mummy	NA	-25.94 ± 0.18	20	-24.49, -27.16	ABC
<i>L. testaceipes</i>	None	Adult (< 6 h post emergence)	Male	-24.40 ± 0.45	27	-19.50, -26.97	BCD
<i>L. testaceipes</i>	4 h @10°C ¹	Adult (< 6 h post-emergence)	Male	-23.95 ± 0.50	20	-19.30, -26.49	CDE
<i>L. testaceipes</i>	4 h @10°C ¹	Adult (< 6 h post-emergence)	Fem.	-23.29 ± 0.61	20	-19.23, -26.53	DE
<i>L. testaceipes</i>	None	Adult (> 12 h post emergence)	Fem.	-22.55 ± 0.62	48	-9.29, -26.74	E
<i>L. testaceipes</i>	None	Adult (> 12 h post emergence)	Male	-20.32 ± 1.32	29	-5.40, -27.26	F

¹Adult specimens that were acclimated for 4 h @ 10 are described in the text as being 6-10 h post-emergence.

Table 3.3. Coldest supercooling points (SCPs) for adults of various aphid and parasitoid species.

Species	SCP (°C)	Reference
<i>Lysiphlebus testaceipes</i> Cresson	-26.13	This paper
<i>Aphidius colemani</i> Viereck	-25.4	Hofsvang and Hågvar 1977
<i>Eretomocerus eremicus</i> (Rose & Zolnerowich)	-25.0	Tullett et al. 2004
<i>Ephedrus cerasicola</i> Stäfy	-26.1	Hofsvang and Hågvar 1977
<i>Schizaphis graminum</i> (Rondani)	-25.98	This paper
<i>Diuraphis noxia</i> Mordvilko	-24.9	Butts 1992
<i>Aphis glycenes</i> Matsumura	-24.9	McCornack et al. 2005
<i>Megoura crassicauda</i> Mordvilko	-24.5	Asai et al. 2002
<i>Myzus persicae</i> (Sulzer)	-24.2	Bale et al. 1988
<i>Sitobion avenae</i> (F.)	-24.2	Knight et al. 1986
<i>Acyrtosiphon pisum</i> (Harris)	-23.7	Asai et al. 2002
<i>Aphis fabae</i> Scopoli	-23.6	O'Doherty 1986
<i>Elatobium abietinum</i> (Walker)	-15.7	Powell 1974

Fig. 3.1. Generalized diagram of supercooling point measuring equipment as used in this experiment.

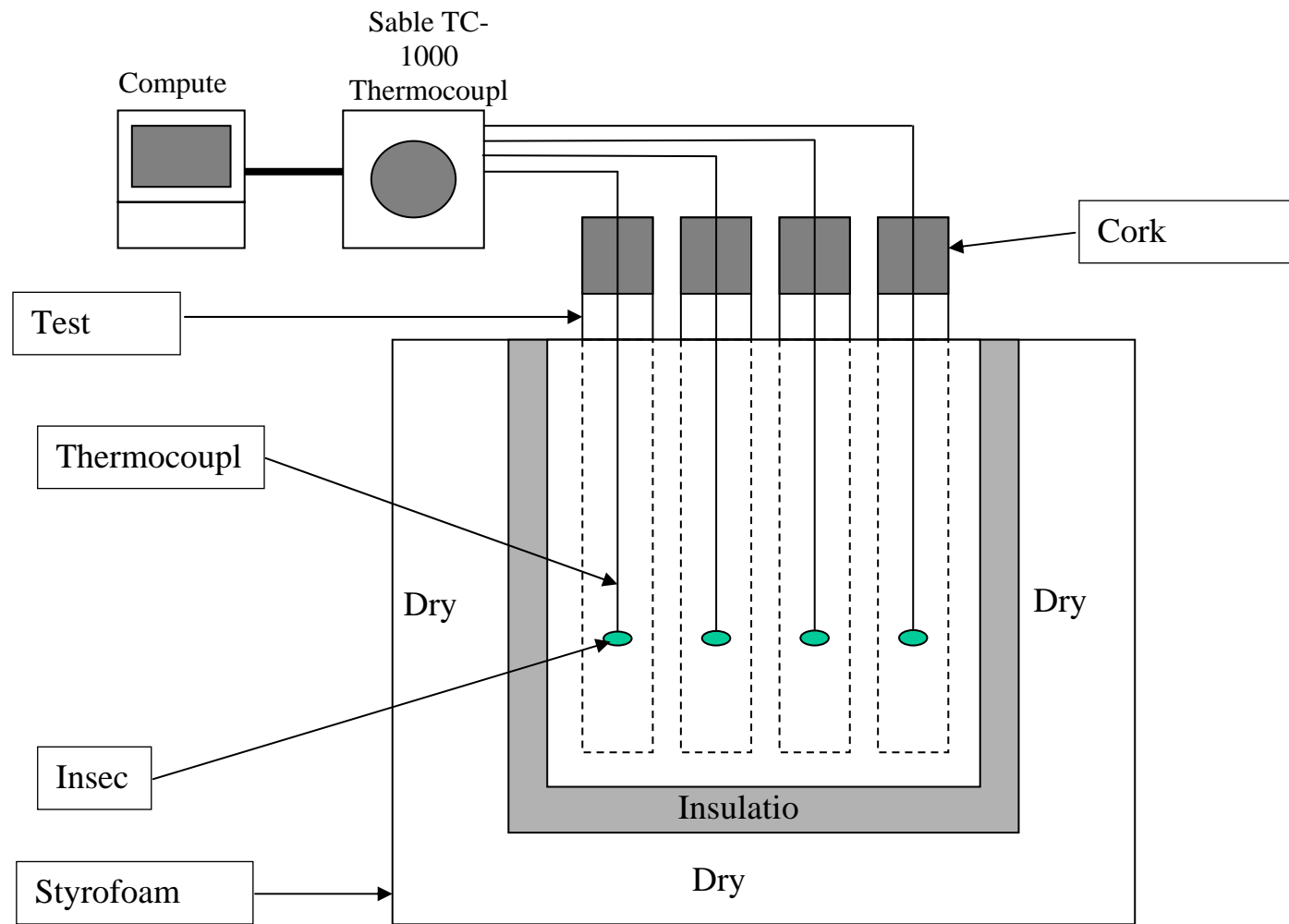


Fig. 3.2. Generalized plot illustrating the exotherm associated with the latent heat of fusion. The onset of the exotherm corresponds with the supercooling point for each specimen (Salt 1961).

Typical Supercooling Plot

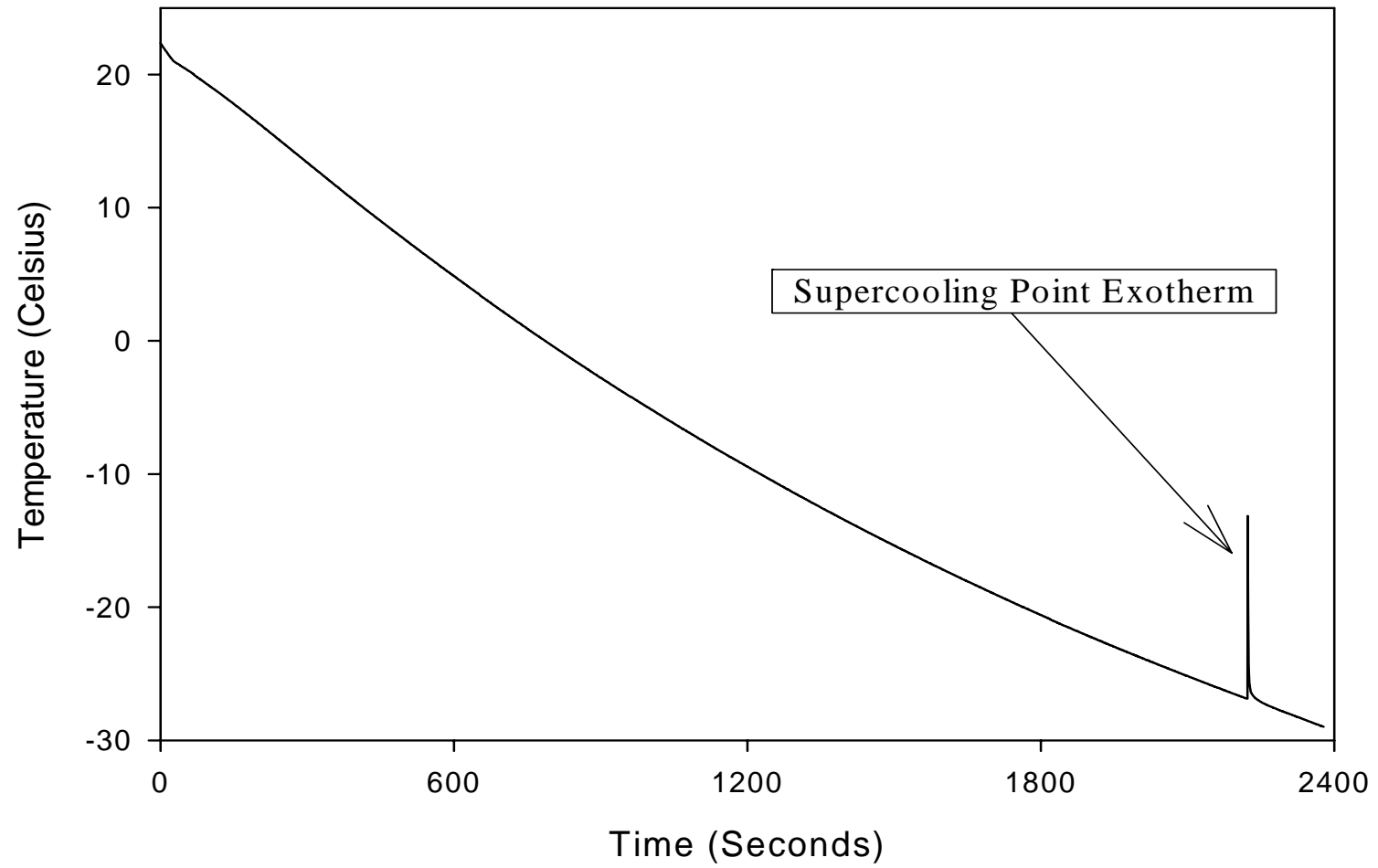
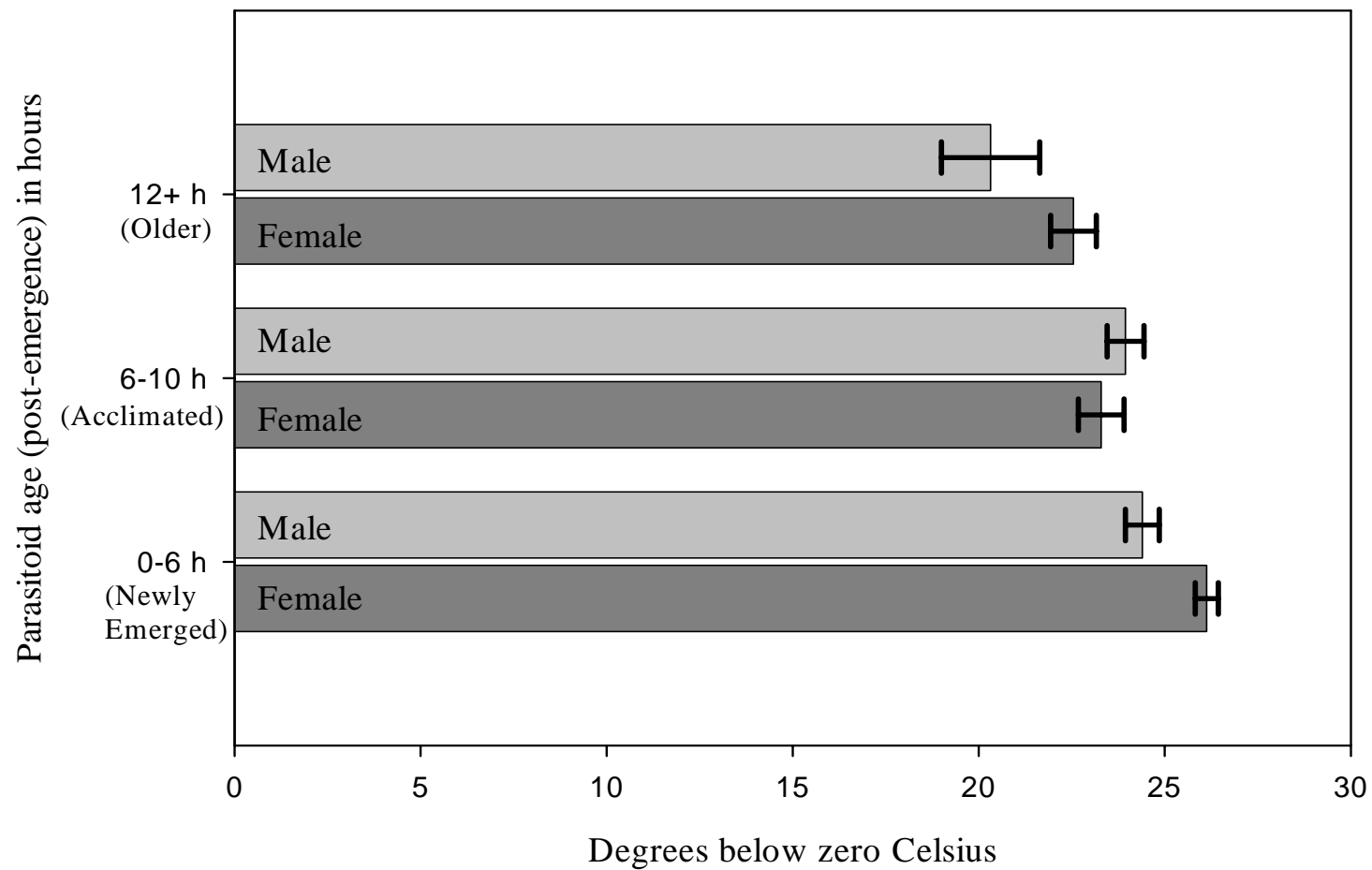


Fig. 3.3. Plot of supercooling points of *Lysiphlebus testaceipes* as related to adult parasitoid age (hours post-emergence from mummy).



CHAPTER IV

COLD HARDINESS OF *LYSIPHLEBUS TESTACEIPES*

Abstract

Lysiphlebus testaceipes (Cresson) mummies and adults were cooled for various periods of time to determine their cold hardiness at temperatures commonly experienced in the Southern Great Plains. Subsequently, surviving parasitoids were exposed to greenbug, *Schizaphis graminum* (Rondani) hosts, to determine whether the temperature extreme they survived adversely affected oviposition. *Lysiphlebus testaceipes* females survived regimens that cooled the parasitoid to 2, -2, and -6°C for 12 h, however at -8°C, all *L. testaceipes* specimens perished. Female parasitoids that survived cooling treatments were able to oviposit when warmed. Some *L. testaceipes* adults were able to survive up to 21d at 5°C and oviposit successfully when warmed to 22°C, however no adults survived more than 7d at -6°C. A few *L. testaceipes* mummies were observed to survive up to 67d at 5°C and 28d at -6°C and still oviposit successfully when warmed to 22°C. These cold temperature survival abilities along with observations that parasitoids are actively foraging at cold winter temperatures in the field provide insights on how *L. testaceipes*, when it is present in sufficient numbers, is able to effectively prevent greenbug populations from increasing in winter wheat agro-ecosystems.

Introduction

Lysiphlebus testaceipes (Cresson) (Hymenoptera: Aphididae), is a nearctic parasitoid that attacks over 100 aphid species, and is an important parasitoid of cereal aphids including the greenbug *Schizaphis graminum* (Rondani) (Homoptera: Aphididae), in the Southern Great Plains (Mackauer and Starý 1967, Krombein et al. 1979, Starý et al. 1988, Pike et al. 2000). This parasitoid has great potential for destroying large numbers of greenbugs (Jones 2001), and has become an important part of greenbug management programs in winter wheat, *Triticum aestivum* L. (Royer et al. 2002, 2005, Giles et al. 2003, Jones et al. unpublished data).

Lysiphlebus testaceipes has been found to provide control of greenbug and other small grain aphid populations on winter wheat from fall to spring in Oklahoma (Jones 2001, Jones et al. 2003, Giles et al. 2003). However these studies did not examine what effects cold winter temperatures (December to February) might have on the performance of this parasitoid. Based on historical temperatures recorded at Oklahoma City, Oklahoma (National Virtual Data System, NOAA; <http://nndc.noaa.gov/>), normal high and low temperatures during the wheat growing season range from -2°C in January to 30°C in September with extremes of 43°C and -21°C (Table 4.1). In order to understand interactions between greenbugs and *L. testaceipes*, it is necessary to describe parasitoid biology over the full range of these temperatures. Much work has been done at warmer temperatures (above 10°C), yet relatively little has been studied when cold weather occurs.

One very important question relative to predicting biological control of aphids in winter wheat has to do with the survivability (cold-hardiness) of *L. testaceipes* during winter months in Oklahoma. That is, what are the temperature thresholds below which *L.*

testaceipes populations are unable to survive or are significantly reduced? Insects exposed to temperatures below the melting point of their body fluid are in danger of being killed by a lethal freezing of that fluid (Salt 1961, Baust 1973, Block 1995). Previous work has indicated that *L. testaceipes* adults and mummies can supercool to about -26° C (Jones et al. unpublished data). However, supercooling ability is not necessarily the best indication of what temperature extremes an insect can survive (Bale 1987). This question about cold-hardiness can be addressed by determining the temperature required to kill 50 percent of the population for a constant time period (LTemp₅₀), and the time required to kill 50 percent of the population at a constant low temperature (LTime₅₀; Salt 1950, Leather et al. 1993). Simple survival of a female parasitoid is not the only issue of importance. The question of “functional” survivability must be considered. This is important because the female parasitoid not only has to survive the cold temperature, but must also be able to function and reproduce afterward. Additionally life stage may be important for survival. It is not currently known which life stage of *L. testaceipes* is best suited for cold temperature survival. Parasitoids in the adult stage are necessary for reproduction to occur, however the pupal or larval stage may be more important for *L. testaceipes* to persist throughout the winter months in the Southern Great Plains.

The objectives of this research were;

- 1) Determine minimum temperatures which *L. testaceipes* adults and mummies (pupal stage) could survive for 12 hours and what temperature was lethal to 50% of the specimens (LTemp₅₀).

2) Determine how long (LTime₅₀) *L. testaceipes* adults and mummies could survive at 5°C (just below the developmental threshold temperature for *L. testaceipes*) and at the LTemp₅₀ temperature (-6°C) for *L. testaceipes*.

3) Examine the ability of parasitoids to reproduce after exposure to cold temperatures.

Materials and Methods

Greenbug and Parasitoid Colonies. Biotype “E” greenbugs colonies were maintained as previously described (Jones et al. 2003, 2005) on wheat (cv ‘2137’) grown in a fritted clay and sphagnum moss mixture. *Lysiphlebus testaceipes* colonies were also maintained as previously described (Jones et al. 2003, 2005). Wheat (cv ‘2137’) grown in 5 cm diameter by 20 cm tall Ray Leach “conetainers™” (Stuewe & Sons Inc., Corvallis, Oregon) was infested with 25-35 3rd instar and older greenbugs from the wheat stock colony (Jones et al 2003). This was done to ensure that parasitoids of known age (\pm 12h) developed in greenbugs reared on wheat. Limiting the number of greenbugs helped ensure that the fitness of emerging parasitoids was not influenced by plant health (Fuentes-Granados et al. 2001). These greenbugs were allowed to settle overnight, after which 5 male/female pairs of *L. testaceipes* parasitoids were released into each conetainer™ cage. After several days, parasitized greenbugs developed into mummies. Cages were inspected daily for mummies that were removed and placed individually into 1.5 ml microcentrifuge vials. These mummies were used immediately for evaluations requiring mummies or were allowed to develop at 22°C with a photo-period of 12:12 (L:D) into adult parasitoids for evaluations requiring adults.

Twelve Hour Cold Hardiness of Adult *L. testaceipes*. Adult *L. testaceipes* parasitoids targeted for evaluations were placed as pairs (male/female) into 1.5ml micro

centrifuge tubes immediately after emerging. These parasitoids were then placed into an environmental chamber and cooled from 22°C at 4°C drops in temperature, every two hours, by manually resetting the chamber temperature, until a defined temperature of 2, -2, -6, or -8°C was attained. Once the desired minimum temperature was attained the parasitoid pairs were maintained at that temperature for 12h, after which temperatures were increased 4°C every 2h until the chamber was at 22°C again. After being returned to 22°C, individual female parasitoids were placed into a large test tube (2.5 x 20 cm) with 10 greenbugs on a wheat seedling grown in a 1.5 ml micro centrifuge tube. The parasitoids were allowed to interact with greenbugs for 24 h, before they were removed. These greenbugs were reared in the test tube at room temperature (about 22° C) for three days and were dissected to determine parasitism and the number of eggs oviposited by the subject parasitoid (Jones et al. 2003). Fifty pairs of *L. testaceipes* adults (as three replications of 17, 17, and 16 pairs for each replication) were evaluated at each temperature.

Twelve Hour Cold Hardiness of *L. testaceipes* Mummies. Recently mummified greenbugs parasitized by *L. testaceipes* were placed singly into 1.5 ml micro centrifuge tubes and then placed into an environmental chamber. These mummies were then assigned to the same cold regimen treatments described for *L. testaceipes* adults. Once the cold cycles were completed, mummies were then held at 22°C (12:12 L:D) until they emerged as adults. Newly emerged female parasitoids were then paired up with a male and exposed to 10 greenbug hosts in test tubes (as previously described) for 24h. Greenbug hosts were dissected three days later to determine successful oviposition. A

total of one hundred mummies in replications of 33, 33 and 34 *L. testaceipes* mummies were evaluated at each temperature.

Long Term Cold Hardiness of *L. testaceipes* Adults. *Lysiphlebus testaceipes* mummies (in 1.5 ml microcentrifuge tubes) were placed into an environmental chamber at 22° C to allow development into adults. Within 12 h after emergence, one hundred of these parasitoids (50 male and 50 female) were then placed individually in 1.5 ml microcentrifuge tubes into another environmental chamber and cooled from 22 to 5°C (just below the developmental threshold for *L. testaceipes*) in steps of 4°C every 2h (the last step was 3°C). Parasitoids were removed from the chamber at intervals of 1, 2, 3, 7, 9, 12, 14, 16, 19, 21, and 22 days, briefly examined (10 microcentrifuge tubes at a time), and replaced into the chamber to determine survival. A total of three replications of 100 newly emerged adult parasitoids each were evaluated for survival at 5°C. Adult parasitoids cooled to 5°C were not assayed for ovipositional ability since the same individuals were followed over time until each one died making oviposition observations impossible.

Another 200 *L. testaceipes* adults (100 females and 100 males) were cooled stepwise as previously described to the approximate LTemp₅₀ (-6°C) of adult *L. testaceipes*. At intervals of 1, 2, 3, 4, 5, and 6 days after being cooled, 16 or 17 males and an additional 16 or 17 females were removed from the incubator. Survival of each parasitoid was recorded, after which surviving female parasitoids were paired with a male parasitoid and evaluated for ovipositional ability as previously described. Three replication of this procedure were made such that a total of one hundred parasitoids (50

male and 50 female) were evaluated (2 replications of 16 males and 16 females and a third replication of 17 males and 17 females) for each time interval.

Long Term Cold Hardiness of *L. testaceipes* Mummies. To evaluate the length of time that *L. testaceipes* mummies could survive when cooled, groups of newly formed mummies (mummy cohorts ranged from 16 mummies to 258 mummies) were cooled to 5°C for 1, 2, 3, 7, 9, 12, 14, 16, 27, 35, 47, 49, 67, or 68 days. For each time period a minimum of 50 mummies were evaluated, whether by repetition of small cohorts or by a single large cohort. Additional groups of mummies (33 to 41 mummies), were cooled stepwise to -6°C and maintained for 1, 2, 7, 14, 21, or 28 days (Table 4.2). When each group of mummies were removed from the environmental chamber, the mummies were warmed to 22°C and held for several days to allow development into adults. Surviving parasitoids were then sexed, paired to allow mating and then evaluated for oviposition ability as previously described.

Field Observations. In order to verify that *L. testaceipes* is indeed able to survive Oklahoma winter temperatures and is active during the wheat growing season, sentinel plants infested with greenbugs were used to determine whether parasitoids were ovipositing in a local wheat field during 2003-2004. These sentinel plants were prepared by taking wheat grown in a conetainer™ as described above, and infesting it with 25-30 greenbugs without regard to greenbug age. The greenbug-infested conetainer™ was then placed into a growth chamber and cooled to 5°C for 24h to help lessen the possible shock of transferring greenbugs reared at 22°C into the field where temperatures could drop below 0°C. Eight sentinel plants were placed in the ground (still in the conetainer™),

and spaced 30m apart along a transect across a 40 ha wheat field in Payne County, Oklahoma.

Sentinel plants were placed in the field from 1 December 2003 until 18 March 2004. Each group of sentinel plants was left in the field for 14d after which each was replaced by a fresh plant. Collected sentinel plants were each caged inside a 5cm by 30cm tall acetate tube that had two mesh windows in the side and a mesh top for ventilation and then returned to the laboratory. Each plant was then maintained for seven days at 22°C (12:12 L:D) to allow parasitoids that may be present to develop into mummies. Mummies were removed every 24h, placed into a 1.5ml microcentrifuge tube and then reared at 22°C until adult emergence. Adult parasitoids were then identified to species using a parasitoid key developed by Pike et al. (1997). Temperature data were obtained from the Oklahoma Mesonet (<http://www.mesonet.org>).

Statistical Analysis. An analysis of variance (ANOVA) was performed using PROC MIXED in PC SAS version 8.2 (SAS Institute 1999) to determine differences in survival for adults and mummies for each temperature and time period. ANOVA was also used to analyze ovipositional data at a significance level of $P = 0.05$. SAS PROC PROBIT was used to determine LTime₅₀ and LTemp₅₀ values.

Voucher Specimens. Voucher specimens of *L. testaceipes* adults, mummies and *S. graminum* adults were deposited in the Department of Entomology and Plant Pathology museum at Oklahoma State University in Stillwater.

Results and Discussion

Twelve Hour Cold Hardiness of *L. testaceipes* Adults and Mummies. About 74% of male and female *L. testaceipes* survived being cooled to 2°C for 12 h (Table 4.2). At -2°C and -6°C survivorship declined to 66.8 and 49.4% respectively. When *L. testaceipes* adults were cooled to -8°C for 12h, all specimens perished. From this data, the LTemp₅₀ was determined to be about -6°C for *L. testaceipes* adults. Of the surviving female parasitoids 70.5% oviposited a mean of 12.0 eggs per female at 2°C. At -2°C, 75.0% of surviving female parasitoids oviposited a mean of 10.4 eggs per female parasitoid, and 87.2% of surviving female parasitoids at -6°C oviposited a mean of 6.4 eggs (Table 4.2).

Eighty-five percent of *L. testaceipes* mummies survived 2°C for 12h with 44% of the adult females that emerged from the surviving mummies ovipositing a mean of 6.9 eggs per parasitoid. Survival dropped to 80% for mummies cooled to -2°C, with 40% of the emerged females ovipositing a mean of 6.4 eggs per parasitoid. Sixty-seven percent of mummies survived being cooled to -6°C, with 35% of the emerged females ovipositing a mean of 6.8 eggs per parasitoid. All mummies that were cooled to -8°C for 12h perished (Table 4.2). Because 67% of the mummies survived being cooled to -6°C, but none survived being cooled to -8°C, and no temperatures were tested in between those temperatures, PROC PROBIT was unable to determine the LTemp₅₀ with any precision. Therefore all we can declare is that the LTemp₅₀ was between -6 and -8°C for *L. testaceipes* mummies.

There may be a direct relationship between the 12h temperature treatment and the mean number of eggs that adult *L. testaceipes* oviposited (Table 4.2). A possible reason

for this might be that more body resources were utilized at the colder temperatures than were utilized at the warmer temperatures. However, *L. testaceipes* oviposition rates for females cooled for longer periods of time did not have a detectable trend (Table 4.3). The presence of a trend in oviposition rates for females in the 12h study was more likely a false trend that would not be seen if the 12h study were conducted with a larger cohort of parasitoids.

Long Term Cold Hardiness of *L. testaceipes* Adults and Mummies. At 5°C, a few *L. testaceipes* mummies were able to survive at least 67 days and the emerging females were able to oviposit successfully (less than 0.2 greenbugs per parasitoid; Table 4.4), though at a much reduced rate (<0.2 eggs per female) (Table 4.3). The majority of the mummies cooled to 5°C were able to survive at least 16d with the LTime₅₀ being calculated at about 683.9h or approximately 28.5d (Tables 4.5 and 4.6). When *L. testaceipes* mummies were cooled to -6°C, survival was somewhat reduced compared to mummies cooled to 5°C. LTime₅₀ was determined to be 300.8h or approximately 12.5d. However some individual specimens were viable after 28d (Table 4.5) parasitizing a mean of 4.0 greenbugs per emerging female (Table 4.4) with a mean of 11.3 eggs per female (Table 4.3).

Lysiphlebus testaceipes adults were only able to survive a maximum of one week at -6°C (Table 4.5). The LTime₅₀ was 66.8h (Table 4.6). Adults that survived -6°C oviposited a mean of 3.8 eggs per female into a mean of 2.4 greenbugs after 4 days, up to 5.8 eggs per female into a mean of 2.7 greenbugs after only 2 days (Tables 4.3 and 4.5). When adult parasitoids were cooled to 5°C, 2.3% were able to survive at least 21 days. At 5°C, it took 250.2h or approximately 10.4d for 50% of the specimens to perish (Table

4.6). *Lysiphlebus testaceipes* adults chilled to 5°C were not assayed for ovipositional ability because individual parasitoids were followed over time until each one died, thus oviposition observations were not possible.

Sentinel Plants. *Lysiphlebus testaceipes* were collected on sentinel plants throughout the collection period from 1 December 2003 until cessation of collections on 18 March 2004 (Fig. 4.1). Numbers of *L. testaceipes* collected did not follow the seasonal decrease in temperature, but rather increased until 2 March 2004 after which collections declined (Fig. 4.1). Additionally, while it was determined that exposure to -8°C killed 100% of *L. testaceipes* mummies and adults in the laboratory, *L. testaceipes* was active in the field though out the winter even though the ambient temperature dropped below -8°C many times (Fig. 4.1). This was probably due to some parasitoids being in various protected microclimates within the wheat field that enabled them to survive these lethal low temperatures (Leather et al. 1993). These microclimates include being sheltered by vegetation, temperature inversions, snow cover, hiding under the soil surface, inclination, and possibly other factors that affect local conditions (Flohn 1969, Danks 1978, Wellington and Trimble 1984).

These results compare well with Archer et al. (1973, 1974) who performed cold storage studies on *L. testaceipes*. They found that *L. testaceipes* mummies could survive up to 90d at 1.7 and 4.4°C, while adults only survived 21days. We found that *L. testaceipes* can survive as a mummy for over 68d at 5°C and over 28d at -6°C. Additionally *L. testaceipes* adults can survive over 21d at 5°C, but are limited to less than 7d at -6°C (Table 4.5). However -8°C was lethal to both adults and immature *L. testaceipes* in the laboratory.

Because *Lysiphlebus testaceipes* can survive for such long periods of time at these cold temperatures, it is well suited for life in the Southern Great Plains. Temperatures in the Oklahoma City, OK area, which should be representative of the Southern Great Plains, drop below the -8°C temperature that caused 100% mortality for *L. testaceipes* in the laboratory. However, these minimums occur for only short periods of time, with the ambient temperature frequently exceeding the developmental threshold of *L. testaceipes* (6.6°C; Royer et al. 2001) most days (Table 4.1, Fig. 4.1).

Because temperatures are frequently warmer than the developmental threshold, and the parasitoid can complete a life cycle even during the coldest month, *L. testaceipes* should not need to enter diapause to overwinter in the Southern Great Plains. Additionally, sentinel plant data demonstrate that even when the temperature drops below -8°C, there are a number of *L. testaceipes* parasitoids that are able to find protected locations that enable them to survive the lethal temperatures. When the temperatures near the developmental threshold, *L. testaceipes* development slows and their life cycle is considerably lengthened (Royer et al. 2001). Additionally, in most winters in the Southern Great Plains, there are many aphid hosts available (personal observation) to utilize for the next generation. In colder regions of the Great Plains, *L. testaceipes* may yet enter diapause to survive the winter since aphid hosts are scarcer, but there is no data yet to support this possibility. This ability to survive cold temperatures as adults and mummies, and successfully attack greenbugs and other cereal aphids throughout the winter months may explain why this parasitoid has been capable of providing biological control of greenbug and other cereal aphids in winter wheat in the Southern Great Plains.

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Table 4.1. Table of temperatures in Celsius for Oklahoma City, Oklahoma (1973-2003) from the National Virtual Data System, part of the National Oceanic & Atmospheric Administration (NOAA) (<http://nndc.noaa.gov/>).

	September	October	November	December	January	February	March	April
High (normal)	29°C	23°C	16°C	10°C	8°C	12°C	17°C	22°C
Low (normal)	17°C	11°C	3°C	-2°C	-3°C	-1°C	4°C	9°C
Maximum	42°C	36°C	31°C	30°C	27°C	33°C	34°C	38°C
Minimum	2°C	-9°C	-12°C	-22°C	-20°C	-19°C	-16°C	-7°C

Table 4.2. Survival of *L. testaceipes* cooled stepwise from 22°C to 2, -2, -6, and -8°C for 12 hours, along with mean percent of females ovipositing when exposed to 10 greenbugs after being warmed stepwise back to 22°C.

Life Stage	Exposure		Number of Parasitoids	Mean Percent Survival			Mean Percent of Females Ovipositing
	(hours)	Temp.		Male	Female	Pooled	
Mummy	12 h	2°C	100 mummies	34%	51%	85%	70.5% (6.9 ⁽¹⁾)
	12 h	-2°C	100 mummies	36%	44%	80%	75.0% (6.4 ⁽¹⁾)
	12 h	-6°C	100 mummies	28%	39%	67%	87.2% (6.8 ⁽¹⁾)
	12 h	-8°C	100 mummies	NA	NA	0%	0.0%
Adult	12 h	2°C	50 male: 50 Female	70.8% a	77.3% a	74.0% a	97.3% (12.0 ⁽¹⁾)
	12 h	-2°C	50 male: 50 Female	65.1% a	68.5% a	66.8% a	97.0% (10.4 ⁽¹⁾)
	12 h	-6°C	50 male: 50 Female	44.7% b	54.1% b	49.4% b	82.9% (6.4 ⁽¹⁾)
	12 h	-8°C	50 male: 50 Female	0.00% c	0.00% c	0.00% c	0.0%

⁽¹⁾ Mean eggs laid per female parasitoid

Table 4.3. Total eggs oviposited by *Lysiphlebus testaceipes* females \pm SE, after being cooled to 5, and -6°C for various periods of time (means within the same column followed by the same letter are not significantly different from each other at $P = 0.05$).

	Life Stage and Temperature			
	Mummies		Adults	
<u>Time</u>	<u>5°C</u>	<u>-6°C</u>	<u>5°C</u>	<u>-6°C</u>
24 (1d)	4.7 \pm 0.3 bcd ⁽¹⁾	3.0 \pm NA a ⁽⁷⁾	NA	4.9 \pm 0.8 a ⁽¹³⁾
48 (2d)	5.3 \pm 1.9 bc ⁽¹⁾	2.2 \pm NA a ⁽⁸⁾	NA	5.8 \pm 1.0 a ⁽¹³⁾
72 (3d)	4.4 \pm 1.2 bcd ⁽¹⁾		NA	5.3 \pm 0.6 a ⁽¹³⁾
96 (4d)				3.8 \pm 0.6 a ⁽¹³⁾
120 (5d)				4.7 \pm 0.3 a ⁽¹³⁾
144 (6d)				0.0 \pm 0.0 a ⁽¹³⁾
168 (7d)	6.4 \pm 1.4 ab ⁽¹⁾	5.1 \pm NA a ⁽⁹⁾	NA	
216 (9d)	9.6 \pm 1.6 a ⁽¹⁾		NA	

288 (12d)	6.9 ± 1.0 ab ⁽¹⁾		NA	
336 (14d)	7.5 ± 0.3 ab ⁽¹⁾	$9.0 \pm$ NA a ⁽¹⁰⁾	NA	
384 (16d)	7.6 ± 0.1 ab ⁽²⁾		NA	
456 (19d)			NA	
504 (21d)		$7.4 \pm$ NA a ⁽¹¹⁾	NA	
648 (27d)	$8.3 \pm$ NA ab ⁽³⁾			
672 (28d)		$11.3 \pm$ NA a ⁽¹²⁾		
840 (35d)	$7.3 \pm$ NA ab ⁽⁴⁾			
1128(47d)	$1.0 \pm$ NA cd ⁽⁵⁾			
>1600 (67d)	$<0.2 \pm$ NA d ⁽⁶⁾			

⁽¹⁾ Three repetitions of 17, 17, and 16 mummies for a total of 50 mummies.

⁽²⁾ One repetition of 258 mummies.

- ⁽³⁾ One repetition of 159 mummies.
- ⁽⁴⁾ One repetition of 229 mummies.
- ⁽⁵⁾ One repetition of 140 mummies.
- ⁽⁶⁾ One repetition of 226 mummies.
- ⁽⁷⁾ One repetition of 41 mummies.
- ⁽⁸⁾ One repetition of 33 mummies.
- ⁽⁹⁾ One repetition of 37 mummies.
- ⁽¹⁰⁾ One repetition of 36 mummies.
- ⁽¹¹⁾ One repetition of 39 mummies.
- ⁽¹²⁾ One repetition of 35 mummies.
- ⁽¹³⁾ Three repetitions of 17, 17, and 16 parasitoids of each sex for a total of 50 males and 50 females.

Table 4.4. Greenbugs parasitized by *Lysiphlebus testaceipes* females \pm SE, after being cooled to 5, and -6°C for various periods of time (means within the same column followed by the same letter are not significantly different from each other at $P = 0.05$).

	Life Stage and Temperature			
	Mummies		Adults	
<u>Time</u>	<u>5°C</u>	<u>-6°C</u>	<u>5°C</u>	<u>-6°C</u>
24 (1d)	2.8 \pm 0.3 ab ⁽¹⁾	1.4 \pm NA a ⁽⁷⁾	NA	2.6 \pm 0.5 a ⁽¹³⁾
48 (2d)	2.7 \pm 0.8 ab ⁽¹⁾	0.5 \pm NA a ⁽⁸⁾	NA	2.7 \pm 0.4 a ⁽¹³⁾
72 (3d)	2.5 \pm 0.1 b ⁽¹⁾		NA	2.9 \pm 0.1 a ⁽¹³⁾
96 (4d)				2.4 \pm 0.8 a ⁽¹³⁾
120 (5d)				4.2 \pm 0.2 a ⁽¹³⁾
144 (6d)				0.0 \pm 0.0 a ⁽¹³⁾
168 (7d)	3.9 \pm 0.6 ab ⁽¹⁾	1.7 \pm NA a ⁽⁹⁾	NA	

216 (9d)	$4.2 \pm 0.2 \mathbf{a}^{(1)}$		NA	
288 (12d)	$3.6 \pm 0.3 \mathbf{ab}^{(1)}$		NA	
336 (14d)	$4.2 \pm 0.2 \mathbf{a}^{(1)}$	$3.0 \pm \text{NA} \mathbf{a}^{(10)}$	NA	
384 (16d)	$3.7 \pm 0.3 \mathbf{ab}^{(2)}$		NA	
456 (19d)			NA	
504 (21d)		$2.9 \pm \text{NA} \mathbf{a}^{(11)}$	NA	
648 (27d)	$4.4 \pm \text{NA} \mathbf{a}^{(3)}$			
672 (28d)		$4.0 \pm \text{NA} \mathbf{a}^{(12)}$		
840 (35d)	$3.9 \pm \text{NA} \mathbf{ab}^{(4)}$			
1128(47d)	$1.0 \pm \text{NA} \mathbf{b}^{(5)}$			
>1600 (67d)	$<0.2 \pm \text{NA} \mathbf{c}^{(6)}$			

⁽¹⁾ Three repetitions of 17, 17, and 16 mummies for a total of 50 mummies.

- ⁽²⁾ One repetition of 258 mummies.
- ⁽³⁾ One repetition of 159 mummies.
- ⁽⁴⁾ One repetition of 229 mummies.
- ⁽⁵⁾ One repetition of 140 mummies.
- ⁽⁶⁾ One repetition of 226 mummies.
- ⁽⁷⁾ One repetition of 41 mummies.
- ⁽⁸⁾ One repetition of 33 mummies.
- ⁽⁹⁾ One repetition of 37 mummies.
- ⁽¹⁰⁾ One repetition of 36 mummies.
- ⁽¹¹⁾ One repetition of 39 mummies.
- ⁽¹²⁾ One repetition of 35 mummies.
- ⁽¹³⁾ Three repetitions of 17, 17, and 16 parasitoids of each sex for a total of 50 males and 50 females.

Table 4.5. Long term percentage survival for *Lysiphlebus testaceipes* \pm SE, cooled to 5, and -6°C for various periods of time (means within the same column followed by the same letter are not significantly different from each other at $P = 0.05$).

	Life Stage and Temperature			
	Mummies		Adults	
Time	5°C	-6°C	5°C	-6°C
24 (1d)	94.0% \pm 3.4 ab ⁽¹⁾	61.9% \pm 7.6 ab ⁽⁷⁾	96.7% ⁽¹³⁾	75.7% \pm 3.1 a ⁽¹⁴⁾
48 (2d)	96.0% \pm 2.8 a ⁽¹⁾	82.4% \pm 6.6 a ⁽⁸⁾	89.0% ⁽¹³⁾	81.6% \pm 3.2 a ⁽¹⁴⁾
72 (3d)	90.0% \pm 4.3 bcd ⁽¹⁾		81.0% ⁽¹³⁾	49.1% \pm 2.3 b ⁽¹⁴⁾
96 (4d)				21.9% \pm 3.1 c ⁽¹⁴⁾
120 (5d)				9.1% \pm 2.7 d ⁽¹⁴⁾
144 (6d)				0.00% \pm 0.0 e ⁽¹⁴⁾
168 (7d)	84.0% \pm 5.2 cde ⁽¹⁾	52.6% \pm 8.2 ab ⁽⁹⁾	72.3% ⁽¹³⁾	
216 (9d)	94.0% \pm 3.4 abc ⁽¹⁾		54.7% ⁽¹³⁾	

288 (12d)	$82.0\% \pm 5.5$ de ⁽¹⁾		45.3% ⁽¹³⁾	
336 (14d)	$76.0\% \pm 6.1$ e ⁽¹⁾	$37.8\% \pm 8.1$ bc ⁽¹⁰⁾	33.3% ⁽¹³⁾	
384 (16d)	$85.3\% \pm 2.2$ e ⁽²⁾		21.7% ⁽¹³⁾	
456 (19d)			11.7% ⁽¹³⁾	
504 (21d)		$37.5\% \pm 7.8$ bc ⁽¹¹⁾	2.3% ⁽¹³⁾	
648 (27d)	$55.9\% \pm 6.5$ f ⁽³⁾			
672 (28d)		$8.3\% \pm 4.7$ c ⁽¹²⁾		
840 (35d)	$41.9\% \pm 3.3$ f ⁽⁴⁾			
1128(47d)	$1.4\% \pm 1.0$ g ⁽⁵⁾			
>1600 (67d)	$<2.4\% \pm 1.7$ g ⁽⁶⁾			

⁽¹⁾ Three repetitions of 17, 17, and 16 mummies for a total of 50 mummies.

⁽²⁾ One repetition of 258 mummies.

- ⁽³⁾ One repetition of 159 mummies.
- ⁽⁴⁾ One repetition of 229 mummies.
- ⁽⁵⁾ One repetition of 140 mummies.
- ⁽⁶⁾ One repetition of 226 mummies.
- ⁽⁷⁾ One repetition of 41 mummies.
- ⁽⁸⁾ One repetition of 33 mummies.
- ⁽⁹⁾ One repetition of 37 mummies.
- ⁽¹⁰⁾ One repetition of 36 mummies.
- ⁽¹¹⁾ One repetition of 39 mummies.
- ⁽¹²⁾ One repetition of 35 mummies.
- ⁽¹³⁾ Three cohorts of 100 adult *L. testaceipes* parasitoids examined every day or two until no parasitoids survive.
- ⁽¹⁴⁾ Three repetitions of 17, 17, and 16 parasitoids of each sex for a total of 50 males and 50 females.

Table 4.6. Time required to kill 50% of *Lysiphlebus testaceipes* population (LTime₅₀) ± 95% confidence interval as determined by SAS PROC PROBIT.

Life Stage and Temperature			
Mummies		Adults	
<u>5°C</u>	<u>-6°C</u>	<u>5°C</u>	<u>-6°C</u>
683.9 h ± 31.7 ⁽¹⁾	300.8 h ± 49.0 ⁽²⁾	250.2 h ± 7.8 ⁽³⁾	66.8 h ± 5.2 ⁽⁴⁾

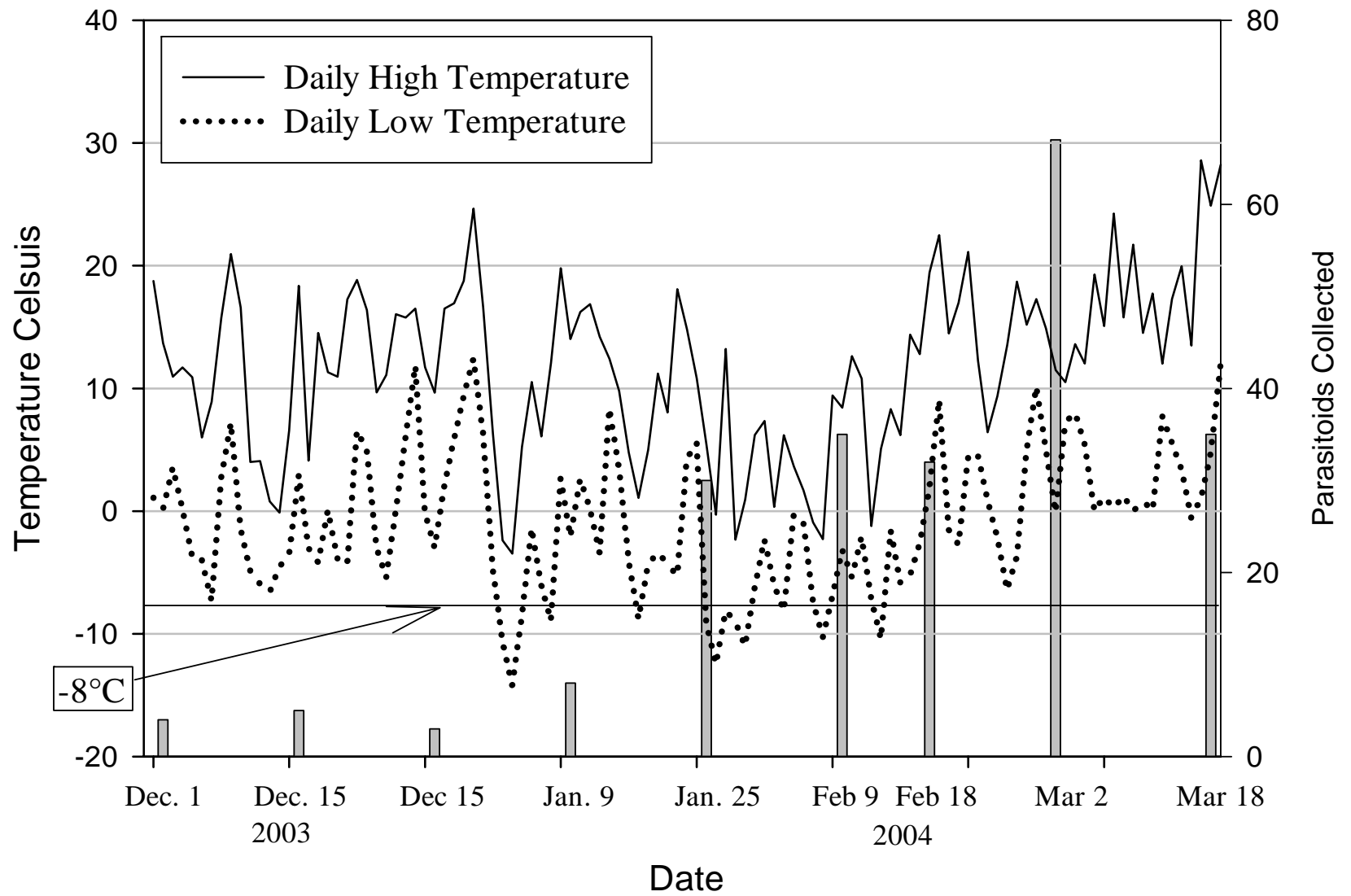
⁽¹⁾ $df = 1, X^2 = 482.8, P < 0.0001$

⁽²⁾ $df = 1, X^2 = 79.6, P < 0.0001$

⁽³⁾ $df = 1, X^2 = 1240.2, P < 0.0001$

⁽⁴⁾ $df = 1, X^2 = 186.8, P < 0.0001$

Fig. 4.1. Sentinel plant collections of parasitoids from a winter wheat field in Payne County Oklahoma for December 2003 through March 2004 (bar graph) superimposed on a plot of minimum and maximum temperatures for Stillwater, Oklahoma obtained from the Oklahoma Mesonet. All parasitoids collected were *Lysiphlebus testaceipes* except for four hyperparasitoids collected on 18 March 2004.



CHAPTER V

PARASITISM OF GREENBUG, *SCHIZAPHIS GRAMINUM*, BY THE PARASITOID *LYSIPHLEBUS TESTACEIPES*, AT WINTER TEMPERATURES

Abstract

Functional responses by *Lysiphlebus testaceipes* (Cresson), a common parasitoid of small grain aphids, on greenbug, *Schizaphis graminum* (Rondani), were measured at seven temperatures (14, 12, 10, 8, 6, 4, and 2°C) during a 24 hour period (12 h Light: 12 h Dark). Oviposition by *L. testaceipes* ceased at temperatures below 4°C. At all experimental temperatures, a type I, rather than a type II or type III functional response, was determined to be the best fit based upon coefficient of determination (r^2) values. *Lysiphlebus testaceipes* was observed to oviposit in greenbugs at temperatures below the developmental temperature of both the greenbug host (5.8°C) and the parasitoid itself (6.6°C). This ability to oviposit at sub-developmental temperatures enables the parasitoid to increase the percentage of greenbugs that are parasitized while the greenbugs are unable to reproduce. The implications of these findings regarding population suppression of greenbugs are discussed.

Introduction

Winter wheat (*Triticum Aestivum* L.) is an important multi-purpose cereal crop grown in the Southern Great Plains. Over twelve million acres are planted annually for grain, forage or as a combination grain/forage crop in Oklahoma and Texas (Epplin et al. 1998, NASS 2005). In this region of the United States, winter wheat is attacked primarily by phloem feeding cereal aphids resulting in reduced forage and grain yields (Gerloff and Ortman 1971, Burton 1986, Niassy et al. 1987, Peters et al. 1988, Kindler et al. 2002, 2003, K. Giles unpublished data). One of the most damaging of the cereal aphids commonly found attacking winter wheat, is the greenbug, *Schizaphis graminum* (Rondani). Greenbug can have a large impact on wheat production. Its economic impact in Oklahoma has ranged from \$0.5 to \$135 million annually (Starks and Burton 1977, Webster 1995).

Greenbug populations can be suppressed below economic injury levels (EIL) through the actions of aphid parasitoids such as *Lysiphlebus testaceipes* Cresson (Hymenoptera: Aphidiidae)(Jones 2001, Giles et al. 2003). *Lysiphlebus testaceipes* is a solitary endoparasitoid whose geographic range is Nearctic, Neotropical, Oceanic, in addition to being Palearctic because of intentional introductions (Mackauer and Starý 1967). It has been observed to attack over 100 aphid species (Mackauer and Starý 1967, Starý et al. 1988, Pike et al. 2000). *Lysiphlebus testaceipes* has been observed to suppress greenbug populations below EIL's in wheat directly through mortality, and indirectly by reducing reproductive potential (Spencer 1926, Eikenbary and Rogers 1974, Giles et al. 2003). Additionally, *L. testaceipes* causes aphids to drop from the plant in an attempt to

avoid parasitism. Once on the ground, aphids are highly subject to desiccation and attack by other natural enemies (Losey et al. 1998).

Because of the relatively moderate climate in Oklahoma and Texas, greenbugs and other cereal aphids are able to feed on wheat throughout fall, winter and spring months (Elliott et al. 2003, Royer et al. 2005). Adult parasitoids have been observed actively foraging on cool sunny days in Oklahoma throughout the winter months (D. B. Jones unpublished data). However, when winter temperatures are at the lower extremes commonly encountered during wheat production, little is known about the relationship between *L. testaceipes* and its greenbug host.

Our previous work on *L. testaceipes* attack rates were based on assumptions by integrated pest management practitioners (Patrick and Boring 1990, Royer et al. 1998). They suggested that parasitoids could not suppress greenbug populations at cool temperatures such as below 14°C because parasitoid development was delayed relative to their aphid hosts. Indeed, studies demonstrating lower developmental thresholds for greenbug (5.8°C; Walgenbach et al. 1988) versus *L. testaceipes* (6.6°C; Royer et al. 2001) and dramatic reductions in attack rates by *L. testaceipes* as temperatures were decreased to 14°C (Jones et al. 2003) support this assumption. However, recent field observations on the suppression of greenbug by *L. testaceipes* during cold winter months suggest that adult parasitoids are actively foraging at temperatures below greenbug developmental thresholds and effectively preventing populations from increasing (Jones 2001, Giles et al. 2003).

The primary objective of this study was to measure the 24 h functional response of *L. testaceipes* on greenbugs infesting winter wheat at 14°C and repeat these

measurements at progressively colder temperatures until *L. testaceipes* failed to parasitize greenbug hosts. Additionally, we investigated the relationship between temperature and the proportion of *L. testaceipes* females that oviposited at each temperature.

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; some were established on grain sorghum (cv ‘SG-925’) and others were established on wheat (cv ‘2137’) grown in a fritted clay and sphagnum moss mixture. Insect colonies and all plants were kept inside double walled fine mesh cages located within a climate-controlled greenhouse (approximately 22°C). The double walled cages prevented contamination of colonies by feral greenbugs and parasitoids while permitting ample airflow. Fresh plants were rotated as needed into cages housing colonies.

Three parasitoid colonies were maintained at 22 ± 1 °C and a photo-period of 12:12 (L:D) in double walled fine mesh cages in growth chambers. *Lysiphlebus testaceipes* was isolated from specimens collected in Caddo county, OK in the spring of 2003 (40 *L. testaceipes* adults isolated from greenbug mummies). Using sub-samples of parasitoid offspring, we verified the parasitoids as *L. testaceipes* by keys (Pike et al. 1997) and PCR analysis (Chen et al. 2002, Jones et al. 2005). Pots of grain sorghum infested by greenbugs were placed in the colonies every 3-4 days to maintain a steady supply of parasitoids. Parasitoid colonies were maintained on grain sorghum because wheat stock plants succumbed relatively quickly to greenbug feeding damage.

Functional Response Evaluations. Wheat seed (cultivar '2137') was planted in 5 cm diameter by 20 cm tall Ray Leach "conetainers™" (Stuewe & Sons Inc., Corvallis, Oregon). When plants were approximately 30 cm tall (about 3-4 weeks), they were thinned to two similar sized tillers that were threaded through a 0.6 cm diameter hole in a 5 cm diameter by 0.6 cm thick circular Plexiglas disk. The disk was fitted into the conetainer™ at soil level and cotton filled up the remaining area of the hole to create a sealed experimental arena floor that prevented access to the soil. A 5 cm diameter by 30 cm tall clear acetate tube cage with two 5 cm holes covered with fine mesh polyester netting in the sides (to allow ventilation) was then fitted around the top of the conetainer™. The top of each tubular cage was also covered with netting that was held in place by a rubber band. Greenbugs from the colonies reared on wheat were introduced by placing second and third instars on wheat tillers in each conetainer™ with a fine brush. By only using similar-aged greenbugs, possible complicating factors such as host age preference by the wasps were avoided. We established greenbugs in conetainers™ at densities that ranged from 5 greenbugs per conetainer™ up to 80 greenbugs per conetainer™ at each of the following seven temperatures in growth chambers: 2, 4, 6, 8, 10, 12, and 14°C. Because greenbugs are somewhat fragile, mortality from handling made it difficult to establish a predetermined density of greenbugs. Additionally, paedogenesis, reproduction by nymphs, occurs in approximately 2% of immature greenbugs (Wood and Starks (1975). Because of these difficulties, we targeted four density ranges (≤ 20 , 21-40, 41-60, and 61-80 greenbugs/conetainer™) at each experimental temperature. This ensured a sufficient range of densities necessary to describe the functional response (Jones et al. 2003). Actual numbers of greenbugs in each

conetainer™ were determined when greenbugs were later dissected. Greenbugs were allowed to acclimate at each temperature for 4 hours before parasitoids were introduced. A minimum of six conetainer™ replicates were evaluated at each temperature and density range. Because all temperatures and densities could not be run at the same time, temperatures and density combinations were run in a random order.

In order to have naïve parasitoids that developed in greenbugs reared on wheat, conetainers™ of wheat were infested with 25-35 3rd instar and older greenbugs from the wheat stock colony. By limiting the number of greenbugs, the fitness of emerging parasitoids was not influenced by plant health (Fuentes-Granados et al. 2001). These greenbugs were allowed to feed overnight, after which 5 male/female pairs of *L. testaceipes* parasitoids were released into each conetainer™ cage. Parasitized greenbugs were allowed to develop into mummies, after which, they were removed from the colony, and placed individually into 1.5 ml microcentrifuge vials. These isolated mummies were allowed to develop until they emerged as adults. Upon emergence, the parasitoids were sexed and paired to allow mating. Only parasitoids that had emerged on the day of the experiment were used in that day's work. Parasitoids destined for evaluation were placed into the growth chamber to acclimatize at each experimental temperature for four hours before being released into designated conetainers™ with greenbugs.

Parasitoids were released as a mated pair in each experimental conetainer™ during the dark cycle. The lights came on the next morning at 6:00 AM and then turned off 12 h later at 6:00 PM, after which both parasitoids in each conetainer™ were removed and their survival recorded. If a female wasp did not survive, data from that conetainer™ were not used. Survival of the male wasp was noted, but did not influence whether data

were discarded. During the 24 h period that the parasitoids were exposed to greenbugs, they were only active during the 12 hour light period and were quiescent when lights were off (D. B. Jones unpublished data). After the removal of parasitoids, conetainers™ were placed in a chamber at 22°C for 2-3 days to allow parasitoid eggs to develop into larvae before dissections were attempted. Subsequently, conetainers™ were held at 5°C to arrest parasitoid development, until all greenbugs were dissected. Eggs of aphid parasitoids are quite difficult to detect, thus delaying dissections until after hatching greatly improved accuracy of data (Hofsvang and Hågvar 1978, van Steenis 1993, Jones et al. 2003). Encapsulation could hinder accuracy, but encapsulation of *L. testaceipes* by *S. graminum* has yet to be observed (D. Jones personal observation).

Dissections were performed in an aqueous solution of 2% saline (NaCl) and 1% dishwashing detergent to act as a surfactant. Greenbugs were dissected by grasping the head with a pair of fine forceps and “pricking” the caudal region with a second pair of fine forceps, opening the body cavity. Contents were then gently squeezed from the greenbug into the dissecting solution and examined for the presence of parasitoid larvae. Though *L. testaceipes* is solitary, superparasitism frequently occurs (Jones et al. 2003). Therefore numbers of larvae present in each greenbug and the total numbers of greenbugs per experimental unit (conetainer™ cage) dissected were recorded. Though some eggs may fail to hatch, the total number of parasitoid larvae present was assumed to be equal to the total number of eggs laid per female in 24 h (Hofsvang and Hågvar 1978, van Steenis 1993).

Statistical Analyses. All statistical analyses were performed using PC SAS version 8.2 (SAS Institute 1999) at a significance level of $P=0.05$. Coefficients of

determination (r^2 values) were calculated using PROC NLIN to determine which functional response model (Type I, II, or III) best described the number of greenbugs parasitized at each temperature over the range of host densities. The following models were evaluated:

$$\text{Type I:} \quad N_A = aTN \quad (\text{Holling 1959a})$$

$$\text{Type II:} \quad N_A = aTN / (1+aT_hN) \quad (\text{Holling 1959b})$$

$$\text{Type III:} \quad N_A = N [1-\exp(-aT / (1+aT_hN))] \quad (\text{Hassell et al. 1977})$$

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 attack rate, and T_h is the amount of time the parasitoid spent handling the host. For the type I models the parameter a , along with the parameters a and T_h for the type II and type III models, were estimated using PROC NLIN (Donnelly and Phillips 2001, Jones et al. 2003). Though these parameters can be measured by observation (Mills and Gutierrez 1999), it was not practical to do so in this experiment.

Typically, functional responses are calculated for only those predators or parasitoids that actually attack their prey or host and are perceived of as normally functioning animals. However in this paper we also estimated functional response for all of the female parasitoids including those that remained alive but did not oviposit. We did this because our observations indicated that as temperatures decreased the proportion of parasitoids that oviposited decreased as well. This decrease in the proportion of ovipositing parasitoids may help to describe *L. testaceipes* biology at sub-optimal temperatures and the resulting dynamics with greenbug populations in field situations. While including non-parasitizing parasitoids in the analyses was not typical of functional

response models, these non-ovipositing parasitoids are viable, potential attackers of aphids that may only need warmer temperatures in order to become active.

Voucher specimens. Voucher specimens of *L. testaceipes* adults and mummies and *S. graminum* adults were deposited in the Department of Entomology and Plant Pathology museum at Oklahoma State University in Stillwater.

Results and Discussion

Parasitism at Low Temperatures. Previous work by Jones et al. (2003) suggested that *L. testaceipes* should be able to oviposit at temperatures below 14°C. This experiment confirmed that assumption as we observed that 23.3% of *L. testaceipes* females assayed successfully oviposited at 4°C (Fig. 5.1). This minimum temperature is very close to observations by Hunter and Glen (1909), who, with limited observations, reported that *L. testaceipes* could oviposit at 3.3° C. This result also compares well with field observations that *L. testaceipes* can be active during typical Oklahoma winter temperatures (Pomeroy and Brun 1999, Giles et al. 2003).

These observations are interesting because *L. testaceipes* is actively ovipositing at temperatures below its developmental threshold of 6.6°C (Royer et al. 2001) and below the developmental temperature threshold of its greenbug host (greenbug developmental threshold = 5.8°C; Walgenbach et al. 1988). Provided adult females are present in wheat fields during the winter, this ability to oviposit at temperatures below the developmental threshold of the host enables the parasitoid to effectively increase its population levels (within greenbug hosts) while the host cannot increase its population. As experimental temperatures increased, so did the percentage of ovipositing females (Fig. 5.1). However, percentages were similar at 8 to 14°C. As environmental temperature increased above the developmental threshold for greenbugs, several factors including numerical and functional responses influence the dynamics between *L. testaceipes* and its host.

Functional Response Calculations. When considering all experimental parasitoids including those that did not oviposit, a type I functional response provided the

best fit at 4, 6, and 8° C (Table 5.1). However, the fit was poor because the coefficients of determination (r^2) were only 0.15, 0.34, and 0.46 respectively. At 10, 12, and 14° C, a type II functional response best described the relationship between greenbug density and the attack rate of *L. testaceipes*. The r^2 values were only marginally better than for a type I (Table 5.1). Comparisons of instantaneous attack rates (a) estimated from Type I functional response models revealed that the 4° C functional response model was not significantly different from the 6° C model, but was significantly different (lower) than the models for all other experimental temperatures (Table 5.2). When instantaneous attack rates (a) were calculated for the type II models, no significant differences were observed (Table 5.2). Handling time (T_h) estimates were also generated for the type II models, however, no significant differences were observed among temperatures.

When those parasitoids that did not oviposit were removed from the calculations, the functional response coefficients of determination improved considerably. Again the r^2 values were only marginally better for type II functional response models over the coefficient of determination values for a type I model. Instantaneous attack rates (a) estimated from Type I functional response models revealed that the 4 and 6° C models were significantly different from the 14° C model, but were not significantly different from the 8, 10, and 12° C models. Conversely the 14° C model was also not significantly different from the 8, 10, and 12° C models (Table 5.3). When instantaneous attack rates (a) and handling time estimates (T_h) were calculated for the type II models, no significant differences were observed (Table 5.3).

Whether we considered only *L. testaceipes* females that oviposited, or all of the experimental parasitoids, type II models provided only a slightly improved fit with regard

to r^2 values (Table 5.2). Additionally the extremely small handling times observed appear to be biologically insignificant and provide little predictive power when describing the relationship between greenbug density and attack rates of *L. testaceipes*. At temperatures below 14° C the type I functional response model appears to best describe attack rates as greenbug population densities increase. The slopes describing these attack rates are quite similar between 8 to 14° C, however the slope is significantly different at 4° C (Figs. 5.2 & 5.3).

Implications for Winter Ecology of *Lysiphlebus testaceipes*. A functional response is defined as the change in attack rate of a parasitoid or a predator exposed to increasing host densities per defined unit of time (Solomon 1949). The results of this experiment demonstrate that at temperatures below 14° C, functional response models are poor predictors of attack rates (Fig. 5.2). Despite this poor predictive ability of the models, we have observed suppression of greenbug and other cereal aphid populations by *L. testaceipes* during the cold winter months in Oklahoma (Jones 2001, Giles et al. 2003).

Our study adds additional information toward understanding why *L. testaceipes* can be such an effective natural enemy in the Southern Great Plains during winter months. When *L. testaceipes* is present in winter wheat fields during the mild autumns (>14° C during August to November), this parasitoid is able to contribute toward suppression of aphid populations by a combination of (1) a high attack rate (Jones 2001, Giles et al. 2003), (2) sterilization of attacked aphids (Spencer 1926, Hight et al. 1972, Eikenbary and Rogers 1974), (3) dislodgment of aphids from the plant (Losey 1998), and (4) its reproductive (numerical) response from attacked aphids (Giles et al. 2003). These factors are also important contributions toward aphid suppression during the mild spring

months from February to May. During December and January, when temperatures are often below 14° C, the reproductive response may be relatively unimportant. As temperatures continue to drop, a developmental advantage occurs with greenbugs (5.8°C; Walgenbach et al. 1988) that have a developmental threshold lower than *L. testaceipes* (6.6°C; Royer et al. 2001).

Providing that temperatures do not drop below the threshold for aphid development, aphids should continue to numerically increase at rates higher than *L. testaceipes*. Despite weak functional response relationships at cool temperatures, a significant proportion of female *L. testaceipes* parasitoids continue to attack greenbugs as temperatures decrease below developmental thresholds for both the host greenbug and the parasitoid (Fig. 5.1). Under these low temperature conditions, *L. testaceipes* adult females can continue to parasitize, sterilize and dislodge greenbugs without significant development or reproduction by the host. Additionally, *L. testaceipes* are longer lived at colder temperatures and are able to inflict mortality for extended periods of time (up to 3 weeks; D. Jones unpublished data).

These characteristics of *L. testaceipes* could enable the parasitoid to keep its population expanding (relative to aphid hosts) even when the weather is not optimal for reproduction and subsequent development. Eventually adult parasitoids will die and/or exhaust their egg load during this period. However, the parasitoid progeny within their greenbug hosts are in a state of reduced or arrested development. The progeny is alive and able to develop once temperatures increase (Archer et al. 1973, Royer et al. 2001). Indeed, we have collected apparently healthy greenbugs from winter wheat fields in January and early February, which were all or mostly all parasitized (Giles et al. 2003).

Understanding interactions between greenbugs and *L. testaceipes* during cold winter weather in the Southern Great Plains requires information on the influence of decreasing temperatures on parasitoid ecology/biology. The observed low r^2 values for functional response models evaluated in our study indicate that attack rates at temperatures below 14°C would be difficult to predict. The actual within-field interactions between *S. graminum* and *L. testaceipes* during the winter will depend upon multiple factors including the relationship between microclimate temperatures and activity (attack by *L. testaceipes*), development, and reproduction. A future model with all of these factors will allow us to validate field collected population dynamics data.

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Table 5.1. Coefficients of determination for functional response regression models for *Lysiphlebus testaceipes* at 2, 4, 8, 10, 12, and 14°C (12:12 L:D) on greenbugs.

Parasitoid		Type I	Type II	Type III
Species ^a	Temperature (±1°C)	r^2	r^2	r^2
<i>L. testaceipes</i>	2 ^b	NA	NA	NA
	4 ^b	0.116	0.150	0.150
	6 ^b	0.335	0.335	0.335
	8 ^b	0.461	0.461	0.461
	10 ^b	0.344	0.398	0.398
	12 ^b	0.283	0.308	0.308
	14 ^b	0.405	0.406	0.406
	2 ^c	NA	NA	NA
	4 ^c	0.655	0.669	0.669
	6 ^c	0.483	0.524	0.524
	8 ^c	0.625	0.625	0.625
	10 ^c	0.667	0.719	0.720
	12 ^c	0.480	0.523	0.524
	14 ^c	0.721	0.730	0.730

^a *Lysiphlebus testaceipes* host densities ranged from 5 to 80 greenbugs per experimental unit. Type I, II and III functional response equations were evaluated using SAS PROC NLIN to generate coefficients of determination (r^2 values), indicating best fit.

^b Functional response r^2 values calculated using all parasitoids at that temperature.

^c Functional response r^2 values calculated using only those parasitoids that oviposited at that temperature.

Table 5.2. Estimates of instantaneous attack rates (a) for all *Lysiphlebus testaceipes* females evaluated calculated from experimental data fit to Type I and II functional response models.

Functional Response Model	Temperature °C	Instantaneous attack rate $a \pm SE^a$	Handling time $Th \pm SE^a$
Type I	2	NA	NA
	4	0.02 ± 0.01 a	NA
	6	0.08 ± 0.02 ab	NA
	8	0.22 ± 0.04 b	NA
	10	0.12 ± 0.03 b	NA
	12	0.14 ± 0.05 b	NA
	14	0.19 ± 0.05 b	NA
Type II	2	NA	NA
	4	0.16 ± 0.66 a	0.67 ± 0.70 a
	6	0.08 ± 0.02 a	0.00 ± 0.00 a
	8	0.22 ± 0.04 a	0.00 ± 0.00 a
	10	0.58 ± 1.16 a	0.10 ± 0.07 a
	12	0.35 ± 0.55 a	0.06 ± 0.08 a
	14	0.21 ± 0.18 a	0.01 ± 0.06 a

^a a and Th estimated by PROC NLIN.

Means sharing the same letter are not significantly different at $\alpha = 0.05$.

Table 5.3. Estimates of instantaneous attack rates (a) for *Lysiphlebus testaceipes* females that successfully oviposited, calculated from experimental data fit to Type I and II functional response models.

Functional Response Model		Instantaneous attack rate $a \pm SE^a$	Handling time $Th \pm SE^a$
Temperature °C			
Type I	2	NA	NA
	4	0.10 ± 0.03 a	NA
	6	0.12 ± 0.03 a	NA
	8	0.29 ± 0.06 ab	NA
	10	0.22 ± 0.04 ab	NA
	12	0.24 ± 0.07 ab	NA
	14	0.34 ± 0.06 b	NA
Type II	2	NA	NA
	4	0.25 ± 0.41 a	0.10 ± 0.12 a
	6	0.61 ± 1.00 a	0.09 ± 0.06 a
	8	0.29 ± 0.17 a	0.00 ± 0.00 a
	10	0.87 ± 1.20 a	0.05 ± 0.03 a
	12	0.68 ± 1.07 a	0.04 ± 0.04 a
	14	0.48 ± 0.35 a	0.01 ± 0.02 a

^a a and Th estimated by PROC NLIN.

Means sharing the same letter are not significantly different at $\alpha = 0.05$.

Fig. 5.1. Bar graph with standard error bars, showing the percent of *Lysiphlebus testaceipes* females who successfully oviposited in greenbug, *Schizaphis graminum*, over a 24h (12:12 L:D) at 2, 4, 6, 8, 10, 12, and 14°C.

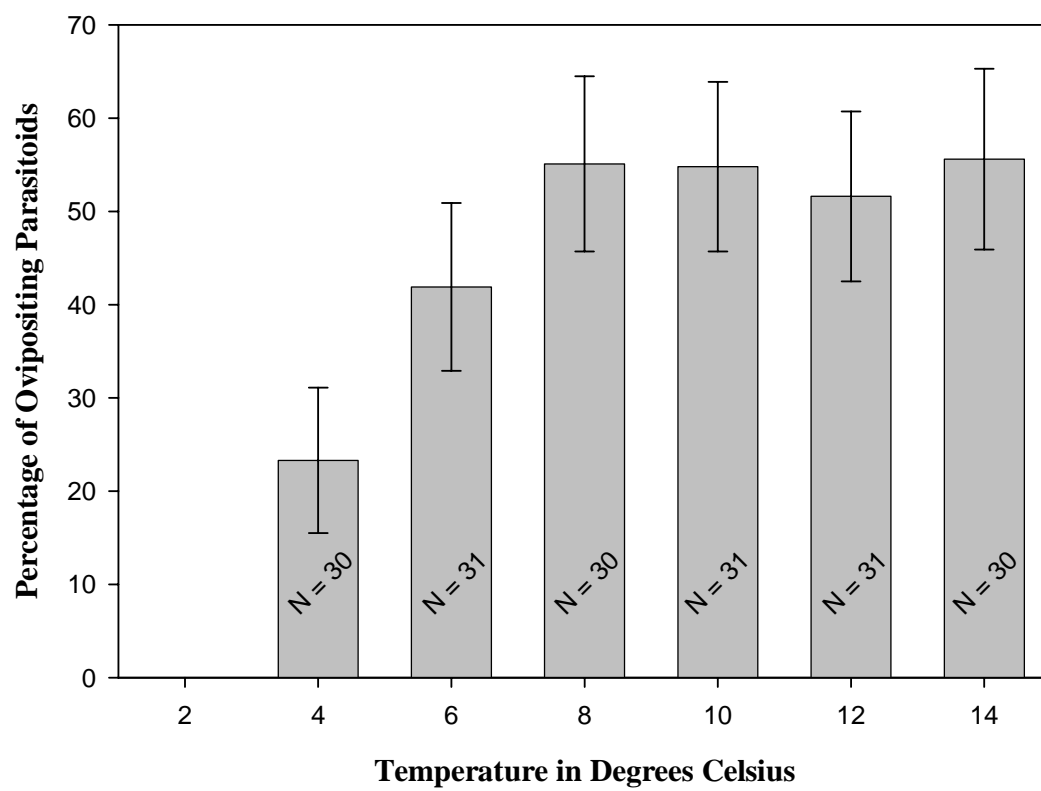


Fig. 5.2. Scatter plots with linear regression trend lines (type I functional response) for *Lysiphlebus testaceipes* attack rates (**excluding** those parasitoids that did not oviposit) at 4, 6, 8, 10, 12, and 14°C (12:12 L:D). The 2°C scatter plot was omitted because no oviposition occurred.

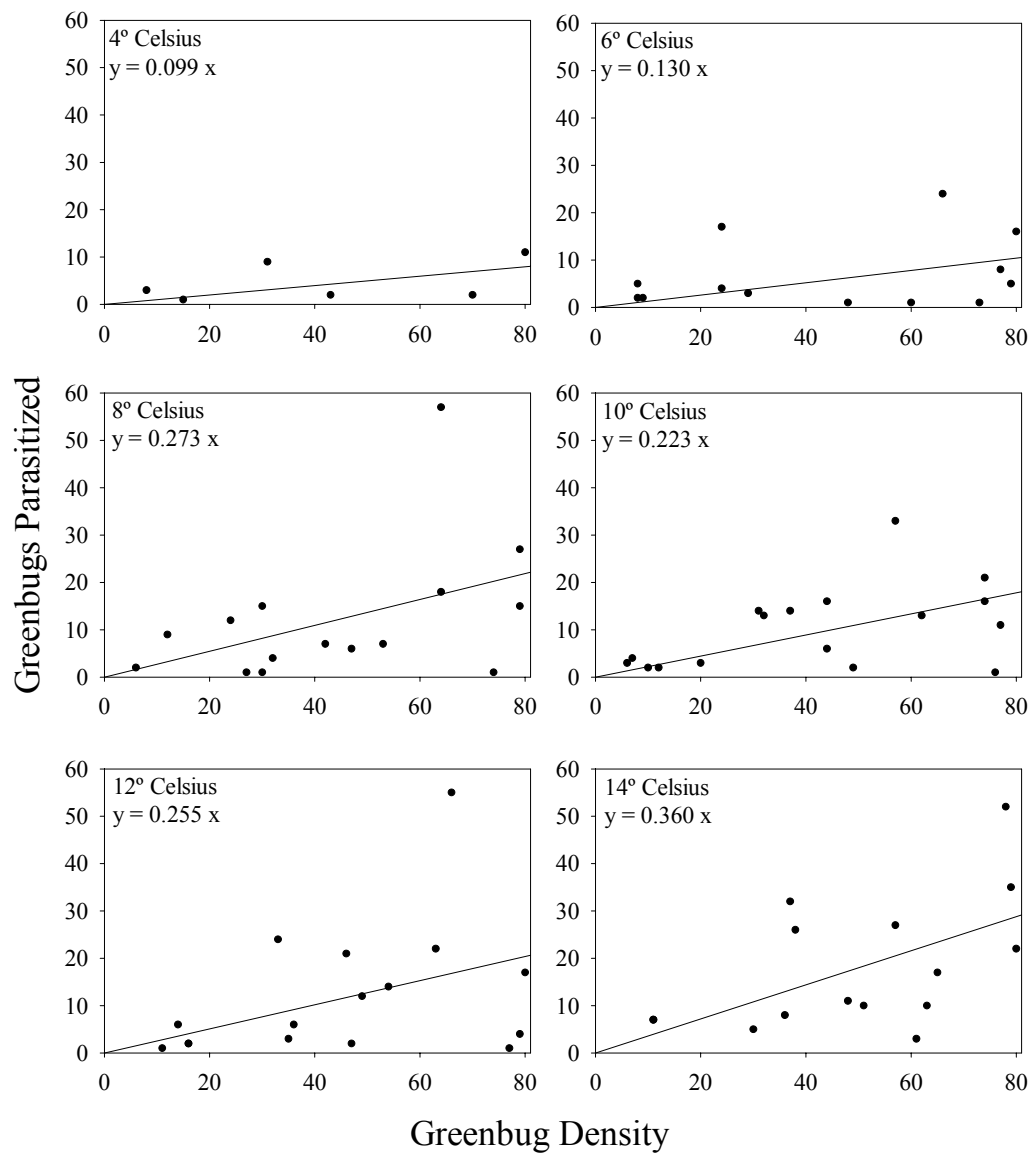
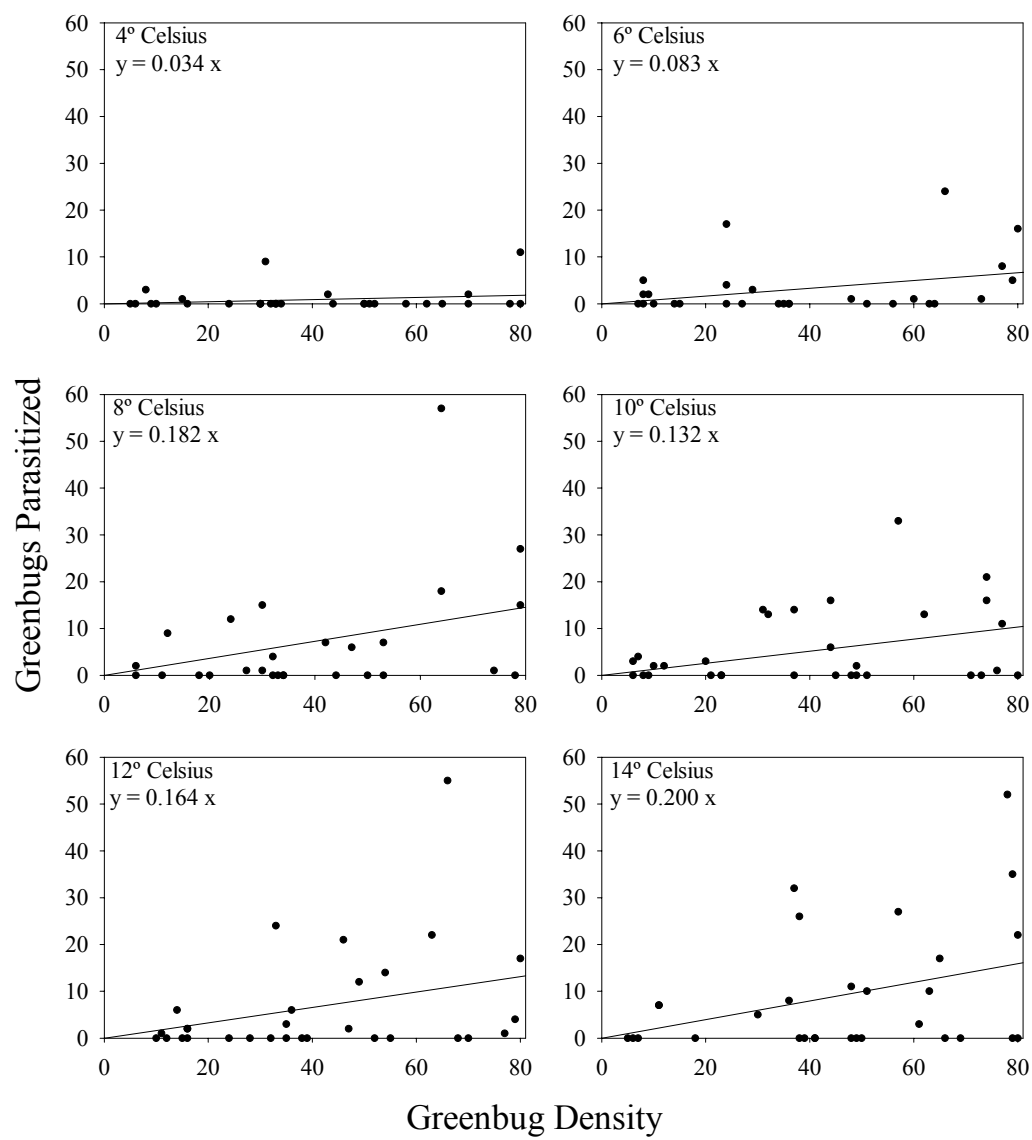


Fig. 5.3. Scatter plots with linear regression trend lines (type I functional response) for *Lysiphlebus testaceipes* attack rates (**including** those parasitoids that did not oviposit) at 4, 6, 8, 10, 12, and 14°C (12:12 L:D). The 2°C scatter plot was omitted because no oviposition occurred.



CHAPTER VI
SUMMARY AND CONCLUSIONS

In Oklahoma, parasitoids experience temperatures that range from ideal, to tolerable, to unsuitable, during the winter wheat growing season. These studies provide new and more accurate knowledge about the winter ecology of the aphid parasitoid *Lysiphlebus testaceipes* Cresson (Hymenoptera: Aphidiidae). Results from my first experiment in this dissertation provides insights about the supercooling point of *L. testaceipes* and its greenbug, *Schizaphis graminum* (Rondani)(Homoptera: Aphididae) host. It was discovered that mean supercooling points for *L. testaceipes* ranged from -20.32°C for older male parasitoids to -26.33°C for mummies acclimated at 10°C . For many insects, acclimation for a short period of time at an intermediate temperature can significantly lower the SCP (Sømme 1982, Lee 1991). Acclimation for four hours at 10°C had no significant effect on mean SCP for *L. testaceipes* mummies or *L. testaceipes* males. However, acclimation did significantly lower the SCP approximately 3°C for *L. testaceipes* females (-26.13 vs. -23.29°C). Acclimated and non-acclimated mummies, greenbug hosts and non-acclimated female *L. testaceipes* adults had the lowest mean SCPs, but were not significantly different from one another ($df = 218$, $t < 1.82$, $P > 0.07$). This lack of significant difference in SCPs was not unexpected since parasitoid mummies and their hosts are closely related with respect to their body resources (Brodeur and Boivin 2004).

These SCPs for greenbug and *L. testaceipes* were similar to similar species including other cereal aphids such as English grain aphid, *Sitobion avenae* (F.), aphid parasitoids *Aphidius colemani* Viereck, and *Ephedrus cerasicola* Stål and the whitefly parasitoid *Eretmocerus eremicus* (Rose & Zolnerowich) (Hofsvang and Hågvar 1977,

Knight et al. 1986, O'Doherty 1986, Butts 1992, Asai et al. 2002, Tullett et al. 2004, McCornack et al. 2005)

A general trend was also detected that shows as the parasitoid ages, its ability to supercool was reduced. Supercooling points for older parasitoids were at significantly higher temperatures than all other treatments. Non-acclimated older male parasitoids (older than 12h post-emergence) spontaneously froze at the warmest mean temperature ($-20.32^{\circ}\text{C} \pm 1.32$). While, non-acclimated older female parasitoids ($>12\text{h}$ post-emergence) had a mean SCP of -22.55°C . Additionally these two age groups had at least a three-fold larger range compared with any of the other age groups.

Supercooling ability can often be attributed to the accumulation of cryoprotectant chemicals and/or the absence on ice nucleating agents (Lee 1991). Sugars such as glucose, trehalose, and fructose and polyols such as glycerol, mannitol, sorbitol are known to provide increased supercooling ability and are commonly found in insects (Sømme 1967, 1969, Tanno 1964, Block and Zettel 1980). Perhaps common sugars such as trehalose constitute a high percentage of *L. testaceipes* hemolymph and provide much of their supercooling ability. The depletion of this sugar or some other resource necessary for the parasitoid to live may be responsible for the SCP to be inversely related to the age of the parasitoid. Another possibility is that as the parasitoids aged they accumulated ice-nucleating agents in their hemolymph as a by-product of normal metabolic processes allowing the parasitoids to freeze at warmer temperatures.

Based on the SCP, *L. testaceipes* could likely survive even the most extreme temperatures experienced in central Oklahoma. However SCP is only an indication of how cold an organism can be before it freezes (Bale 1993). The parasitoid may perish at

much warmer temperatures or be rendered unable to function in a normal manner much in the same manner that the English grain aphid, a potential host of *L. testaceipes*, has a cold tolerance of -14.6° C, about 12° C above its SCP (Parish and Bale 1991).

Lysiphlebus testaceipes is commonly found at latitudes that experience much colder temperatures than Oklahoma (Royer et al. 2001). Supercooling ability is only a base value that indicates what temperature *L. testaceipes* might be able to endure. How these parasitoids survive the winter at the colder latitudes has yet to be answered.

In the second experiment the ability of *L. testaceipes* to withstand cold temperatures and whether surviving affected their ability to reproduce once temperatures warmed was examined. In this experiment -6°C for 12h was the lethal temperature for 50% of *L. testaceipes* adults and mummies. Negative eight degrees Celsius was lethal for all subject parasitoids. Being chilled did not affect the ability of the parasitoids to function once temperatures were made favorable again after the cold treatment with female parasitoids being able to oviposit a mean of 6 or more eggs.

Knowing the minimum temperature that *L. testaceipes* can survive is but a part of the overall explanation of how this parasitoid can survive conditions on the Southern Great Plains. Longevity at cold temperatures is also important. *Lysiphlebus testaceipes* mummies could survive for 67d at 5°C, and up to 28d at -6°C. The time it took to kill 50% of the test insects (LTime₅₀) was approximately 28.5d at 5°C and 12.5d at -6°C. Adult *L. testaceipes* were not as hardy. Adults cooled to 5°C survived up to 21d with a LTime₅₀ of approximately 10.4d. Adults cooled to -6°C perished within 7d with the LTime₅₀ being only 66.8h. All life stages of *L. testaceipes* were able to successfully

reproduce following cold treatments indicating that any damage that may have altered ovipositional ability was usually enough to be fatal to the parasitoid.

A final part of the second experiment positioned sentinel plants infested with greenbugs in a wheat field in Payne County, Oklahoma during the 2003-2004 winter wheat growing season. From this data, it was determined that *L. testaceipes* was indeed actively parasitizing host greenbugs even during the coldest months of the year. Temperatures were frequently below the -8°C threshold that killed 100% of *L. testaceipes* mummies and adults in the laboratory, yet some parasitoids were able to survive and oviposit once conditions improved. This was probably due to some parasitoids being in various protected microclimates within the wheat field that enabled them to survive these lethal low temperatures (Leather et al. 1993).

Because temperatures are frequently warmer than the developmental threshold, and the parasitoid can successfully reproduce even during the coldest month, *L. testaceipes* should not need to enter diapause to overwinter in the Southern Great Plains. Additionally, sentinel plant data demonstrate that even when the temperature drops below -8°C, there are a number of *L. testaceipes* parasitoids that are able to find protected locations that enable them to survive the lethal temperatures. When the temperatures near the developmental threshold, *L. testaceipes* development slows and their life cycle is considerably lengthened (Royer et al. 2001). Additionally, in most winters in the Southern Great Plains, there are many aphid hosts available (personal observation) to utilize for the next generation. In colder regions of the Great Plains, *L. testaceipes* may yet enter diapause to survive the winter since aphid hosts are more scarce, but there is no data yet to support this conjecture. The ability to survive cold temperatures as adults and

mummies and successfully attack greenbugs and other cereal aphids throughout the winter months may explain why this parasitoid frequently provides biological control of greenbug and other cereal aphids in winter wheat in the Southern Great Plains.

The third experiment was initiated in response to observations I made on a previous experiment. The previous work suggested that *L. testaceipes* should be able to oviposit at temperatures below 14°C (Jones et al. 2003). The third experiment confirmed that assumption as we observed that 23.3% of *L. testaceipes* females assayed successfully oviposited at 4°C. This minimum temperature is very close to observations by Hunter and Glen (1909), who, with limited observations reported that *L. testaceipes* could oviposit at 3.3° C. This result also compares well with field observations that *L. testaceipes* is active during typical Oklahoma winter temperatures (Pomeroy and Brun 1999, Giles et al. 2003).

The experiment also demonstrated that *L. testaceipes* can successfully oviposit at temperatures below its developmental threshold of 6.6°C (Royer et al. 2001) and below the developmental temperature threshold of its greenbug host (greenbug developmental threshold = 5.8°C; Walgenbach et al. 1988). Provided adult females are present in wheat fields during winter, this ability to oviposit at temperatures below the developmental threshold of the host enables the parasitoid to effectively increase its population levels (within greenbug hosts) during the time when the host cannot increase its population.

Whether we considered only *L. testaceipes* females that oviposited, or all of the experimental parasitoids, the type I functional response model (Solomon 1949) appears to best describe attack rates as greenbug population densities increase at temperatures below 14° C. However, the results of this experiment demonstrate that at temperatures below

14° C, functional response models are poor predictors of attack rates. Despite the poor predictive ability of the models, *L. testaceipes* has been observed to suppress greenbug and other cereal aphid populations during the cold winter months in Oklahoma (Jones 2001, Giles et al. 2003).

When coupled with previous research, the three studies in this dissertation provide an understanding of why *L. testaceipes* is an effective natural enemy in the Southern Great Plains during winter months. When *L. testaceipes* is present in winter wheat fields during mild autumns (>14°C during August to November), the parasitoid is able to contribute toward suppression of aphid populations by a combination of (1) a high attack rate (Jones 2001, Giles et al. 2003), (2) sterilization of attacked aphids (Spencer 1926, Hight et al. 1972, Eikenbary and Rogers 1974), (3) dislodgment of aphids from the plant (Losey 1998), and (4) its reproductive (numerical) response from attacked aphids (Giles et al. 2003). These factors are also important contributions toward aphid suppression during the mild spring months from February to May. During December and January, when temperatures are often below 14° C, the reproductive response may be relatively unimportant. However, because *L. testaceipes* is able to withstand temperatures as low as -8°C, and apparently has the ability to seek shelter from colder temperatures in the field, it should be considered for biological control of small grain aphids in the production of winter wheat even when temperatures are sub-optimal.

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Major Field: Entomology

Scope and Method of Study: The purpose of this study was to investigate cold weather ecology of the greenbug parasitoid, *Lysiphlebus testaceipes* Cresson (Hymenoptera: Aphidiidae). These investigations included (1) determination of the supercooling point for *L. testaceipes* and its greenbug host, *Schizaphis graminum* (Rondani) (Homoptera: Aphididae); (2) determination of the 12 hour lethal low temperature (LTemp₅₀) for *L. testaceipes* mummies and adults; (3) determination of the lethal time (LTime₅₀) at just below the developmental threshold for *L. testaceipes* mummies and adults (5°C), and at the 12 hour LTemp₅₀ (-6°C); (4) an investigation of the 24 hour functional response of *L. testaceipes* on greenbug at 2, 4, 6, 8, 10, 12, and 14°C; (5) an investigation of parasitoid activity in the field using greenbug infested sentinel plants to detect oviposition by *L. testaceipes* and other parasitoids during the 2003-2004 winter wheat growing season in Payne county, Oklahoma.

Findings and Conclusions: *Lysiphlebus testaceipes* was measured to have a supercooling point of approximately -26°C, but that supercooling point became significantly warmer as the parasitoid aged (12+ hour old males supercooled at approximately -20°C), the greenbug host had a supercooling point of -26°C as well. The 12 hour LTemp₅₀ for *L. testaceipes* mummies and adults was approximately -6°C, with -8°C being sufficient to kill all test specimens. The LTime₅₀ ranged from 66.8h for adults at -6°C to 683.9h for mummies at 5°C. Functional Response studies revealed that a type I functional response equation was sufficient to describe attack rates for *L. testaceipes* on greenbug at all study temperatures, however all attacks ceased at 2°C. Sentinel plant investigations revealed that *L. testaceipes* was the primary parasitoid active in the winter wheat field in Payne county Oklahoma. Additionally, *L. testaceipes* was actively parasitizing greenbugs and other small grain aphids throughout the winter of 2003-2004.

ADVISER'S APPROVAL: Kristopher L. Giles