EFFECTS OF INTERSPECIFIC COMPETITION AND NARROW-SPECTRUM INSECTICIDES ON THE SURVIVAL AND DEVELOPMENT OF *DIAERETIELLA RAPAE* IN OKLAHOMA WINTER CANOLA

By

WILLIAM PAUL JESSIE

Bachelor of Science in Zoology
Oklahoma State University
Stillwater, Oklahoma
2008
Bachelor of Science in Natural Resource Ecology/Mgmt.
Oklahoma State University
Stillwater, Oklahoma
2009
Master of Science in Entomology and Plant Pathology
Oklahoma State University
Stillwater, Oklahoma
2013

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
December, 2017

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Dissertation Approved:

Dr. Kristopher Giles

Dissertation Adviser

Dr. J.P. Michaud

Dr. Norm Elliott

Dr. Mark Payton

Dr. Tom Royer

.

ACKNOWLEDGEMENTS

This research was partially funded by the USDA-NIFA AFRI Sustainable Biofuels Initiative (Kring et al. #2011-67009-30141); lead institution University of Arkansas and the Oklahoma Center for the Advancement of Science and Technology (OCAST). I would like to thank my advisor, Dr. Kristopher Giles, for allowing me to conduct this research and, along with Dr. Tom Royer, Dr. Norm Elliott, Dr. JP Michaud, and Dr. Mark Payton, for providing advice, support, and enthusiasm. I would like to acknowledge the faculty, staff and students in the Department of Entomology and Plant Pathology for being exceptionally welcoming and enjoyable. Thank you to the members of the Integrated Pest Management Laboratory, both past and present, for assistance with all the research we have conducted and for creating an excellent working environment. I would also like to thank Casi Jessie for all of her assistance with this and other projects, general maintenance of my functionality and sanity, and the tremendous number of contributions she has made to my daily happiness over the last 14 years.

Name: WILLIAM JESSIE

Date of Degree: DECEMBER, 2017

Title of Study: EFFECTS OF INTERSPECIFIC COMPETITION AND NARROW-SPECTRUM INSECTICIDES ON THE SURVIVAL AND DEVELOPMENT OF *DIAERETIELLA RAPAE* IN OKLAHOMA WINTER CANOLA

Major Field: ENTOMOLOGY

Abstract: *Diaeretiella rapae* plays a significant role in aphid suppression across its range. This parasitoid wasp specializes on crucifer-feeding aphids, now common in a rapidly expanding crop (winter canola) in the US Southern Great Plains. *Diaeretiella rapae* is currently found almost exclusively in winter canola during the spring when aphid intensities are high, yet does not frequently maintain aphid populations below economic thresholds. The minimal role that *D. rapae* plays in regulating aphids may be influenced by competing natural enemies and/or disruptive management approaches. The abundance and generalist behavior of *L. testaceipes* may result in frequent competitive interactions between these two parasitoids, either through extrinsic interference (if *D. rapae* can discriminate parasitized hosts), or intrinsic (i.e., larval) competition inside aphid hosts. Additionally, *D. rapae* may face significant mortality in the face of frequent applications of broad-spectrum insecticides common in winter canola. This mortality source could be minimized by the use of selective insecticides, such as flonicamid and sulfoxaflor, which have specificity to hemipteran pests and little to no effect on natural enemies. Laboratory experiments were designed to determine the outcomes of competition between *D. rapae* and *L. testaceipes* on *L. pseudobrassicae* and *M. persicae* hosts on winter canola, and on *Rhopalosiphum padi* hosts on winter wheat. Separate experiments were conducted in the field and laboratory to determine lethal and sub-lethal effects of flonicamid and sulfoxaflor on preimaginal *D. rapae*. Results indicate a reduced percentage of canola aphids are parasitized when *D. rapae* forages simultaneously with *L. testaceipes*, suggesting the proximity of canola fields to winter wheat may enhance colonization of canola by *L. testaceipes* and reduce the suppressive effects of *D. rapae* on aphids in this crop. Results from experiments with selective insecticides revealed application of flonicamid resulted in effective suppression of aphids while retaining higher rates of parasitism post-treatment when compared with other insecticides. Furthermore, the minimal sub-lethal effects of flonicamid on *D. rapae* suggest this insecticide is compatible with biological control. By using selective insecticides, such as flonicamid, populations of *D. rapae* in winter canola may be conserved and thus offset the negative effects of competition with *L. testaceipes*.

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CHAPTER I

INTRODUCTION

Winter canola (*Brassica napus* L.) (Brassicales: Brassicaceae) has experienced frequent and severe aphid (Hemiptera: Aphididae) outbreaks annually since widespread cultivation in the US Southern Great Plains began at the start of the 21st century (Franke et al. 2009, Royer and Giles 2017). Damage has been mitigated by the use of wide-spectrum insecticides (organophosphates, pyrethroids, neonicotinoids) as seed and foliar treatments (Royer and Giles 2017), but reliance on chemical control is often economically and environmentally costly. Indeed, it is common for producers to regularly treat aphid populations with insecticides in winter canola.

Biological control is one of the most valuable services provided by wildlife (Debach and Rosen 1991, Gutierrez et al. 1999, Losey and Vaughan 2006) and documenting the impacts of natural enemies in agricultural landscapes is a critical first step towards incorporation of biological control into integrated pest management (IPM) programs. The development of such programs is prefaced by studies on basic natural enemy and pest ecology and their interactions with pest management practices. One of the most important natural enemies of aphids in winter canola is *Diaeretiella rapae* (McIntosh) (Hymenoptera: Braconidae). As a specialist parasitoid of crucifer-feeding aphids, this species is important for aphid management in several cropping systems worldwide (Mackauer and Kambhampati 1984, Bahana and Karuhize 1986, Neuville et al. 2016). Currently, this species is commonly found parasitizing aphids in winter canola (French et al. 2001, Elliott et al. 2014, Jessie 2017), but infrequently maintains aphid populations below economic

thresholds. Recent studies of parasitoids in Oklahoma winter crops revealed that *D. rapae* is outnumbered by *Lysiphlebus testaceipes* (Cresson) by over 250:1 (Jessie 2017). This predominant parasitoid is commonly found suppressing aphids in neighboring winter wheat (*Triricum aestivum* L.) (Poales: Poaceae) fields but also in large numbers in canola fields. Although recent surveys of winter wheat and canola found no evidence of host overlap (Elliott et al. 2014), the extremely high abundance of *L. testaceipes* and its relative lack of host-specificity may result in frequent competitive encounters in diverse landscapes. Both *L. testaceipes* and *D. rapae* are capable of using *R. padi* and *M. persicae* hosts, which are common in winter wheat and winter canola crops, respectively (Pike et al. 2000), and thus competition between these two species may shape the structure of parasitoid communities.

Frequent applications of broad-spectrum insecticides in winter canola can have severe impacts on populations of *D. rapae*. The incorporation of selective (i.e., pest-specific) insecticides for curative treatments may allow for improved natural enemy survival which can increase overall aphid suppression. Additionally, such compounds may facilitate responses of natural enemies to resurgent pest populations and prevent additional outbreaks (Javed and Matthews 2002, Ragsdale et al. 2007). Two selective insecticides registered for use in canola are sulfoxaflor and flonicamid, which have specific activity against hemipteran pests. Studies on non-target effects of these chemicals on beneficial insects, such as predators, parasitoids, and pollinators, have revealed species-specific variability that can depend on the route of exposure (i.e., direct vs residual contact). Specific information on how these insecticides affect natural enemies have only recently been published (Robideau 2015, Colares et al. 2016, Barbosa et al. 2017), and information on *D. rapae* responses to these materials is not yet available.

Research Aim

The aim of this research was to identify and understand the factors limiting successful biological control of canola aphids by *D. rapae* parasitoids. The potential interference of *L. testaceipes* with *D. rapae* was one focus, given that *L. testaceipes* could potentially disrupt the

development and/or foraging behavior of *D. rapae* in winter canola. A holistic knowledge of factors limiting *D. rapae* populations will assist in developing more comprehensive management plans for winter canola pest management, and may ultimately reduce chemical inputs. To this end, studies were designed to determine the degree to which interspecific competition and insecticide use may impede successful biological control of winter canola aphids. Furthermore, no studies to date have been performed on the ability of *D. rapae* to survive and develop within aphids treated with flonicamid and sulfoxaflor, two novel insecticides that are potentially compatible with conservation biological control. Sub-lethal effects of insecticidal seed treatments and chemical spray adjuvants are well known, but no information exists on how the preimaginal survival of *D. rapae* may be influenced by these narrow-spectrum insecticides.

Objectives

A. Interspecific Competition

I. Quantify the host discrimination behavior of *D. rapae* and *L. testaceipes* when provided with heterospecifically-parasitized hosts.

II. Document the outcomes of intrinsic competition between *D. rapae* and *L. testaceipes* in multiparasitized hosts.

III. Quantify parasitism outcomes when *D. rapae* and *L. testaceipes* forage simultaneously with, or subsequent to, heterospecific competitors.

B. Insecticides

IV. Quantify field-level parasitism in winter canola fields before and after applications of sulfoxaflor, flonicamid, and a pyrethroid.

V. Examine stage-specific survival of preimaginal *D. rapae* following exposure to sulfoxaflor and flonicamid in laboratory microcosms.

VI. Determine sublethal effects of these insecticides on pre-imaginal parasitoids when applied to the aphid host.

Explanation of Dissertation Format

This introduction (Chapter I) is followed by a review of the relevant literature on Oklahoma winter crops, parasitoids, and selective insecticides (Chapter II). Chapter III includes laboratory-based experiments (Objectives I-III) to describe competitive interactions between *D. rapae* and *L. testaceipes* on predominant aphids in the Oklahoma winter crop landscape. Chapter IV contains a second study examining the effects of selective insecticides on *D. rapae* in a series of field and laboratory experiments (Objectives IV-VI). A general conclusion is included in Chapter V to present summarized findings and concluding remarks. Appendices I-XI contain descriptive data recorded during experiments.

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CHAPTER II

REVIEW OF LITERATURE

Winter Wheat

Winter wheat (*Triticum aestivum* L.) is an annual, cool season grass grown commercially across the Great Plains for forage and grain since the early 20th century. Winter varieties are those adapted to resist damage from below-freezing temperatures through increases in cellular sucrose concentrations and dormancy until early spring when warmer temperatures promote tiller and node formation (Newton 1922, Wise et al. 2011). In Oklahoma, winter wheat is typically planted in late August through September, and harvest is completed by late June (Edwards et al. 2015). In 2016, more than 2 million hectares were planted across Oklahoma, and 42 states contribute to a total of over 14 million hectares planted across the United States each year (USDA NASS 2017). Oklahoma is the second largest producer of winter wheat and over 60% is grown for both grain and as forage for cattle (Hossain et al. 2004). Dual-purpose wheat provides high-quality forage for livestock during late fall through early spring when other forage sources are dormant, and allows producers to offset low grain prices (Hossain et al. 2003). Because this crop is one of the only sources of green vegetation throughout winter months, it is frequently utilized by arthropods for food and shelter. Primary pests of winter wheat include aphids, cutworms and armyworms (Lepidoptera: Noctuidae), Hessian fly (*Mayetiola destructor* Say, Diptera: Cecidomyiidae), wire worms (Coleoptera: Elateridae), grasshoppers (Orthoptera), and mites (Acari) (Royer and Giles 2016).

The most frequent and damaging of these pests are the aphids *Schizaphis graminum* Rondani and *Rhopalosiphum padi* L. Royer et al. (2015) reports more than 70 host plants used by *S. graminum*, and outbreaks in Oklahoma winter wheat occur approximately every 6 years (Burton et al. 1985). *Rhopalosiphum padi* has occasional outbreaks and may be more damaging to winter wheat than is *S. graminum* due to transmission of barley yellow dwarf virus. This disease can cause significant damage, especially when young plants are infected in the fall (Hunger et al. 2012). As a holocyclic aphid, it is known to overwinter on woody vegetation such as *Prunus* spp. and disperses into wheat following temperature and photoperiod cues (Dixon 1971), but may overwinter without a holocycle in mild climates. Oklahoma populations of *S. graminum* are anholocyclic and overwinter in wheat fields and other graminacous habitats.

In unmanaged fields, cattle grazing can reduce aphid populations and barley yellow dwarf incidence by as much as 87% and 70%, respectively (Ismail et al. 2003). However, management of these pests has historically relied on routine applications of insecticides combined with curative treatments when aphids were detected (Wratten et al. 1990). Due to the low price of hard red winter wheat, producers in Oklahoma are encouraged to plant insecticide-treated seed and to scout fields frequently to ensure the judicious use of foliar insecticides (Royer et al. 2005 and 2015). When aphid populations reach economic thresholds, producers have several foliar insecticide options. Those registered for winter wheat include pyrethroids, carbamates, organophosphates, sulfoxamines, diamides, spinosyns, and butenolides (Royer and Giles 2016). Most foliar applications occur as a low cost generic pyrethroid included in top-dress fertilizer applications in late February to early March. Efficient sampling plans for winter wheat allow producers to respond to aphid populations effectively, as economic thresholds now incorporate key natural enemy abundances (Giles et al. 2003).

More recently, the integrated management of winter wheat aphid pests has relied heavily on biological control (Giles et al. 2008, Royer et al. 2015). Winter wheat supports a diverse assemblage of natural enemies, as it remains the largest source of green vegetation during

Oklahoma winters. Large populations of lady beetles have been documented imposing significant top-down effects on aphid pests (Kring et al. 1985, Rice and Wilde 1988, Michels et al. 2001). Significant aphid mortality is consistently exerted by the aphid parasitoid *Lysiphlebus testaceipes* (Cresson). In studies of aphid abundance and parasitism rates, Giles et al. (2003) described the relationship between the proportion of aphid mummies per wheat tiller and overall within-field parasitism rates. This data was used to develop rapid, reliable sampling plans that estimate aphid suppression (Royer et al. 2005), and has resulted in significant reductions in pesticide applications (Edwards et al. 2015). As few as four mummified aphids in a sample of 15 wheat tillers can indicate that effective aphid suppression is imminent, thus preventing the unnecessary use of foliar insecticides (Giles et al. 2003).

A key factor influencing biological control efficacy appears to be the diversity of vegetation associated with winter wheat in the larger landscape (Rice and Wilde 1988, Brewer and Elliott 2004, Giles et al. 2008, Nassab et al. 2013). *Lysiphlebus testaceipes* is known to respond positively to increased heterogeneity at larger scales than other Aphidiinae species such as *Diaeretiella rapae* (McIntosh) (Ahern 2000, Pike et al. 2000, Brewer et al. 2008). During the spring, supplemental food resources (i.e., nectar and pollen) in the habitats neighboring winter wheat can significantly increase natural enemy longevity and foraging efficiency, resulting in lower average pest abundance (Brewer and Elliott 2004, Bianchi et al. 2006, Giles et al. 2008, Schellhorn et al. 2015, Gurr et al. 2017). The recent adoption of winter canola into the winter wheat landscape may provide valuable resources to *L. testaceipes* and other natural enemies, but few studies have addressed the ecological implications of widespread canola adoption for this parasitoid species (Jessie 2017).

Winter Canola

Canola (*Brassica napus* L.) refers to any number of rapeseed cultivars selectively bred for reduced erucic acid in the seed, thus rendering a more palatable and healthier cooking oil with low levels of saturated fat (Harland 2009, Boyles et al. 2012). Its growing demand has made it one of the largest oilseed crops worldwide. Rapeseed, the plant from which canola varieties are derived,

has been cultivated for cooking and industrial uses for centuries (Raymer 2002, Boyles et al. 2012). Following the development of lower-viscosity synthetic lubricants, demand for rapeseed oil decreased and producers in Canada relied solely on the cooking oil market. Rapeseed production worldwide now tops 36 million hectares and continues to grow annually (FAOSTAT 2017). Domestic canola production has also risen from 60 thousand hectares harvested in the early 1990's to over 850 thousand hectares harvested in 2017 (USDA NASS 2017).

Winter-hardy canola varieties first appeared during the late 1980's, and were introduced to Oklahoma in the early 21st century primarily as small experimental plots (Boyles et al. 2012). Winter canola has been selectively bred to survive prolonged low temperatures and moisture via vernalization in compact rosettes (Kacperska 1984). Winter canola leaves also produce more epicuticular waxes than spring canola (Desneux and Ramirez-Romero 2009), which reduce moisture loss from plant surfaces. This waxy protection can limit the mobility of some insects (Eigenbrode and Espelie 1995) and reduce the foraging efficiency of some parasitoids (Chang et al. 2004, Gentry and Barbosa 2006). As temperature and moisture increase during early spring, winter canola rosettes begin to bolt and produce elongate racemes of clustered flowers (Musil 1950). During this flowering and seedpod forming period, plants are susceptible to damage and yield loss from herbivorous insects and protective measures may be required. Multiple applications of insecticides have been commonly used to prevent outbreaks of pests, particularly aphids (Franke et al. 2009).

In the south central United States, winter-adapted canola is now grown in rotation with winter wheat every 1-3 years to diversify continuous wheat systems and optimize management of grassy weeds (Franke et al. 2009). The profitability of this multiyear crop rotation relative to continuous wheat production has facilitated rapid annual increases in production area (DeVuyst et al. 2009, Bushong et al. 2012); and in Oklahoma, canola is frequently grown in an annual rotation with wheat (Boyles et al. 2009). Despite the many biological and ecological differences between winter canola and wheat, they can both be produced using similar small-grain production

equipment. Originally touted as a long-anticipated rotational crop for otherwise continuous winter-wheat systems, the market value of winter canola has occasionally surpassed that of winter wheat, allowing producers to benefit from crop diversification (DeVuyst et al. 2009). Although winter survival is heavily dependent on agronomic practices and environmental conditions, there is genetic variability in winter survival among available cultivars. Yield potential, heat and drought tolerance, disease resistance, and herbicide resistance are all important factors in cultivar selection. Many recently released cultivars are hybrid varieties, which can produce larger seeds to increase uniformity in planting and yield potential (Boyles et al. 2012). Most cultivars planted in Oklahoma are glyphosate resistant, as long-term management of grassy weeds is an important factor for maintaining high yield (Godsey and Boyles 2012).

Despite frequent and severe aphid outbreaks, Oklahoma quickly became the second largest domestic producer of canola, and in 2014, over 100 thousand hectares of winter canola were planted (USDA NASS 2017). Although the profitability of this crop has facilitated adoption by Oklahoma producers seeking a crop to rotate in traditionally continuous winter wheat systems, surveys of canola growers in 2009 revealed the threat of insect pests remained a primary concern (Franke et al. 2009). Following winter canola's emergence in early fall, the crop is vulnerable to a number of pest species including caterpillars, false chinch bugs (*Nysius raphanus* Howard), aphids, and thrips (Thysanoptera). The diamondback moth (*Plutella xylostella* L.) is also a frequent pests of winter canola during the overwintering rosette stage, when larvae can damage young seedlings despite neonicotinoid seed treatments (Boyles et al. 2012).

Three aphid species were documented in early experimental trials of winter canola: *Lipaphis pseudobrassicae* (Davis) (commonly and erroneously referred to as *L. erysimi* Kaltenbach [Blackman and Eastop 2006]), *Myzus persicae* (Sulzer), and *Brevicoryne brassicae* L. (French et al. 2001). Of these, the crucifer-specialists *B. brassicae* and *L. pseudobrassicae* are the most frequent and damaging, whereas green peach aphids are common but infrequently reach outbreak populations. Furthermore, crucifer-specialists are capable of sequestering defensive secondary

metabolites from the plant into their own tissues to defend against natural enemies (Kazana et al. 2007, Kos et al. 2011). Jessie et al. (2015) found that specialized aphids are indeed capable of imposing significant developmental costs to predators in the Southern Great Plains, which may negatively influence biological control in this crop. The generalist aphid *M. persicae* does not sequester plant volatiles but instead excretes them in honeydew (van Emden et al. 1969).

Although aphid infestations were often limiting to profitable winter canola production (Franke et al. 2009), the widespread planting of canola seed treated with neonicotinoids has reduced crop losses from early-season infestations of *L. pseudobrassicae* (Royer and Giles 2010, Giles et al. 2011). This has also facilitated delaying foliar insecticide applications until spring, which can improve colonization of canola fields by beneficial predators and parasitoids. Chown and Giles (2006) observed that late-season aphid infestations resulted in significant damage to reproductive portions of canola plants and subsequently, late-season insecticide applications became common in Oklahoma (Franke et al. 2009). As of 2009, the most common curative insecticides used against aphids were bifenthrin, λ-cyhalothrin, and parathion-methyl (Franke et al. 2009).

Recent studies of insect activity in winter canola revealed the most common natural enemies are aphid parasitoids (*D. rapae*, *L. testaceipes*, and *Aphelinus spp*.), followed by chrysopids (*Chrysoperla* spp.) and coccinellids (*Coccinella septempunctata* L. and *Hippodamia convergens* Guérin-Méneville) (Jessie 2017). Frequently, these beneficial insects are observed arriving in winter canola many weeks later than aphids, which permits aphid the opportunity to outpace the suppressive effects of predators and parasitoids. Neuville et al. (2016) found that delayed arrival of *D. rapae* to cabbage fields (*B. oleracea* convar. *Acephala*) significantly reduced biological suppression of *B. brassicae*. Large aphid populations in winter canola likely attract and benefit predator and parasitoid populations, and this crop may serve as a source crop for a diverse group of natural enemies. However, applications of broad-spectrum insecticides against aphids during the spring likely lowers abundance of these natural enemies in canola habitats; thus, canola fields may actually function as a lethal sink for predators and parasitoids. *Diaeretiella rapae* and

some Aphelinidae would be expected to respond positively to increasing acreage of winter canola in Oklahoma, as these species are crucifer specialists and typically operate at much smaller scales than does *L. testaceipes* (Brewer et al. 2008). However, the currently limited availability of winter canola and its yearly rotation with winter wheat results in a highly fragmented ephemeral habitat, which is likely less supportive of *D. rapae* populations.

Aphid Parasitoids

Wasp species in order Hymenoptera are primarily parasitic on other animals. Most often, these insects are parasitoids, wherein to complete development, the insect must kill their host. Wasps in the subfamily Aphidiinae (Hymenoptera: Braconidae) exclusively parasitize aphids (Hemiptera: Aphididae) and have been critically important components of biological control programs worldwide (Starý 1969, Waage and Hassell 1982, Schmidt et al. 2003). Many members of the family Aphelinidae (Hymenoptera: Chalcidoidea) are also exclusively parasitoids of aphids and scale insects (Hemiptera: Diaspididae). Often characterized by their specificity, these groups of parasitoids exhibit varying degrees of host and host-plant affiliation, which can be influenced by environmental and physiological factors (Desneux et al. 2009). Despite relative host-plant specificity, geographic and host ranges of aphid parasitoid species often overlap, resulting in competition among species for shared hosts. Not surprisingly, research on Aphidiinae has therefore focused on parasitoid host ranges and distributions for much of the 19th and early 20th centuries (Starý 1970).

Descriptions of parasitoid taxonomy and basic biology occurred concurrently with studies of their potential for biological control during the early 20th century, particularly as new observations of successful biological control efforts were published by researchers at the University of California in Berkeley (Smith 1919, DeBach et al. 1955, Doutt 1958). Since then, their utility in biological control programs has been widely evaluated against several insect pests (Hågvar and Hofsvang 1991). Currently, the economic potential and unique life histories of this group stimulates wide scientific interest and study (Godfray 2016).

Development

The life cycle of an aphid parasitoid begins when a female wasp successfully oviposits in a suitable host. Typically, female wasps lay eggs singly, but may self-superparasitize (i.e., lay multiple eggs) when host densities are low (Van Alphen and Visser 1990, Kant et al. 2011), in the presence of conspecifics (Godfray 1994) or, more rarely, exhibit facultative gregariousness (i.e., multiple species in a host) (Mackauer and Chow 2015). During oviposition, eggs and venom are deposited along with teratocytes, which rapidly absorb host fluids shortly after oviposition and begin to attack host embryos (Falabella et al., 2000). Teratocytes are critically important for successful manipulation of host metabolism (Li et al., 2002), and release nutrients from host embryos which are then available to the parasitoid larva (Falabella et al., 2000). During four larval stadia, the immature wasp consumes the internal material of its host (Kant 2012). Only the first and fourth larval instars are mandibulate. First instar larvae are believed to use their mandibles in combat against supernumerary larvae, whereas the second and third instars lack mandibles, instead feeding on fluids within the hemocoel (Broussal 1966, Couchman and King 1977). Because parasitoid larvae essentially exist within aquatic habitats, respiration occurs through passive cutaneous exchange with a closed tracheal system (Fischer 1971).

The fourth and final larval instar is also mandibulate, and feeds actively on all remaining host tissues, leaving the digestive tract and nervous system to the very last. This activity leaves only the cuticle of the host intact, and the wasp larva's movements push the cuticle outward to form a spherical 'mummy' around the penultimate instar (Godfray 1994). As the host's soft tissues are now completely absent, the wasp larva respires via trachea within the air-filled mummy. Development from egg to pupation lasts approximately four days, dependent upon host suitability and environmental conditions. Prior to pupation, the larva uses its mandibles to cut a slit in the aphid cuticle ventrally, and attaches the mummified aphid to a substrate using labial silk glands (Couchman and King 1977). Silk glands are then used to create a protective cocoon around the pupa within the aphid mummy. Adult wasps emerge after approximately four days through a

circular opening on the host's dorsum created by the adult's mandibles. As the female exits the mummy, chemical cues associated with the aphid cuticle inform subsequent foraging decisions (Van Emden et al. 1996). Following a brief period of grooming, mate-searching behavior begins and adults will search for nectar and honeydew sources. Following mating, female parasitoids begin searching for hosts.

Adult Stage

Host location involves several successive steps, including habitat and host searching, host recognition, and host acceptance. Proximate models consider these behaviors hierarchical, with discrete progression from step to step (Doutt 1959, Vinson 1976, Mackauer et al. 1996). However, parasitoid foraging behavior is variable among and within species, suggesting a complexity akin to optimal foraging theory wherein several alternative behaviors exist at each step and can be used to ensure maximal fitness gains (Stephens and Krebs 1986, Bell 2012). Each step is dependent upon several host and habitat cues, including chemicals produced by the host, such as sex, alarm, and aggregation pheromones as well as honeydew and frass (Ruther et al. 2002). In addition, parasitoids may use chemical cues from host plants, particularly herbivore-induced plant volatiles (Read et al. 1970, Dicke and Sabelis 1988, Lewis and Martin 1990, Vet and Dicke 1992, Storeck et al. 2000, van Emden et al. 2008).

Read et al. (1970) found that plant chemicals are an initial attractant for parasitoid wasps to habitats containing host aphids. Because foraging efficiency depends on host searching and handling times (Hudak et al. 2003), once suitable habitats have been located, within-patch foraging commences (Read et al. 1970, Michaud and Mackauer 1994, Mackauer et al. 1996). The size and density of hosts as well as parasitoid age and number of within-patch competitors determine host acceptance, suitability, and sex allocation decisions (Flanders 1942, Godfray 1994, Jervis 2005). Larger (i.e., older) hosts are more frequently attacked than smaller aphids (Kant et al. 2011, Tazerouni et al. 2011); yet parasitoid fecundity may be maximized when smaller hosts are selected. For *Aphidius ervi* Haliday parasitizing *Acyrthosiphon pisum* (Harris), reproductive performance

was greatest when females began development in second instar hosts, despite adult dry mass being greatest when third and fourth instar hosts were parasitized (Sequeria and Mackauer 1992). The ultimate evaluation of host acceptance and suitability occurs through a series of antennation and ovipositor probing behaviors (Vinson 1976, Mackauer et al. 1996). During probing, females are assess host quality and, based on the information received, either oviposit or resume foraging for a more suitable host (Mackauer et al. 1996).

Female aphidiids must make important decisions during foraging to maximize their fitness during relatively short lifespans. Depending on host handling time, host quality, and the female's physiological state, lifetime fitness may be maximized by adjustments of attack and ovipositional rates, as well as egg fertilization rates. For example, encountering sequential aphid patches, *A. ervi* increased attack and ovipositional rates when the second patch contained higher quality hosts, and reduced these rates when patch sequences were switched (Michaud 1996).

Host quality at the time of oviposition can influence parasitoid larval growth and development (Sequeria and Mackauer 1994). The lifetime fecundity of female parasitoids is a function of the number of eggs upon adult emergence, body size, and longevity. Adult longevity is affected by body size, food resource availability, mating success, and abiotic factors such as temperature and humidity (Hafez 1961). As host density increases, females may increase their attack rate and expend more resources, which result in decreased longevities (Kant and Minor 2017).

Sex allocation is also an important component of reproductive behavior. When foraging in patches with a high density of hosts or competitors, *D. rapae* females produce more male offspring (Kant et al. 2011, Kant and Minor 2017). However, populations of parasitoids tend to remain female biased, which is preferred in biological control programs because females are responsible for finding and parasitizing hosts. Producing more sons than there are females to mate results in increased local mate competition, which can increase rates of inbreeding and affect species persistence in the environment (Godfray 1994, Ode and Hardy 2008). However, the effects of

inbreeding are likely unimportant for aphidiid parasitoids, as deleterious traits in hapodiploid systems would result in greater mortality for haploid males (Mackauer and Völkl 2002, Salin et al. 2004).

Diaeretiella rapae. *D. rapae* was first described by McIntosh (1855) as *Aphidius rapae*, and later revised by Starý (1960) who placed the species under its own genus as *Diaeretiella rapae*. Thought to have originated in the Western Palearctic, this species is now found worldwide and contributes to suppression of aphid populations in several cruciferous crops (Brassicaceae) (Hafez 1961, Read et al. 1970, Mackauer and Kambhampati 1984, Bahana and Karuhize 1986, Elliott et al. 1994, Gabrys et al. 1998, Devi et al. 1999, Neuville et al. 2016). A strong olfactory response to cruciferous plant volatiles has been demonstrated in *D. rapae* (Read et al. 1970, Sheehan and Shelton 1989); and this response is conditioned by chemical cues received by emerging females as they contact the mummy cuticle (Ferguson 2014). However, despite the attraction to volatiles, females prefer to parasitize sparse colonies of *B. brassicae* rather than dense ones (Lopez et al. 1990).

Pike (2007) reported that *D. rapae* utilizes nearly 70 aphid species across its range, and is the only primary parasitoid of *B. brassicae* (Pike et al. 1999). Several authors have documented the importance of allyl isothiocyanate in host location for *D. rapae* (Cole 1980, Vaughan et al. 1996). This compound is a volatile metabolite produced by cruciferous plants following herbivore feeding and damage. Both experienced and inexperienced female wasps are innately attracted to crucifer synomones, and to aphid kairomones to a lesser extent (Reed et al. 1995, Ferguson 2014). Previous studies (e.g. Read et al. 1970, Sheehan and Shelton 1989) reporting attraction to host plants themselves likely simulated herbivore-feeding kairomones by cutting plant material prior to parasitoid exposure (Reed et al. 1995). Despite the importance of these volatiles for *D. rapae* host location, this species is also known to attack aphids in crops where allylisothiocyanate is not emitted such as cereal and solanaceous crops (Pike et al. 1999). However, *D. rapae* is not known to respond innately to volatiles produced in these systems (Lester and Holtzer 2002).

Exotic *D. rapae* collected from the Palearctic were introduced to Colorado in the early 1990s for control of a recently invading aphid pest of winter wheat, *D. noxia*, because native populations of *D. rapae* were not effective (Wraight et al. 1993). Following these introductions, a greater number of *D. rapae* were recovered from *D. noxia* (Elliott et al. 1995). Similar releases in Wyoming resulted in what appeared to be establishment of the exotic strains, as the proportion of *D. noxia* populations containing *D. rapae* parasitoids increase from 0% in 1991 to 100% in 1998 (Brewer et al. 2001). However, it is unclear whether these were exotic populations or reflect gradual adaption by local strains. The effectiveness of these introductions illustrate the genetic variability that can exist in geographically distinct populations of parasitoids (Baker et al. 2003). However, more recent studies on *D. noxia* natural enemies found relatively low parasitism rates by both *D. rapae* and a native cereal parasitoid wasp, *L. testaceipes* in Colorado and Texas (Michels et al. 2001, Lee et al. 2005). In Wyoming, higher rates of *D. noxia* parasitism have been detected primarily by *Aphelinus* spp. (Brewer et al. 1998).

As a koinobiont, *D. rapae* develops inside an aphid host as it continues to feed and provide supplemental nutrition to the immature wasp. Developmental thresholds for *D. rapae* have been reported from 2.1 - 3.5°C in *B. brassicae* and *D. noxia* hosts (Campbell et al. 1974, Bernal and González 1993). The total developmental period (egg to adult) ranges from 24 days at 15°C to 10 days at 30°C, and adult female longevity ranges from 14 days at 15°C to 5 days at 30°C (Bernal and González 1995, Basheer et al. 2014). The temperature range tolerated by the host species is also an important factor in *D. rapae*'s response to temperature. Souza et al. (2017) found *D. rapae* were able to withstand temperature extremes when developing in *L. pseudobrassicae* but not when developing in *M. persicae*. This benefit is conferred to the parasitoid through the host's own adaptations to thermal stress.

Prior to statewide winter canola production, *D. rapae* was occasionally found parasitizing cereal aphids in Oklahoma sorghum (*Sorghum bicolor* L.) and winter wheat, primarily *R. padi* and *R. maidis* (Fitch) (Gilstrap et al 1984, French et al. 2001, Giles et al. 2003). Recently, however,

Elliott et al. (2014) evaluated parasitism rates of common aphids in winter canola and winter wheat, and found that *D. rapae* emerge only from winter canola aphids. With the rise of winter wheat and canola rotations more suitable hosts for *D. rapae* became available, including *B. brassicae*, *L. pseudobrassicae,* and *M. persicae*, and this parasitoid now forages primarily in cruciferous crops. When given the choice between winter canola and winter wheat, *D. rapae* exhibits a strong preference for winter canola (Ferguson 2014). However, recent surveys of insect activity in crop and non-crop habitats revealed very low abundance of *D. rapae* (Jessie 2017).

Lysiphlebus testaceipes. *Lysiphlebus testaceipes* was first collected from aphids in Florida by John Comstock and described as *Trioxys testaceipes* (Cresson 1879). This species is a solitary koinobiont common within its native Nearctic and Neotropical ranges. Introductions of *L. testaceipes* as a biological control agent have occurred several times in Palearctic regions, where populations have now established (Starý et al. 1988, Žikić et al. 2015). The host range of *L. testaceipes* is wider than that of *D. rapae*, and it has been observed attacking over 100 aphid species on an equally broad range of host plants (Mackauer and Starý 1967, Starý et al. 1988, Pike et al. 2000).

Both host plant and rearing environments influence the acceptance of aphid hosts by *L. testaceipes*. When provided with *Aphis fabae* (Scopoli) on both broad been and sugar beet plants, *L. testaceipes* parasitized significantly more aphids on broad bean plants (Albittar et al. 2016). Interestingly, when presented with *M. persicae* alone, parasitism rates were relatively low; but when supplied with both *A. fabae* and *M. persicae* together, parasitism of *M. persicae* increased. This altered foraging may be a result of chemical cues from acceptable hosts confounding host acceptance behaviors (Meisner et al. 2007). Furthermore, the suitability of *M. persicae* varied depending on the host plant. When reared on broad bean, survival of *L. testaceipes* was higher than when parasitizing *M. persicae* on sugar beets (Albittar et al. 2016). Although *L. testaceipes* successfully develops in *M. persicae* hosts, acceptance ranges from only 4-7% (Carnevale et al. 2003, Silva et al. 2008).

Studies on developmental thresholds reveal that total preimaginal development can require over 49 days at 10ºC, whereas at temperatures above 25ºC, *L. testaceipes* can reach adulthood in approximately 9 days (Elliott et al. 1994, Royer et al. 2001). Their ability to survive low temperatures is a key component of their effectiveness in winter, as temperatures frequently dip below freezing even though warm sunny days are common. Mummies of *L. testaceipes* can survive over three months at 5°C, and nearly a month at -6°C (Jones 2005). Adult *L. testaceipes* have also been observed successfully ovipositing into *S. graminum* at temperatures as low as 3.33°C (Hunter and Glenn 1909).

The importance of *L. testaceipes* in the regulation of cereal aphids (particularly *S. graminum*) has been highlighted by several studies (Spencer 1926, Fisher et al. 1999). In the Southern Great Plains, *L. testaceipes* responds to even small aphid populations and is a key component of *S. graminum* biological control throughout the winter wheat growing season (Jones 2001, Giles et al. 2003, Jones 2005). In field cage studies, *L. testaceipes* was able to suppress *S. graminum* populations when initial parasitism rates were less than 2% (Jones 2001). Interestingly, *L. testaceipes* adults can remain active throughout winter months and survive temperatures as low as -8ºC (Jones 2005).

Factors Influencing Parasitoids in Agroecosystems

Parasitoid wasps are widely recognized for their contributions to biological control in agricultural systems (Mills 2000). Studies of their biology, behavior, and physiology are often focused on how these aspects of their ecology influence their abilities to suppress pest populations (Waage and Hassell 1982, Mackauer et al. 1990). Aphid parasitoids are affected by local and landscape diversity (Landis et al. 2000, Roland 2000), intraguild interactions (Rosenheim et al. 1995, Snyder and Ives 2001), abiotic factors (Stilling 1993) and cultural disturbances such as crop harvest and insecticide applications (Desneux et al. 2007). Because aphid hosts frequently occur as patchily distributed resources, multiple species of parasitoids often compete for a limited number of hosts (Klomp 1964, Kindlmann and Dixon 1999, Brodeur and Rosenheim 2000).

Competition among Parasitoids

Extrinsic competition among adult parasitoids is likely limited by different host preferences and the solitary nature of many parasitoid species. However, host overlap is surprisingly common among endophytic parasitoids (Brodeur and Rosenheim 2000, Harvey et al. 2013). When host ranges overlap, intrinsic competition may occur, wherein larvae compete either directly or indirectly within the parasitized host. The outcome of intrinsic competition is largely determined by the timing of oviposition events, as the second species to parasitize in multiparasitism scenarios is less likely to outcompete an older first instar (Tillman and Powell 1992, De Moraes and Mescher 2005). However, second instar larvae may be at a disadvantage, as mandibulate first instars can readily attack and consume larger amandibulate second instars (McBrien and Mackauer 1991, Danyk and Mackauer 1996)

Some species may have a competitive advantage over another depending on host suitability and the feeding ecology and behavior of larvae and adults. For instance, parasitoid species with rapidly hatching eggs have an advantage over those that hatch slower. Similarly, Hågvar (1988) found *Ephedrus cerasicola* Starý to out-compete *Aphidius matricariae* Haliday when ovipositing up to two days after *A. matricariae*. It is suggested that *E. cerasicola* is able to eliminate competitors through substances injected with the egg upon oviposition (Hågvar 1988). Previous studies found similar results when *E. cerasicola* competed with *A. colemani* (Hågvar and Hofsvang 1988). Studies on competition between *A. ervi* and *A. smithi* Sharma & Subba Rao revealed a significant reduction in parasitism of *Acyrthosiphon pisum* (Harris) by *A. smithi* when foraging simultaneously with *A. ervi* (Chua et al. 1990). In addition, they found that significantly more *A. ervi* survived to adulthood in cases of multiparasitism. McBrien and Mackauer (1990) found first and fourth instar larvae of *A. ervi* are competitively superior to both older and younger larvae of *A. smithi*. This competitive advantage in both extrinsic and intrinsic competition scenarios may explain the displacement of *A. smithi* by *A. ervi* in the pea aphid - alfalfa system in the Pacific northwest (Chua et al. 1990).

Slansky (1986) suggested preference for early instars as hosts is driven, in part, by the decreased likelihood that younger hosts have already been parasitized. Despite the costs of multiparasitism and the mechanisms for host discrimination, the long-range chemical cues that attract parasitoids to patchily distributed hosts increases the likelihood of competitive interactions (Brodeur and Rosenheim 2000). In general, parasitoid wasps are limited in their ability to discriminate hosts recently (< 24h) parasitized by heterospecific competitors. The external markers used by parasitoids are thought to be species-specific, and interspecific host discrimination is likely to result from the physiological changes that occur within host tissues post-oviposition (Vinson 1984, Tillman and Powell 1992, Nufio and Papaj 2001). Thus, host discrimination depends upon both the physiological changes induced by the first parasitoid and the ability of the second to detect such changes (Tillman and Powell 1992). In two closely related species, *A. ervi* and *A. smithi*, discrimination between unparasitized and parasitized hosts appears to result from external markers (McBrien and Mackauer 1991). The competitively superior species *A. ervi* tended to favor multiparasitism, whereas *A. smithi* more frequently superparasitized, results consistent with observed dominance of *A. ervi* in larval competition experiments (McBrien and Mackauer 1990). Intraspecific competition can also result in superparasitism, which often improves host quality (Rasekh et al. 2017). Bai and Mackauer (1990) found superparasitism by *A. ervi* resulted in larger adults than singly parasitized aphids. Kant and Minor (2017) found *D. rapae* produces fewer female offspring when foraging with conspecifics.

In the US Southern Great Plains, several aphid parasitoid species co-occur within a limited diversity of winter crops. Winter wheat and canola are the primary sources of green vegetation during winter, and aphid resources are frequently limited. French et al. (2001) found *D. rapae* commonly utilizing cereal aphids in winter wheat. However, a recent survey of aphids and parasitoids in winter canola and wheat found that these two species partitioned their habitat (Elliott et al. 2014). Aphids collected in winter wheat were found to be parasitized only by *L. testaceipes* and those from winter canola, only by *D. rapae* and *Aphelinus* spp. (Elliott et al. 2014). *L.*

testaceipes was the most abundant insect collected from both of these crop habitats (Jessie 2017). *Lysiphlebus testaceipes* is an indiscriminate forager known to attack many non-host aphids, and has been recovered from aphids on winter canola host plants (French et al. 2001). The frequency and outcomes of competitive encounters between these parasitoids has not been studied, but there exists potential host overlap in both crops, as *L. testaceipes* is known to parasitize *M. persicae*, and *D. rapae* is an occasional parasitoid of both *R. padi* and *D. noxia* in winter wheat (Pike et al. 2000).

Effects of Insecticides on Parasitoids

The effects of insecticides on natural enemies has been widely studied, particularly the reduced toxicities of some materials, and their utility in integrated control programs (Stern and van den Bosch 1959). Over the last 15 years, persistent carbamates and organophosphates have been replaced in most cropping systems with shorter-residual synthetic pyrethroids in an effort to reduce the environmental impacts of broad-spectrum insecticides. During this time, agrochemical companies have focused on the discovery of more selective insecticides with unique modes of action that suppress pests while conserving beneficial insects (e.g. flonicamid, sulfoxaflor, pymetrozine, triazamate). The effects of these newer compounds on parasitoids remain largely unknown and they were therefore a subject of interest in the present study.

Insecticides may affect non-target species through direct or indirect contact. Recently, seed coatings with systemic neonicotinoids are widely employed to protect seedlings. These seed treatments can have deleterious effects on foraging parasitoids (Moscardini et al. 2014). Herbivores that feed upon plants grown from treated seed can then be subject to predation or parasitism by natural enemies, which are then exposed to the insecticide secondarily.

Pyrethroids. One of the most widely used groups of insecticides, pyrethroids were first developed in the 1920's and modern pyrethroid compounds suitable for agricultural use were developed in 1973 (Elliott et al. 1973). These compounds are based on the structure of pyrethrins, a group of insecticidal compounds produced by plants in the *Chrysanthemum* genus (Casida and Quistad 1995). They work by preventing the closure of the voltage-gated sodium channels in insect

axons (IRAC MoA: 3A). As the sodium channels remain open, a constant inflow of sodium ions into the neuron causes persistent action potentials resulting in excitation, convulsions, paralysis, and death (Soderlund et al. 2002).

Synthetic pyrethroids are rapidly metabolized and have a relatively high oral LD_{50} for mammals (Soderlund et al. 2002). Low rates of pyrethroid insecticides can be lethal for plant pests such as aphids, but also inflict significant mortality on non-pest insects including pollinators, predators, and parasitoids. Pyrethroids exhibit a relatively indiscriminate action on all arthropods, and parasitoids are generally more susceptible to pyrethroid toxicity than are their herbivorous hosts (Croft and Brown 1975). Because of their long history of use in agriculture, pyrethroids have been widely studied for their effects on non-target species.

Pyrethroid insecticides are known to have significant lethal and sublethal effects on parasitoids, but the specific consequences of exposure are dependent upon the specific compound (Delpuech et al. 2005, Desneux et al 2007). Parasitoids exposed to many common pyrethroid formulations as larvae are unable to complete development, and treatment of pupal stages often results in significantly decreased survival and longevity (Delorme 1976, Hsieh and Allen 1986, Krespi et al. 1991). Cônsoli et al. (1998) reported a 35% reduction in fecundity when the parasitoid *T. pretiosum* was exposed to λ-cyhalothrin. Treatment of honeydew patches with deltamethrin resulted in a strong repellant effect on *A. rhopalosiphi* (Longley and Jepson 1996).

The foraging ability of *A. ervi* was significantly reduced after exposure to low rates of λ-cyhalothrin (Desneux et al. 2004). In addition, treated females exhibited less antennation and reduced ovipositional activity. Interestingly, when treated with deltamethrin, *D. rapae* and *A. matricariae* appeared to be unaffected by the treatment (Desneux et al 2004). Furthermore, a combination of deltamethrin and *D. rapae* reduced populations of *M. persicae* in field cages better than did either alone (Desneux et al. 2005). These variable effects highlight the need for detailed studies on the effects of insecticides on aphid parasitoid ecology and behavior. Compatibility

between chemical and biological control agents could permit chemical control of pest outbreaks without disruption of long-term, sustainable pest management programs.

Neonicotinoids. Neonicotinoids are a class of insecticides developed in the early 1990's that are now one of the most commonly used insecticides worldwide (Jeschke et al 2010). Structurally similar to nicotine, these compounds act as agonists of post-synaptic acetylcholine receptors (IRAC MoA: 4A, Tomizawa and Casida 2004). Selectivity is conferred through structural and organizational differences in the nicotinic acetylcholine receptors between insects and mammalian nervous systems, but the specific architecture has not been fully described (Tomizawa and Casida 2004).

Frequently used as seed coatings, the systemic activity of neonicotinoids in plants provides long-term protection from herbivores, particularly for slow-growing crops or those that experience dormancy shortly after planting (Laurent and Rathahao 2003). Seed treatments are often preferred to foliar applications, as they involve a selective application of much smaller pesticide quantities and have fewer non-target effects (Hull and Beers 1985, Albajes et al. 2003). However, these systemic insecticides can be found in all plant tissues, including pollen and nectar sources used by foraging pollinators and natural enemies (Lundgren 2009, Alburaki et al. 2017). In Oklahoma, neonicotinoid seed treatments (imidicloprid, thiamethoxam, or clothianidin) are commonly recommended for use in winter canola (Royer and Giles 2017).

The effects of neonicotinoids on aphid parasitoids have not been carefully examined. The aphid parasitoid *L. testaceipes* has been shown to consume extra-floral nectar from seed-treated flowering plants. Although not lethal, female wasps exposed to thiamethoxam through extra-floral nectaries had reduced attack rates and erratic host acceptance behaviors (Moscardini et al. 2014). In addition, the proportion of female offspring produced by these females was significantly lower than control females. Stapel et al. (2000) found *M. croceipes* was less responsive to host-plant odors after consuming imidicloprid through extra-floral nectaries. Naveed et al. (2010) monitored parasitism of *Bemesia tabaci* (Genn.) by aphelinid parasitoids in seed-treated cotton (*Gossypium*

arboretum L.) fields, finding consistently lower levels of within-field parasitism compared to untreated plants. Studies on the effects of imidicloprid on *L. fabarum* mummies and adults exposed to imidicloprid resulted in approximately 60 and 90 percent mortality, respectively (Sabahi et al. 2011).

Sulfoxamines. Similar to neonicotinoids, the sulfoxamines are a new class of insecticides that also act as agonists of post-synaptic nicotinic acetylcholine receptors (IRAC MoA: 4C). However, sulfoxaflor (Methyl[1-(2-trifluoromethylpyridin-5-yl)ethyl]-N-cyanosulfoximine), the only registered member of this group, has selective activity against hemipteran and thysanopteran pests (Babcock et al. 2011). It is believed that sulfoxaflor's interaction with acetylcholine receptors is distinct from those of neonicotinoids (Watson et al. 2011). Because sulfoxaflor exhibits both systemic activity in plants and greater selective activity for sap-feeding insects, this chemical may provide a better alternative to neonicotinoid seed treatments (de Little et al. 2016, Wang et al. 2016). Unfortunately, sulfoxaflor is considered highly toxic to *Apis mellifera* (L.) and may not alleviate concerns surrounding the widespread use of neonicotinoids (Zhu et al. 2017). Though not widely used in Oklahoma winter crops, its potential compatibility with natural enemies may allow for its integration into canola and/or wheat pest management decisions.

In the relatively few studies performed since its development, sulfoxaflor has been found relatively non-toxic to natural enemies. Brar et al. (2017) found the LC_{50} of sulfoxaflor was three times greater for the psyllid parasitoid *Tamarixia radiata* Waterston than for its host *Diaphorina citri* Kuwayama. However, studies of *Eretmocerus mundus* (a parasitoid of *Bemesia tabaci*) found direct sprays of mummies with recommended field rates resulted in a 94% reduction in adult emergence and 100% adult mortality after 72 hours (Fernández et al. 2015). In a separate experiment, residual contact by adults resulted in 100% mortality within 72 hours, resulting in a 'harmful' IOBC toxicity rating (Fernández et al. 2015).

Much of the published research on sulfoxaflor examines its effects on predaceous natural enemies. In studies of Coccinellidae, mortality of adult *H. convergens* treated with sulfoxaflor were

not significantly different from controls (Tran et al. 2016). Colares et al. (2017) found *H. convergens* survival was nearly 100% after application of sulfoxaflor to adults at up to twice the recommended field rate. Garzón et al. (2015) reported similar results with another lady beetle species, *Adalia bipunctata.* Larval coccinellids appear to be more susceptible to sulfoxaflor. Robideau (2015) provided sulfoxaflor-treated aphids to developing *Coccinella septempunctata* L. and *H. convergens* larvae, finding only 15% and 45% of individuals completed development, respectively. Ingestion of sulfoxaflor-contaminated prey by *H. convergens* larvae at 1x and 2x recommended field rates resulted in 40% and 30% larval survival after 24 hours, respectively (Colares et al. 2017).

Studies on Chrysopidae reveal the opposite effect, with larvae appearing to be less susceptible to sulfoxaflor than are adults (Garzón et al. 2015, Tran et al. 2016, Barbosa et al 2017). Exposure of larvae to sulfoxaflor residues resulted in 7% to 10% survival (Garzón et al. 2015, Tran et al. 2016), whereas adult exposure resulted in 0% to 43% survival (Garzón et al. 2015, Barbosa et al 2017). The varying effects of sulfoxaflor on predator and parasitoid larvae and adults suggests its incorporation into integrated pest management (IPM) programs should not precede evaluations of its toxicity to non-target species, including natural enemies and pollinators.

Flonicamid. The selective feeding blocker, flonicamid (N-cyanomethyl-4-trifluoromethy-l-nicotinamide), was first released to the world market in 2005. It belongs to a relatively new type of insecticide, the chordotonal organ modulators. Such insecticides belong to two groups, the TRPV channel modulators which include the pyridine azomethine derivatives (IRAC MoA: 9) and those which do not act on TRPV channels, the sole member of which is flonicamid (IRAC MoA: 29). Although the specific mode of action has not been identified, it inhibits salivation and ingestion by preventing stylet penetration into the plant (Morita et al. 2007).

The effects of flonicamid on immature and adult parasitoids have not been thoroughly studied, but results thus far indicate a high level of variability depending on the species evaluated and the method of exposure. Jansen et al. (2011) reported a significant reduction in fecundity and

survival of *Aphidius rhopalosiphi* (DeStefani-Perez) females exposed to flonicamid residue in the laboratory, but no significant differences on field-treated plants. Moens et al. (2012) found flonicamid reduced survival, parasitism rate, and longevity of *Microplitis mediator* (Haliday) when treated as adults. Furthermore, adult emergence from treated cocoons was reduced, suggesting that flonicamid has an impact on the pupal stage of *M. mediator* as well.

Fernandez et al. (2015) reported an IOBC toxicity rating of 'harmless' after finding an approximately 5% reduction in adult emergence and parasitism rate of *E. mundus* parasitoids treated with flonicamid as mummies. When treated through residual contact as adults, toxicity was slightly higher, with a 23% increase in mortality and a 9% decrease in parasitism rate. Studies on residual toxicity of flonicamid to *Leptomastix dactylopii* (Howard), a parasitoid of citrus mealybug, found no significant lethal or sublethal effects of the compound (Cloyd and Dickinson 2006). Effects on pollinators were similar with less than 10% mortality in *A. mellifera* when treated with formulated flonicamid (Thomazoni et al. 2009).

Because of the unique mode of action, aphids exposed to flonicamid do not die immediately, but rather are subject to the effects of starvation. In fact, starving aphids may still serve as suitable food to predators and as hosts to parasitoids. When coccinellids were provided with an *ad-libitum* diet of flonicamid-treated aphids for the duration of preimaginal development, no significant differences in survival or developmental duration were detected (Robideau 2015). *Chrysoperla carnea* larvae had survival similar to controls when exposed to flonicamid through contaminated prey or treated leaf discs (Barbosa et al. 2017). Similarly, adults exposed to flonicamid residues had no significant increase in mortality when compared to control treatments (Barbosa et al. 2017) The rapid cessation of aphid feeding and low toxicity to natural enemies suggests this chemical may be an ideal candidate for many IPM programs that target conservation of natural enemies. However, flonicamid, should be evaluated for the non-target biological and ecological effects it may have in the targeted agroecosystem, as its variable effects highlight the need for more detailed studies on its compatibility with biological control agents.

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CHAPTER III

MANUSCRIPT ONE

Competitive Interactions between *Diaeretiella rapae* and *Lysiphlebus testaceipes*

Introduction

Wasps in the subfamily Aphidiinae (Hymenoptera: Braconidae) are exclusively solitary endoparasitoids of aphids that frequently contribute to the regulation of aphid populations in field and greenhouse crops (Hågvar and Hofsvang 1991, Brewer et al. 2008, Brennan 2016). Interestingly, although often erroneously, parasitoid wasps are often considered more important for aphid population control than are predatory natural enemies (Schmidt et al. 2003). Practitioners of biological control have long equated greater diversity of natural enemies with improved levels of biological control, and that competition for shared hosts among multiple parasitoid species should increase herbivore suppression (DeBach and Sundby 1963, DeBach 1966, Evans 2016). Most often, studies of intrinsic competition between parasitoid species highlight consequences for parasitoid populations rather than net effects on biological control (e.g. Sidney et al. 2010, Cebolla et al. 2017). Competition among species shapes community structure and function (Force 1985, Bueno et al. 1993, Brodeur and Rosenheim 2000), and competition among natural enemies has important implications for biological control (Bogran et al. 2002, van Veen et al. 2006). The outcomes of competitive interactions may influence not only within-season pest populations, but also guild structure and species persistence (Chua et al. 1990, McBrien and Mackauer 1990). However, there are conflicting views on whether the effects of interspecific competition are meaningful for biolog-

ical control (Briggs 1993), as empirical evidence is limited and often conflicting (Force 1974, Bogran et al. 2002).

The eggs and larvae of aphid parasitoids are highly adapted to host physiology; they must overcome host immune responses and monopolize internal resources while keeping hosts alive long enough to complete larval development. When parasitoids share hosts, competition for host resources may take one of two forms: 1) adults may engage in scramble competition for hosts which may include aggressive behavior toward competitors (extrinsic competition), or 2) larvae may engage in larval combat within multiparasitized hosts (intrinsic competition) (Vinson and Iwantsch 1980). With few exceptions, only one adult parasitoid emerges per aphid host and all supernumerary larvae are eliminated.

Winter wheat (*Triticum aestivum* L.; Poaceae) is planted on over 8.1 million hectares across the US Southern Great Plains annually, and its aphid pests are regularly suppressed by both native and introduced natural enemy species (USDA NASS 2017, Brewer and Elliott 2004, Giles and Walker 2009). The native aphid parasitoid *Lysiphlebus testaceipes* (Cresson) is arguably the most important of these species, particularly in Oklahoma and Texas where its parasitism rates have been incorporated into aphid sampling plans and insecticide treatment decision thresholds (Kring and Gilstrap 1983, Giles et al. 2003). The efficacy of *L. testaceipes* in suppression of *Schizaphis graminum* Rondani (Hemiptera: Aphididae) in Oklahoma is attributable to various behavioral and physiological adaptations which allow responses to low aphid populations even during cold winter months (Arnold 1981, Jones 2005). Although considered a host and habitat generalist, *L. testaceipes* is consistently the most abundant parasitoid in Oklahoma wheat systems (French et al. 2001, Elliot et al. 2014, Jessie 2017), significantly outnumbering other species such as *Diaeretiella rapae* (McIntosh).

In Oklahoma, *L. testaceipes* and *D. rapae* have historically co-occurred in both wheat and sorghum (*Sorghum bicolor* L.) agroecosystems (French et al. 2001). The recent adoption of winter canola (*Brassica napus* L.; Brassicaceae) in the US Southern Great Plains now provides a seasonal

(fall-spring) cycle of several preferred host species for *D. rapae*, which is primarily a parasitoid of cruciferous (i.e. brassicaceous) aphids. However, biological control of crucifer aphids by *D. rapae* is not reliable in winter canola, and producers typically rely on chemical control measures (Franke et al. 2009, Royer and Giles 2017). Recent surveys of aphids and parasitoids found no parasitism of cereal aphids by *D. rapae*, and no parasitism of canola aphids by *L. testaceipes* (Elliott et al. 2014). Thus, *D. rapae* appears to be restricted to its preferred cruciferous habitat, ostensibly because it is conditionally attracted to volatiles emitted from cruciferous host plants (Reed et al. 1995, Ahern 2000, Ferguson 2014). As winter canola is planted and harvested at similar times as winter wheat, we may expect habitat-partitioning to occur between *D. rapae* and *L. testaceipes* across much of central and western Oklahoma where these crops are frequently planted in close proximity. However, the extremely high abundance of *L. testaceipes* in winter canola may result in frequent competitive encounters (Jessie 2017, WPJ pers. obs.). Both parasitoid species are capable of using one or more aphid host species common in winter wheat and canola crops (Pike et al. 2000), and competition between these two species may be shaping guild structure.

Data on competition between competing parasitoids is essential for describing the combined effects of these natural enemies on aphid populations. The aim of this study was to evaluate interspecific competition between *D. rapae* and *L. testaceipes* on three common aphids in the winter crop landscape (wheat and canola). My objectives were to: 1) quantify host discrimination behavior of *D. rapae* and *L. testaceipes* when provided with heterospecifically-parasitized hosts; 2) document outcomes of intrinsic competition between *D. rapae* and *L. testaceipes* on *L. pseudobrassicae*, *M. persicae*, and *R. padi* hosts; and 3) quantify parasitism outcomes of *D. rapae* and *L. testaceipes* when foraging independently and when foraging with heterospecific competitors.

Methods

Insect Colonies

Three separate colonies of *Rhopalosiphum padi* L., *Lipaphis pseudobrassicae* (Davis), and *Myzus persicae* Sulzer were reared in the laboratory at 24.4±0.9°C and 43±1.2% RH within double-walled mesh cages under fluorescent lighting (40 watt and 2,000 lumen) set at 16:8 (L:D). *Rhopalosiphum padi* were collected from winter wheat fields throughout north-central Oklahoma during the fall of 2014 and placed onto a single susceptible winter wheat plant (cv 'Jagger') in a 10cm diameter plastic pot (0.5L volume) to be screened for the presence of parasitoids. Unparasitized *R. padi* were then transferred to 14cm diameter pots (1.8L volume) with approximately 50, week-old winter wheat seedlings within colony cages. This procedure was repeated for *L. pseudobrassicae* and *M. persicae* collected from winter canola fields. Canola aphids were then reared on individually potted 21-day old susceptible winter canola plants (cv 'Wichita'). All colony plants were potted in a 1:1 mixture of potting soil and fritted clay absorbent material and fertilized with a 20:20:20 (N:P:K) water-soluble fertilizer upon planting. Plants were kept under both fluorescent lighting and high-pressure sodium lighting (400 watt and 50,000 lumen) to maintain plant vigor. Fresh canola or wheat plants were replaced weekly in their respective aphid colonies and watered as needed.

Separately, parasitized aphids were collected from winter wheat and winter canola fields throughout north-central Oklahoma during the fall of 2014. Aphids were returned to the laboratory and isolated on seedlings of their respective host plant; emerging adult wasps were identified to species using morphological keys (van Achterberb 1997) before being released into designated wasp colony cages. Two colonies of *D. rapae* were established for each of the three aphid species on their respective host plant (*R. padi* on wheat and *L. pseudobrassicae* and *M. persicae* on canola), and three colonies of *L. testaceipes* were established on *R. padi* only, as this wasp species was incapable of long-term establishment on either canola aphid species. Freshly infested plants from aphid colonies were added to parasitoid colonies bi-weekly. One week prior to the start of

laboratory experiments, aphid-infested 10cm pots of winter wheat or winter canola were placed into parasitoid colonies for approximately 12hr. Pots were then removed from the cages, isolated, and cleaned of adult parasitoids to establish mummy cohorts. All mummies (approximately 40) forming on these plants were removed and placed into small emergence chambers. The chambers consisted of an opaque 50mL centrifuge tube with the bottom removed, attached to a second, transparent 50mL centrifuge tube containing a cotton ball moistened with a 10% honey solution (Fig. 3.1). Adult wasps remained in the emergence chamber for 48hr to ensure mating success. Adult females were then transferred to experimental units using a cartridge aspirator (Klittich et al. 2016).

Host Acceptance Behavior

Host acceptance for each aphid species on their respective host plant (*L. pseudobrassicae* and *M. persicae* on canola and *R. padi* on wheat) was documented for *D. rapae* and *L. testaceipes* females during observations of single attacks in 5mL glass vials. Aphids were either un-attacked (i.e., unparasitized) or previously attacked by a female wasp of the opposite species (heterospecific wasp). The objective was to investigate interspecific interactions and therefore, no conspecific treatments were included. Each treatment combination (Table 1) was designated a single vial to prevent confounding effects of plant/aphid volatile cross-contamination. Vials were topped with a cotton ball and contained host plant material (3cm canola leaf or wheat leaf portion) with a single second or third instar aphid; the aphid was allowed to settle on its host plant for 1hr. A single 48hr-old mated female wasp (prepared as described above) was added to the vial using a cartridge aspirator, and observed for up to 15min. The number of probes (i.e. the number of ovipositor contacts) each female made with her ovipositor on the aphid was recorded. The female was removed from the vial when she walked away from the aphid (approx. 1cm distance). Female wasps not approaching or probing the aphid after 15min were removed from the experiment and excluded from analysis. Following each successful observation period, aphids were isolated on their

respective host plant ('isolation plant'), a winter canola or winter wheat seedling, and subsequent mummies were isolated until adult parasitoids emerged.

Forty-five females of both wasp species were observed on previously un-attacked *R. padi*, *L. pseudobrassicae*, and *M. persicae*. An additional 45 females of each species were observed attacking aphids previously attacked by a heterospecific wasp. These treatments (Table 3.1) were included to determine if *D. rapae* or *L. testaceipes* females can discriminate aphids previously attacked by a heterospecific female, and to document the outcomes of larval competition in multiply parasitized hosts. Other treatments were established identical to the previously un-attacked treatments, but with the aphid attacked by the second wasp species immediately following host acceptance by the initial wasp species and its subsequent removal from the arena. Therefore, *L. testaceipes* females were observed probing both previously un-attacked aphids or *D. rapae*-attacked aphids and *D. rapae* females were observed probing both previously un-attacked aphids or *L. testaceipes*-attacked aphids. These observations were made in the laboratory at 24.4±0.9°C and 43±1.2% RH.

The number of probes and proportion of *D. rapae* or *L. testaceipes* emerging in each treatment were compared using generalized linear mixed models (GLIMMIX) with Kenward-Roger approximations of degrees of freedom. Least-square means were used to make pair-wise comparisons when treatment effects were found to be significant ($\alpha = 0.05$). All analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC).

To determine the proportion of aphids that were multiparasitized in the attacked aphid treatments, a subset of 15 aphids from each treatment were removed from their plant after 4d and dissected to determine numbers of wasp larvae. The number of wasp larvae 4d after parasitism was assumed equal to the number of eggs laid by the female (Bueno et al. 1993). By comparing the number of larvae among single- and two-species treatments, multiparasitism could be inferred. The potentially confounding effects of superparasitism were minimized by restricting the number of encounters each parasitoid was allowed (≤ 15min). We compared the number of probes and

subsequent numbers of wasp larvae among each aphid species and exposure (un-attacked or previously attacked) combination for *D. rapae* and *L. testaceipes* using generalized liner mixed models (GLIMMIX) with Kenward-Roger approximations of degrees of freedom. When treatment effects were significant ($\alpha = 0.05$), least-square means were used to make pair-wise comparisons among treatments. All analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC).

Interspecific Competition Scenarios

Discrimination of previously parasitized hosts is usually the result of female responses to physiological changes occurring within the aphid approximately 24h post-parasitism (Mackauer 1990). Therefore, a second experiment was conducted to examine the outcomes of simultaneous and staggered interspecific interactions. Simultaneous competition scenarios consisted of pairs of heterospecific wasps (*D. rapae* and *L. testaceipes*) foraging together, whereas staggered competition scenarios consisted of two sequential periods of solitary foraging by heterospecific competitors. Treatments in which a single female of each species foraged independently in isolation were included as controls.

Experimental units consisted of individual seven d-old winter wheat or 14 d-old winter canola plants potted in 10cm pots. Pots were topped with a fine sand substrate followed by a white filter paper fitted around the plant base to allow visual inspection of dead aphids and wasps. Potted plants were infested with the respective aphid species (either 10 *R. padi*, *M. persicae*, or *L. pseudobrassicae*), covered with a clear, 10 x 15cm plastic cylinder with a mesh-vented top, and maintained under laboratory conditions (24.4±0.9°C and 43±1.2% RH). After allowing aphids to settle for 1hr, female wasps were introduced according to treatment (Table 3.2). For 'no competition' scenarios, individual *D. rapae* or *L. testaceipes* females were introduced to experimental units and allowed to forage for 24h. For simultaneous competition scenarios, a single *D. rapae* and a *L. testaceipes* female were introduced to the same experimental unit and allowed to forage together for 24h. For 'staggered competition' scenarios, a single *D. rapae* or *L. testaceipes* female was introduced to the experimental unit and allowed to forage for 24h, after which they

were removed and replaced with a heterospecific female, which was also provided a 24h period to attack aphids. All wasps were removed from experimental units using a cartridge aspirator after foraging. This resulted in the following scenarios for each of the three aphid species: 1) *D. rapae* foraging alone (DrAlone), *D. rapae* followed by *L. testaceipes* (DrFirst), *D. rapae* and *L. testaceipes* foraging simultaneously (Dr+Lt), *L. testaceipes* followed by *D. rapae* (LtFirst), and *L. testaceipes* foraging alone (LtAlone) (Table 3.2).

Any experimental unit in which an adult parasitoid could not be located was excluded from the experiment. Following exposure to parasitoids, aphids were reared and, after mummies formed, they were isolated in 5mL glass vials topped with a cotton ball. Upon emergence, adult wasps were identified to species and sexed. Unemerged adults were not identified to species, and survival was therefore not reported separately for each parasitoid species. The percent parasitism, mean number of adult *D. rapae*, mean number of adult *L. testaceipes*, and the proportion of adults surviving (emerging from mummified aphids) resulting from each competition treatment were compared for each aphid species using generalized mixed model ANOVAs (GLIMMIX) with Kenward-Roger approximations of degrees of freedom. Least-square means were used to make pair-wise comparisons when treatment effects were found to be significant ($\alpha = 0.05$). All analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC).

Results

Host Acceptance Behavior

Diaeretiella rapae. The probing behavior of *D. rapae* females was significantly affected by aphid species ($F_{1,263} = 9.33$, $p = 0.0001$) and parasitoid exposure ($F_{1,263} = 4.82$, $p = 0.0290$), but not by the interaction of these two factors ($F_{2,263} = 0.84$, $p = 0.4321$; Table 3). The number of probes made on *R. padi* previously attacked by *L. testaceipes* was significantly lower than on previously un-attacked *R. padi* ($p = 0.0316$) or *L. pseudobrassicae* and *M. persicae* treatments ($p \leq 0.0022$; Fig. 3.2A). More probes were made by *D. rapae* on previously un-attacked *L. pseudobrassicae*

than either attacked or previously un-attacked *R. padi* ($p \leq 0.0258$), but this was not significantly different from either attacked or previously un-attacked *M. persicae* treatments ($p \geq 0.1903$).

The number of larvae found in each aphid species was significantly affected by both aphid species and parasitoid exposure ($F_{2,84} = 6.07$, $p = 0.0053$; Table 3.3). The number of larvae found in *R. padi* was significantly greater when previously attacked by *L. testaceipes* than when attacked only by *D. rapae* ($p < 0.0001$) (Fig. 3.3A). Multiparasitism was also detected in *M. persicae*, the number of larvae being higher than in previously un-attacked *L. pseudobrassicae* and *R. padi* ($p = 0.0203$), but not statistically different from previously un-attacked *M. persicae* ($p = 0.0795$). A similar number of larvae were found in previously previously un-attacked *L. pseudobrassicae* and *L. pseudobrassicae* attacked by *L. testaceipes* ($p = 0.5557$). No instances of superparasitism were detected for *D. rapae* (i.e. more than one larva when only attacked by a single parasitoid).

The proportion of adult *D. rapae* successfully emerging from aphids differed among aphid species and parasitoid exposure treatments ($F_{2,174} = 26.24$, $p < 0.0001$; Table 3.3). The proportion of adult *D. rapae* emerging from *R. padi* previously attacked by *L. testaceipes* was lower than all other treatments ($p < 0.0001$; Fig. 3.4A). No significant differences were detected between *L. testaceipes* attacked and unexposed *L. pseudobrassicae* ($p = 0.6567$) or similarly treated *M. persicae* ($p = 0.6567$; Table A1).

Lysiphlebus testaceipes. The number of probes made by *L. testaceipes* was affected by aphid species ($F_{2,263} = 134.96$, $p < 0.0001$; Table 3.4), but not aphid exposure ($F_{1,263} = 0.00$, $p = 0.9987$) and the interaction between these two factors was not significant ($F_{2,263} = 0.08$, $p = 0.9233$; Table 3.4). Fewer probes were made on *L. pseudobrassicae* and *M. persicae* than *R. padi* ($p < 0.0001$), but probes were similar between all *D. rapae attacked* and unexposed aphids (Fig. 2B).

There was a significant interaction between aphid species and parasitoid exposure in terms of the number of larvae per aphid ($F_{2,84} = 6.74$, $p = 0.0019$; Table 3.4). The number of larvae found in un-attacked *M. persicae* was similar to un-attacked *L. pseudobrassicae* ($p = 0.3587$). The number of larvae found in *M. persicae* previously attacked by *D. rapae* was significantly higher than in

attacked *L. pseudobrassicae* ($p = 0.0022$). Overall, only two incidences of superparasitism were detected, both in *R. padi* attacked only by *L. testaceipes.*

The proportion of adult *L. testaceipes* successfully emerging was affected by aphid species ($F_{2,174} = 289.89$, $p < 0.0001$), but not by parasitoid exposure ($F_{1,174} = 1.74$, $p = 0.1883$) and the interaction term was not significant ($F_{2,174} = 0.76$, $p = 0.4677$; Table 3.4). No *L. testaceipes* emerged from *L. pseudobrassicae* regardless of parasitoid exposure (Fig. 3.4B). *Lysiphlebus testaceipes* emerged from *M. persicae* not previously attacked by *D. rapae*, but this was not observed on *L. pseudobrassicae* hosts. There were no significant differences in the proportion of adult *L. testaceipes* emerging from parasitized or previously un-attacked *R. padi* ($p = 0.5681$; Table A2).

Interspecific Competition Scenarios

Lipaphis pseudobrassicae. The total proportion of aphids parasitized was significantly affected by competition scenarios on *L. pseudobrassicae* aphids ($F_{4,124.3} = 147.52$, $p < 0.0001$; Table 3.5). When *L. testaceipes* foraged alone, there was no parasitism of *L. pseudobrassicae* (Fig. 3.5). Significantly fewer *L. pseudobrassicae* were parasitized in the Dr+Lt and LtFirst scenarios when compared with DrAlone and DrFirst scenarios ($p < 0.0001$). However, no significant differences were detected between the DrAlone and DrFirst competition scenarios ($p = 0.6865$), or LtFirst and Dr+Lt scenarios ($p = 0.0711$; Table A3).

The number of adult *D. rapae* emerging from *L. pseudobrassicae* was also affected by competition scenarios ($F_{3,100.9} = 19.13$, $p < 0.0001$). Fewer *D. rapae* emerged from Dr+Lt and LtFirst scenarios than DrAlone and DrFirst scenarios ($p \leq 0.0001$; Fig. 3.6). More *D. rapae* emerged on average in the LtFirst scenario when compared with Dr+Lt, but these were not significantly different ($p = 0.0714$). No differences in the number of *D. rapae* emerging from DrAlone and DrFirst scenarios were detected ($p = 0.7163$; Table A4).

No differences in *D. rapae* adult sex ratios were observed for any competition scenario ($F_{3,108} = 1.30$, $p = 0.2770$; Fig. 3.7). No *L. testaceipes* emerged from mummified aphids in any

competition scenario (Fig. 3.8 and 3.9). The overall proportion of adults surviving was also not different for any competition scenario ($F_{3,100.6} = 0.01$, $p = 0.9994$; Fig. 3.10).

Myzus persicae. The proportion of aphids parasitized was significantly different among competition scenarios on *M. persicae*-infested plants ($F_{4,124.8} = 100.75$, $p < 0.0001$; Table 3.5). In the LtAlone scenario, less than 1% of *M. persicae* were parasitized, which was lower than all other scenarios ($p < 0.0001$; Fig. 3.5). Percent parasitism was lower in the Dr+Lt scenario than in the DrAlone or DrFirst scenarios ($p \leq 0.0365$), but parasitism levels were similar between the DrFirst and LtFirst scenarios ($p = 0.4634$). Additionally, parasitism levels were highest in the DrAlone scenario ($p \leq 0.0452$).

The number of *D. rapae* emerging was significantly different among competition scenarios ($F_{3,95.38} = 7.20$, $p = 0.0002$). Fewer *D. rapae* emerged from scenarios where interspecific competition occurred compared to DrAlone ($p \leq 0.0049$; Fig. 3.6). Fewer *D. rapae* emerged from Dr+Lt scenarios than any other scenario, but this was not significantly different from staggered competition scenarios ($p \geq 0.1128$). The proportion of female *D. rapae* emerging from *M. persicae* was affected by the competition scenarios ($F_{3,101} = 6.49$, $p = 0.000$; Table 3.5). A lower proportion of emerging *D. rapae* were female in the LtFirst scenarios ($p \leq 0.0010$; Fig. 3.7). No significant differences in *D. rapae* sex ratios were observed between DrAlone, DrFirst, or Dr+Lt scenarios ($p \geq 0.6119$; Table A5).

The number of *L. testaceipes* emerging from *M. persicae* was significantly different across competition scenarios ($F_{3,96.5} = 1.64$, $p = 0.1863$; Table 3.5). Fewer *L. testaceipes* emerged from the DrFirst scenarios than LtAlone ($p = .0436$), but overall only 15 *L. testaceipes* emerged from *M. persicae* across all scenarios (Fig. 3.8; Table A6). Sex ratios of *L. testaceipes* were not affected by competition scenarios ($F_{3,6} = 0.19$, $p = 0.9017$; Fig. 3.9). The overall proportion of adults surviving differed among competition scenarios ($F_{4,95.63} = 4.02$, $p = 0.0047$; Table 3.5). A lower proportion of adults emerged from mummies in the DrFirst competition scenario ($p \leq 0.0481$; Fig. 3.10). No differences in survival were found among the other treatments (≥ 0.3558; Table A8).

Rhopalosiphum padi. In competition scenarios with *R. padi* hosts, the total proportion parasitized differed significantly among competition scenarios ($F_{4,124.3} = 3.36$, $p = 0.0119$; Table 3.5). Percent parasitism was highest in the Dr+Lt scenario, but this was not different from other scenarios with *L. testaceipes* ($p>0.0538$; Fig. 3.5). Parasitism levels were lowest in the DrAlone scenario, but were not significantly different from the DrFirst scenario ($p = 0.1668$).

The number of *D. rapae* successfully emerging from *R. padi* was significantly different across competition scenarios ($F_{3,97.2} = 158.02$, $p < 0.0001$). All scenarios with interspecific competition resulted in fewer *D. rapae* emerging than in the DrAlone scenario ($p < 0.001$; Fig. 3.6). The number of *D. rapae* emerging from scenarios with *L. testaceipes* did not differ ($p \geq 0.0896$). The sex ratios of emerging *D. rapae* were also unaffected by competition scenario ($F_{3,75.35} = 0.95$, $p = 0.4192$; Fig. 3.7).

The number of *L. testaceipes* emerging was significantly affected by competition scenario ($F_{3,108} = 11.49$, $p < 0.0001$), though treatment effects were not as great as for *D. rapae*. Fewer *L. testaceipes* emerged from competition scenarios when compared to LtAlone ($p \leq 0.0005$; Fig. 3.8). DrFirst scenarios had the lowest number of *L. testaceipes* emerging, and this was significantly different from all other scenarios ($p \leq 0.0313$). Overall, the sex ratios were not significantly different among competition scenarios for *L. testaceipes* ($F_{3,101.3} = 1.78$, $p = 0.1554$; Table 3.5). The DrFirst scenario had the lowest proportion of females emerging, but this was not statistically different from other scenarios ($p \geq 0.0526$). The total proportion surviving was affected by competition scenario ($F_{4,115.6} = 3.53$, $p = 0.0094$). Significantly fewer adults emerged from mummies in the DrFirst scenario than from those in other interspecific competition scenarios ($p \leq 0.0112$), but this was not significantly different from DrAlone ($p = 0.0814$; Fig. 3.10).

Discussion

Aphid hosts exist as patchily distributed resources in both space and time (Kindlmann and Dixon 1999). As a result, an aphid parasitoid is unlikely to have a steady supply of preferred hosts, and will often have to utilize less preferred and/or less suitable hosts. It is therefore expected that

multiple species of parasitoids will inevitably compete for a limited number and diversity of shared hosts (Klomp 1964, Brodeur and Rosenheim 2000). The outcomes of these competitive interactions will depend on the timing of parasitism events (Tillman and Powell 1992, De Moraes and Mescher 2005), the competitive abilities of each parasitoid species (McBrien and Mackauer 1990, Cebolla et al. 2017), and the suitability of each aphid host for each parasitoid species (Harvey et al. 2013). In our studies, we found varying effects of these three factors. When competing on cereal aphid hosts, *L. testaceipes* was clearly the superior competitor, as the proportion parasitized was substantially lower for *D. rapae* when *L. testaceipes* competitors were present. This could be due to discrimination of *L. testaceipes*-parasitized hosts, but instances of multiparasitism (more larvae in "exposed" versus "unexposed" aphid hosts) were frequently detected in *R. padi*. Furthermore, no significant differences in the number of *D. rapae* emerging from interspecific competition scenarios was detected, suggesting the timing of oviposition events was not an important factor and discrimination may not occur after 24hr. If host discrimination were a contributing factor, we would expect differences between the staggered and simultaneous competition scenarios.

Regardless of when parasitism occurred, *L. testaceipes* was significantly more likely to emerge from a multiparasitized *R. padi* than was *D. rapae*. Studies of pea aphid (*Acyrthosiphon pisum* Harris) parasitoids reveal that intrinsically superior competitors are capable of disrupting the otherwise effective parasitism of an inferior species (McBrien and Mackauer 1990, Bueno et al. 1993, Danyk and Mackauer 1996). Other studies indicate that the superior competitor is more likely to emerge from multiparasitized hosts regardless of oviposition timing (Chua et al. 1990). The effects of these interactions are likely inconsequential for cereal aphid biological control in the Southern Great Plains, as the contribution made by the inferior competitor, *D. rapae*, is minimal (Elliott et al. 2014). Similar to multi-parasitoid introduction in classical biological control, the superior competitor will likely displace the inferior and provide a greater overall suppression of its host (Ehler 1990, Brodeur and Rosenheim 2000). More consequential for biological control in the

landscape is the potential for interference by *L. testaceipes* with successful parasitism of canola aphids by *D. rapae*.

The apparent competitive advantage of *D. rapae* on canola aphids may reflect a lower acceptance rate of both *L. pseudobrassicae* and *M. persicae* by *L. testaceipes*. The proportion of *L. pseudobrassicae* and *M. persicae* with a *L. testaceipes* larva (0.0 and 0.13, respectively) was roughly proportional to the number of *L. testaceipes* emerging from host acceptance studies (0.0 and 0.10, respectively). Furthermore, the number of *L. testaceipes* emerging from *M. persicae* was similar across all competition scenarios (Fig. 8). This indicates that when *L. testaceipes* does make the decision to oviposit in *M. persicae*, development is likely to be successful. However, when hosts were limited and *D. rapae* attacked *M. persicae* previously parasitized by *L. testaceipes*, only *D. rapae* adults emerged. Thus, when oviposition events are made by each parasitoid species in rapid succession (< 24h), *D. rapae* does in fact have a competitive advantage within canola aphid hosts. This advantage was not observed in competition scenarios, and it is unclear whether *D. rapae* discriminated against *L. testaceipes*-parasitized *M. persicae* in these experiments, as aphids were not dissected to document multiparasitism.

The lack of a clear competitive advantage in these scenarios could result from either the 24hr delay in the oviposition by *D. rapae* or host discrimination due to aphid physiological changes after the 24hr delay in DrFirst and LtFirst competition scenarios. However, the similarity in the number of *L. testaceipes* emerging from these and the Dr+Lt scenarios indicates *D. rapae* could discriminate recently parasitized *M. persicae* and preferentially attacked previously un-attacked hosts. It is also possible that the experimental units allowed some aphids to go un-attacked by parasitoids, as the proportion parasitized among all scenarios were frequently below 80%. Further studies of larval competition and host discrimination between these two parasitoids on shared *M. persicae* hosts might shed light on the proximate mechanisms responsible. Such studies have been crucial for understanding competitive interactions in other groups of parasitoid natural enemies. For example, Tillman and Powell (1992) and DeMoraes et al. (2005) found significantly different

outcomes of host discrimination and intrinsic competition depending on the sequence and timing of parasitoid oviposition.

Interestingly, *L. testaceipes* appeared to disrupt the foraging behavior of *D. rapae*. Significantly fewer *D. rapae* emerged from Dr+Lt and LtFirst scenarios with *L. pseudobrassicae* hosts, but total parasitism and the number of *D. rapae* emerging were similar between DrAlone and DrFirst scenarios, indicating *L. testaceipes* probing of *L. pseudobrassicae* does not disrupt *D. rapae* larval development but instead affects the foraging and/or oviposition behavior of *D. rapae*. The reduced parasitism in LtFirst scenarios suggests that although *L. testaceipes* is unlikely to oviposit in *L. pseudobrassicae*, *D. rapae* makes fewer ovipositions when foraging 24hr after *L. testaceipes*. Independently, this may be explained by recognition of decreased host quality from *L. testaceipes* probing behavior. Bai and Mackauer (1991) found internal cues to be more important for host discrimination than external pheromone markers. Because these internal cues are typically the result of physiological changes within hosts 24-48hr after oviposition (Mackauer 1990), we would not expect to observe these effects when parasitoids attack in rapid succession. When competing simultaneously, host discrimination would similarly be unlikely, and the disruption of *D. rapae* oviposition likely originates extrinsically.

Aggressive behaviors have been observed in aphid parasitoids (Mackauer 1990). During preliminary observations of simultaneous foraging by these two parasitoid species, encounters of adult wasps resulted in both species walking away from the site of the encounter rather than physical attacks. Although not physically damaging, such interactions may still result in less time spent attacking aphids. This may also explain the similar results observed on *M. persicae* hosts, but further examinations of extrinsic competition between *D. rapae* and *L. testaceipes* are needed to identify behaviors and mechanisms responsible for this disruption.

Typically, parasitism rates are minimally affected by parasitoid competition because both competing parasitoids successfully develop on shared hosts (Bueno et al. 1993). Displacement of one species may even benefit biological control when the superior competitor is the superior

parasitoid (i.e. has the greatest numerical response). For example, displacement of *Aphytis lingnanensis* Compere by *A. melinus* DeBach also resulted in a reduction of shared *Aonidiella aurantii* Maskell hosts in areas where biological control had previously been ineffective (Murdoch et al. 1996). In the Southern Great Plains, *L. testaceipes* does not contribute to biological control of *L. pseudobrassicae* or *M. persicae* (Elliott et al. 2014). Therefore, the effects of *L. testaceipes* attacking aphids in winter canola may be detrimental to biological control.

Altogether, these results indicate that *L. testaceipes* may negatively affect *D. rapae* in winter canola. There is anecdotal evidence of *L. testaceipes* attacking *M. persicae* in winter canola fields, but conclusive determination of competition outcomes in the field are needed. Recently developed molecular techniques present novel methods for the quantification of multiparasitism in the field. Gariepy and Messing (2012) were able to document field levels of multiparasitism by assaying mummified aphids for the presences of DNA from multiple parasitoid species. Similar studies have demonstrated the ability to detect both *D. rapae* and its hyperparasitoid's DNA in empty mummy cases (Varennes et al. 2014). Utilizing these methods to quantify the occurrence of multiparasitism in Oklahoma winter crops may reveal proximate mechanisms shaping the parasitoid community in these habitats.

Because *L. testaceipes* readily attacks both *M. persicae* and *L. pseudobrassicae*, but does not often produce offspring, this parasitoid may also be influencing aphid reproductive rates. Kaiser and Heimpel (2016) found the offspring of recently parasitized aphids subsequently produced young at a greater rate than the offspring of previously un-attacked aphids. Shortly after parasitism, the parasitoid's venom results can result in reduced competition among aphid embryos and increased availability of nutrients for developing nymphs. If substances are injected by *L. testaceipes* during ovipositor probing that produce similar effects in *M. persicae* or *L. pseudobrassicae*, the activities of *L. testaceipes* in winter canola may actually increase the fecundity of these aphids.

Seasonal aphid outbreaks in winter canola provide abundant hosts for *D. rapae*, but may not occur at a large enough scale to allow a sufficiently large enough numerical response to significantly reduce aphid populations. Although winter canola acreage in the Southern Great Plains in increasing, winter wheat remains the dominant crop (USDA NASS 2017). Canola habitats therefore exist as relatively small, highly fragmented, and dispersed resources for *D. rapae*. Our results support the findings of Elliott et al. (2014) that suggest *D. rapae* is not utilizing aphids in winter wheat. Although their survey of parasitoid fauna indicated no host or habitat overlap, parasitism of *R. padi* by *D. rapae* is likely to occur, albeit at very low rates. The inability of *D. rapae* to compete with *L. testaceipes* on cereal aphid hosts may limit their population's potential, but cereal habitats may still hold reservoir populations of *D. rapae* which can migrate to winter canola. Populations of *D. rapae* are relatively small and comprise less than one percent of the total parasitoid fauna, compared to *L. testaceipes* which makes up over 55% of aphid parasitoids found in canola, wheat, and uncultivated habitats (data from Jessie 2017). Most of the *L. testaceipes* collected by Jessie (2017) were found in winter wheat habitats, but these populations appear to regularly move into neighboring canola fields, perhaps in search of floral resources. In fact, nearly 30% of all *L. testaceipes* collected were from winter canola fields. The overwhelming abundance of *L. testaceipes*, and its ability to disrupt parasitism of *M. persicae* and *L. pseudobrassicae* by *D. rapae*, may contribute to the low parasitism rates of these aphids.

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Tables and Figures

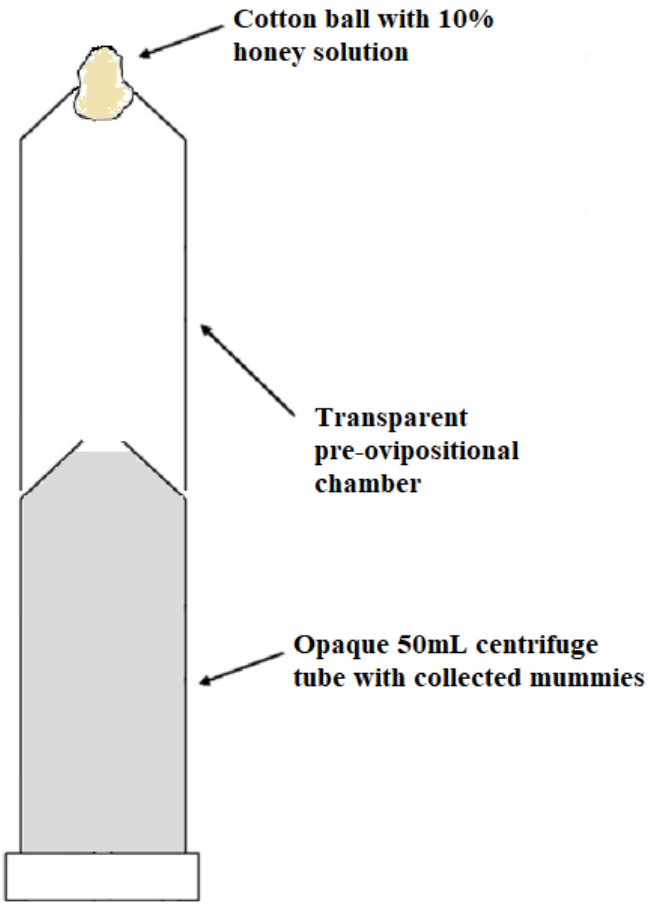

Figure 3.1. Emergence and pre-ovipositional chambers for parasitoid wasps.

Table 3.1. Treatment descriptions for host acceptance experiments replicated on *R. padi, L. pseudobrassicae,* and *M. persicae* hosts.

First Species	Second Species	Replications
D. rapae	-	45
D. rapae	*L. testaceipes*	45
L. testaceipes	*D. rapae*	45
L. testaceipes	-	45

Table 3.2. Treatment descriptions for competition scenarios replicated on *R. padi, L. pseudobrassicae,* and *M. persicae* hosts.

First 24hr	Second 24hr	Treatment	Replications
D. rapae	-	DrAlone	28
D. rapae	*L. testaceipes*	DrFirst	28
Simultaneous	-	Dr+Lt	28
L. testaceipes	*D. rapae*	LtFirst	28
L. testaceipes	-	LtAlone	28

Table 3.3. Results from analysis (PROC GLIMMIX) of aphid species and parasitoid exposure on mean number of probes, larvae, and adult *Diaeretiella rapae* within 5mL vials at 24.4±0.9°C, 43±1.2% RH.

Response variable[a]	**Source of variation**[b]	**df**	**F**	***p***
Number of Probes	Aphid Species	2, 263	9.33	0.0001
	Parasitoid Exposure	1, 263	4.82	0.0290
	Species*Exposure	2, 263	0.84	0.4321
Number of Larvae	Aphid Species	2, 84	5.60	0.0052
	Parasitoid Exposure	1, 84	19.72	<.0001
	Species*Exposure	2, 84	6.07	0.0035
Number of *D. rapae*	Aphid Species	2, 174	29.33	<.0001
	Parasitoid Exposure	1, 174	34.95	<.0001
	Species*Exposure	2, 174	26.42	<.0001

[a]Survival is the proportion of adults successfully emerging from mummies. Dead pupae were not identified.

[b]Aphid species were *Lipaphis pseudobrassicae*, *Myzus persicae*, or *Rhopalosiphum padi*. Parasitoid exposure consisted of aphids atacked by a single parasitoid species, or aphids attacked by two parastitoids.

Table 3.4. Results from analysis (PROC GLIMMIX) of aphid species and parasitoid exposure on mean number of probes, larvae, and adult *Lysiphlebus testaceipes* within 5mL vials at 24.4±0.9°C, 43±1.2% RH.

Response variable[a]	Source of variation[b]	df	F	*p*
Number of Probes	Aphid Species	2, 263	134.96	<.0001
	Parasitoid Exposure	1, 263	0.00	0.9987
	Species*Exposure	2, 263	0.08	0.9233
Number of Larvae	Aphid Species	2, 84	50.28	<.0001
	Parasitoid Exposure	1, 84	123.27	<.0001
	Species*Exposure	2, 84	6.74	0.0019
Number of *L. testaceipes*	Aphid Species	2, 174	289.89	<.0001
	Parasitoid Exposure	1, 174	1.74	0.1883
	Species*Exposure	2, 174	0.76	0.4677

[a]Survival is the proportion of adults successfully emerging from mummies. Dead pupae were not identified.

[b]Aphid species were *Lipaphis pseudobrassicae, Myzus persicae,* or *Rhopalosiphum padi.* Parasitoid exposure consisted of aphids atacked by a single parasitoid species, or aphids attacked by two parastitoids.

Table 3.5. Results from analysis (PROC GLIMMIX) of competition scenario on percent parasitism, adult emergence, and proportion female for each aphid species within laboratory microcosms at 24.4±0.9°C, 43±1.2%

Response variable[a]	Aphid Species[b]	df	F	*p*
Proportion Parasitized	*Lipaphis pseudobrassicae*	4, 128.4	147.52	<0.0001
	Myzus persicae	4, 124.8	100.75	<0.0001
	Rhopalosiphum padi	4, 124.3	3.36	0.0119
Number of *D. rapae*	*Lipaphis pseudobrassicae*	3, 100.9	19.13	<0.0001
	Myzus persicae	3, 95.4	7.20	0.0002
	Rhopalosiphum padi	3, 97.4	158.02	<0.0001
Proportion Female *D. rapae*	*Lipaphis pseudobrassicae*	3, 108.0	1.30	0.2770
	Myzus persicae	3, 101.0	6.49	0.0005
	Rhopalosiphum padi	3, 75.4	0.95	0.4192
Number of *L. testaceipes*	*Lipaphis pseudobrassicae*	-	-	-
	Myzus persicae	3, 96.5	1.64	0.1863
	Rhopalosiphum padi	3, 108.0	11.49	<0.0001
Proportion Female *L. testaceipes*	*Lipaphis pseudobrassicae*	-	-	-
	Myzus persicae	3, 76.6	14.67	<0.0001
	Rhopalosiphum padi	3, 101.3	1.78	0.1554
Survival	*Lipaphis pseudobrassicae*	3, 100.6	0.01	0.9994
	Myzus persicae	4, 95.6	4.02	0.0047
	Rhopalosiphum padi	4, 115.6	3.53	0.0094

[a]Survival is the proportion of adults successfully emerging from mummies. Dead pupae were not identified.

[b]Aphid species were *Lipaphis pseudobrassicae*, *Myzus persicae*, or *Rhopalosiphum padi.* Competition scenarios were DrAlone, DrFirst, Dr+Lt, LtFirst, or LtAlone.

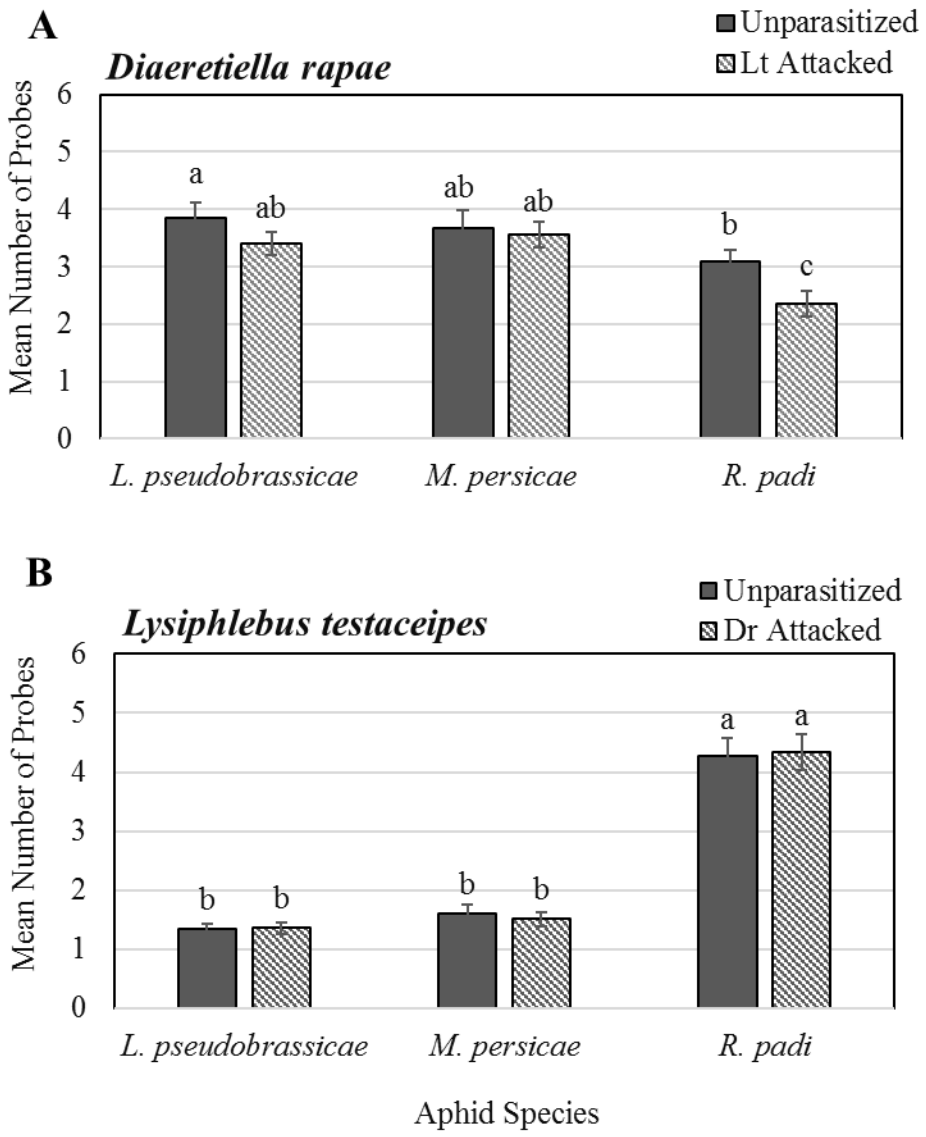

Figure 3.2. Probing behavior of *Diaeretiella rapae* (A) and *Lysiphlebus testaceipes* (B) provided with previously un-attacked aphids and aphids previously attacked by a heterospecific competitor.

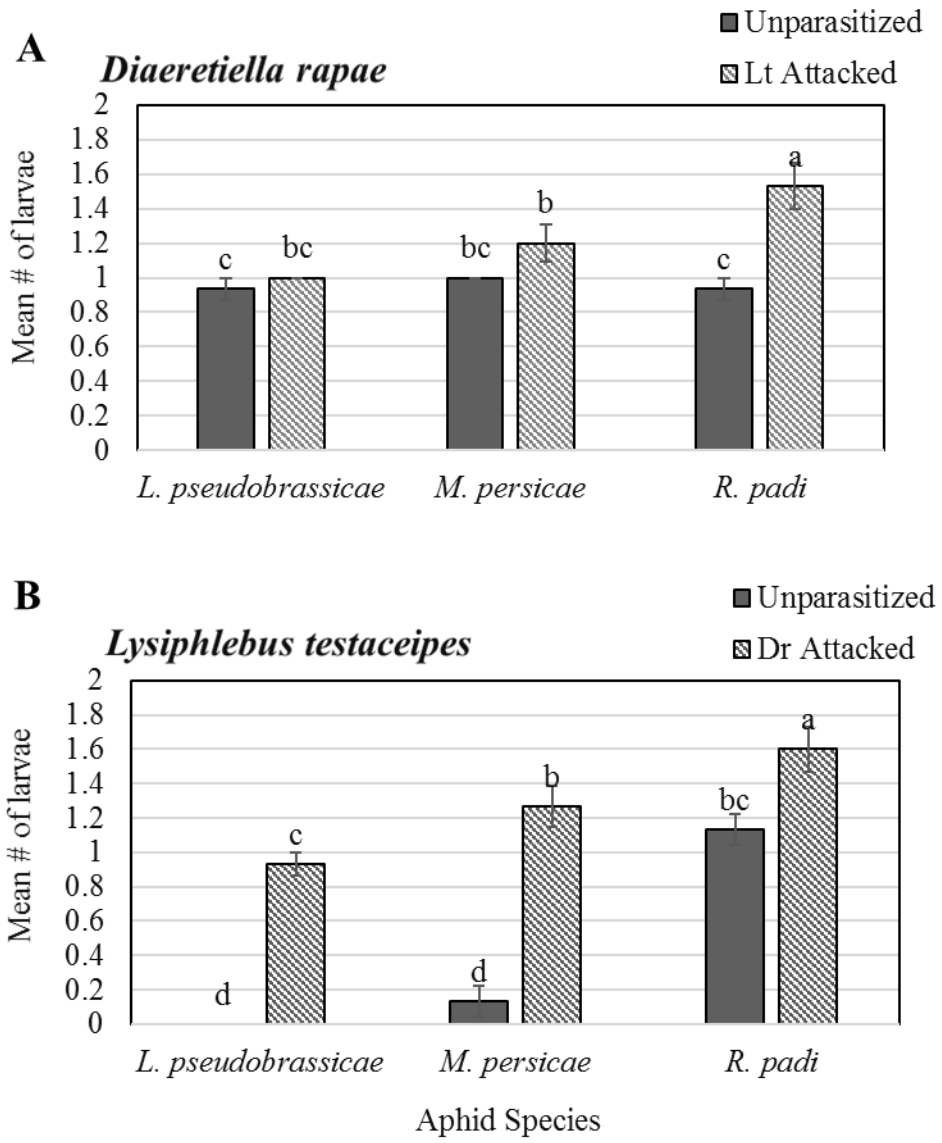

Figure 3.3. Number of larvae found within previously un-attacked aphids and aphids previously attacked by a heterospecific competitor for *Diaeretiella rapae* (A) and *Lysiphlebus testaceipes* (B).

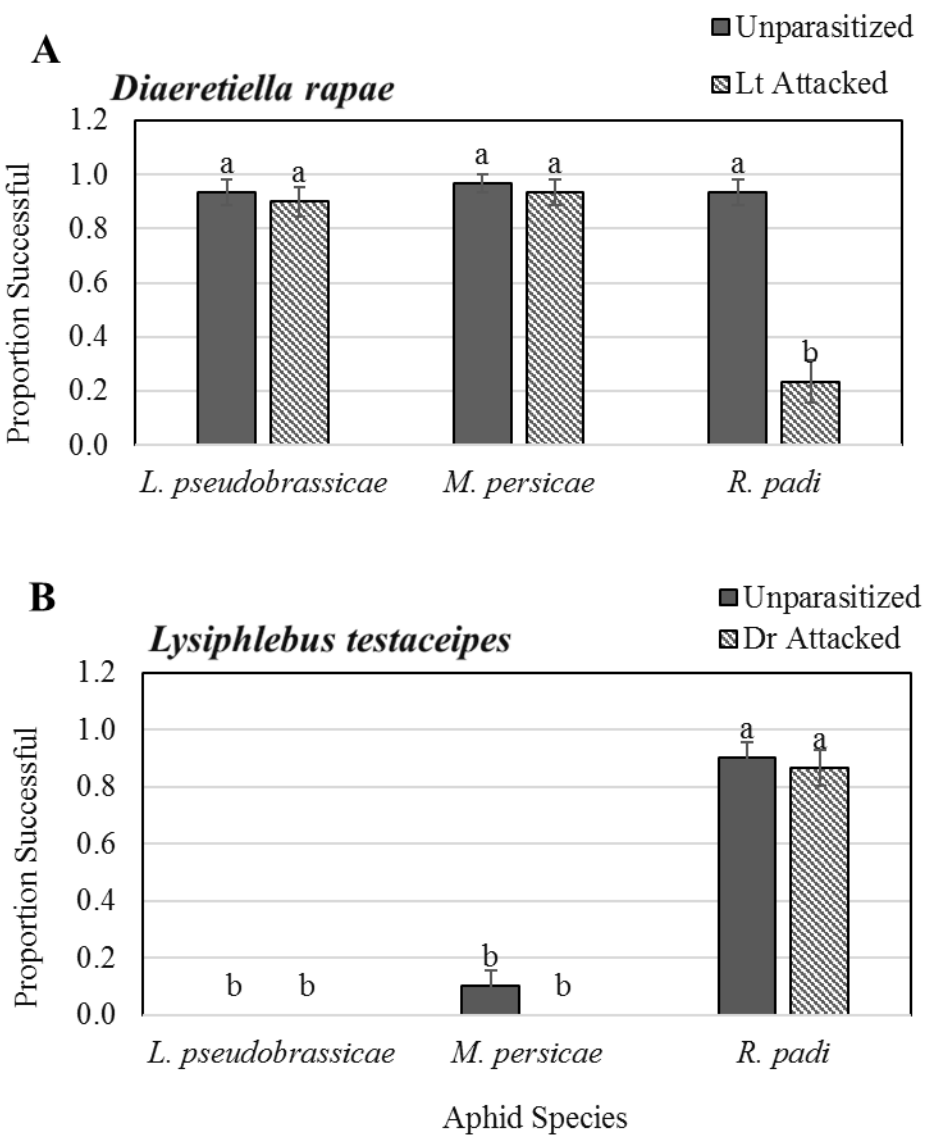

Figure 3.4. Proportion of *Diaeretiella rapae* (A) and *Lysiphlebus testaceipes* (B) emerging from previously un-attacked aphids and aphids previously attacked by a heterospecific competitor.

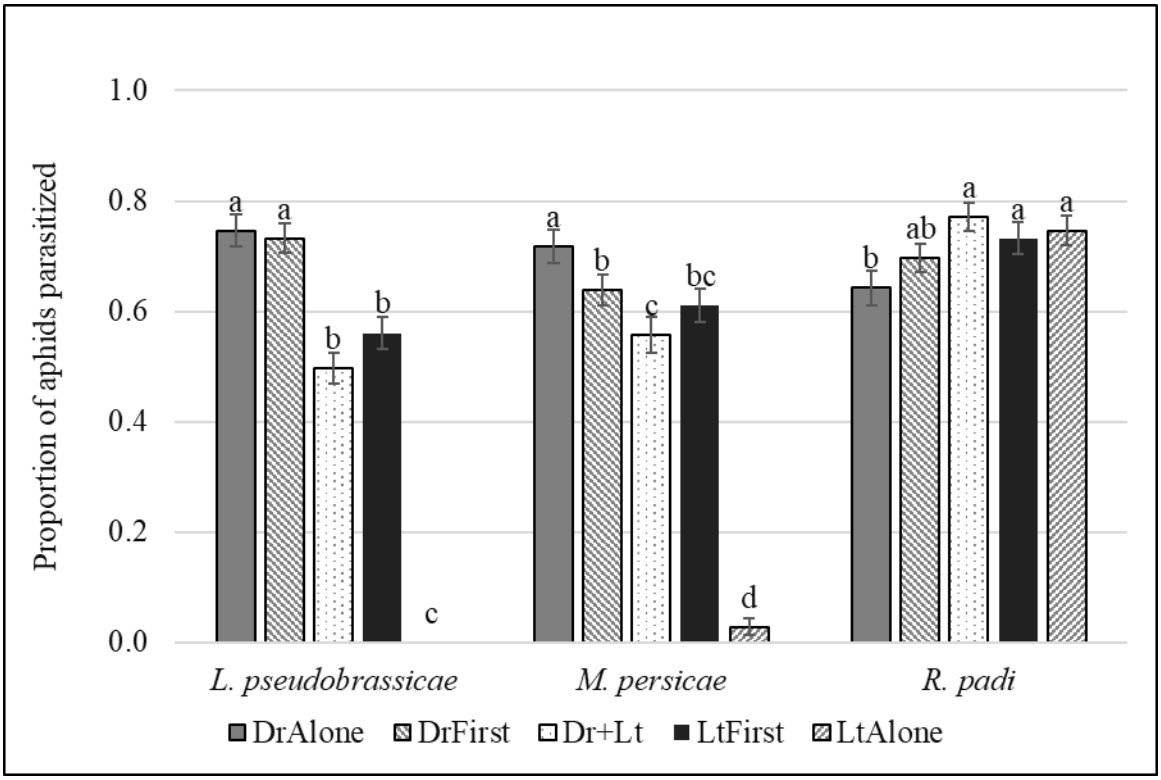

Figure 3.5. Mean proportion of aphids parasitized in each competition scenario on *L. pseudobrassicae*, *M. persicae*, and *R. padi* aphid hosts.

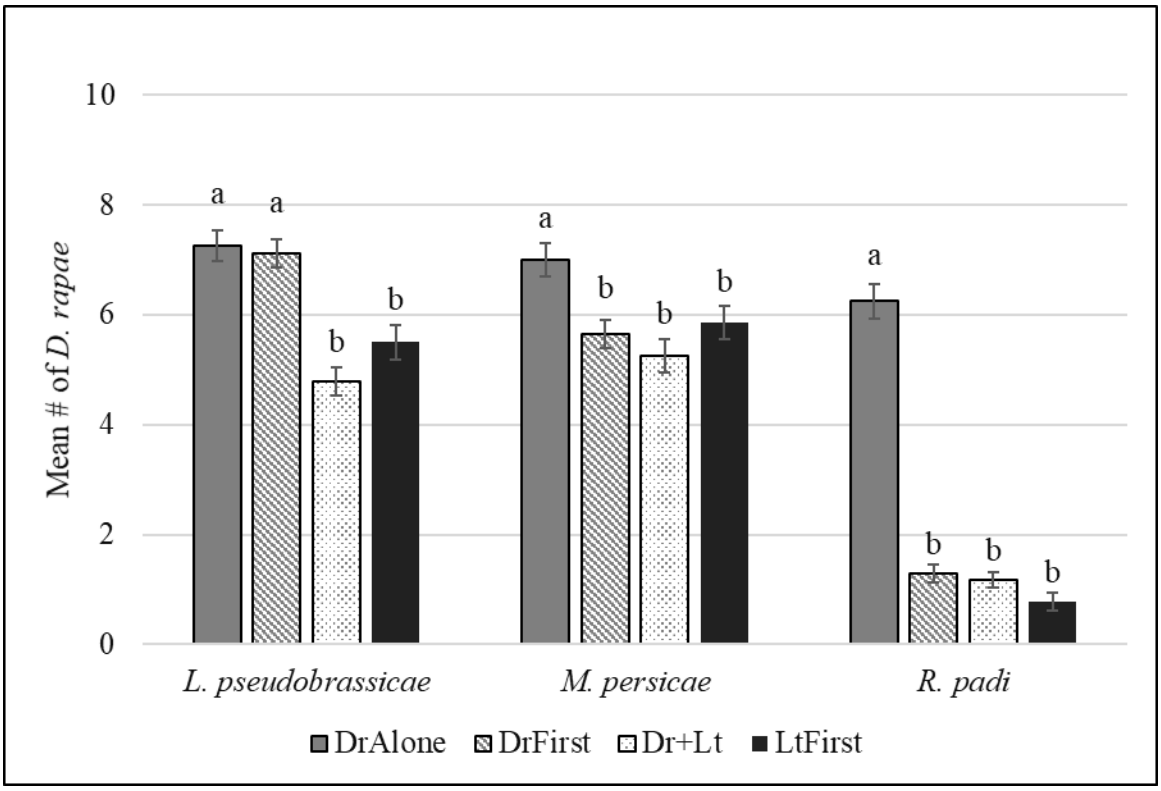

Figure 3.6. Mean number of adult *Diaeretiella rapae* emerging from mummies collected from each competition scenario on *L. pseudobrassicae*, *M. persicae*, and *R. padi* aphid hosts.

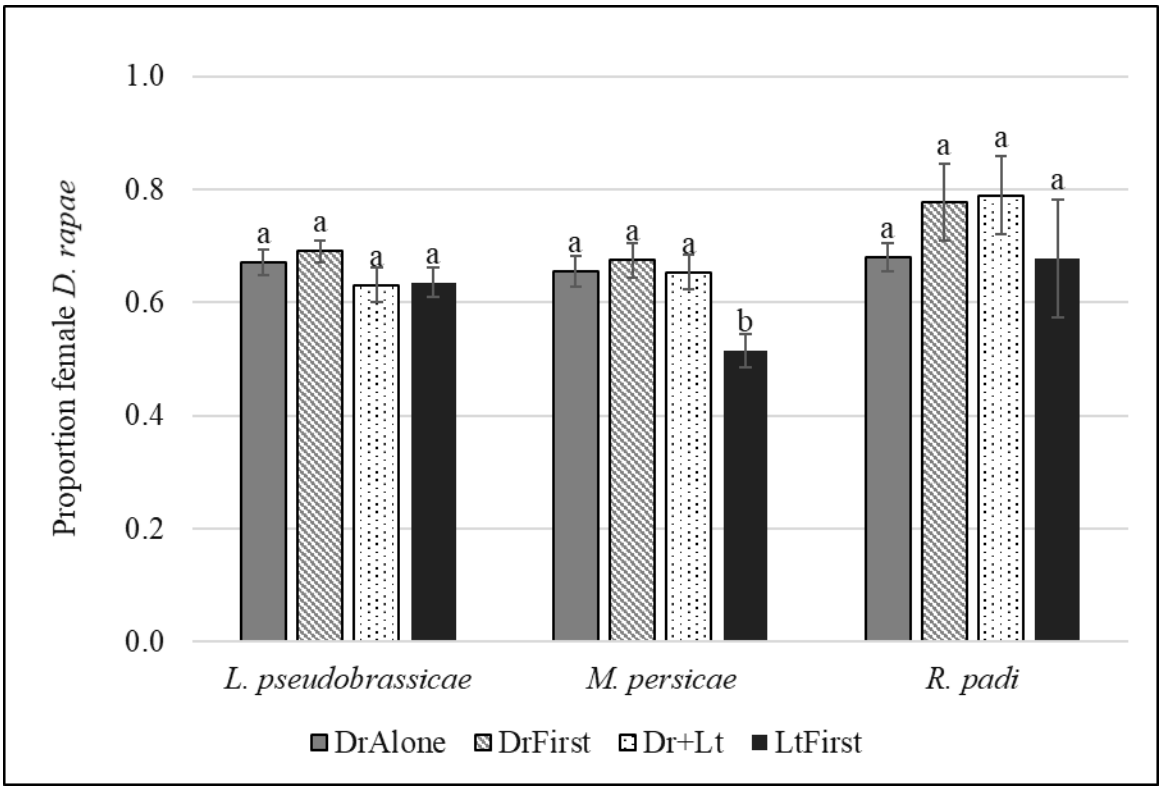

Figure 3.7. Mean proportion of *Diaeretiella rapae* adults that were female within each competition scenario on *L. pseudobrassicae*, *M. persicae*, and *R. padi* aphid hosts.

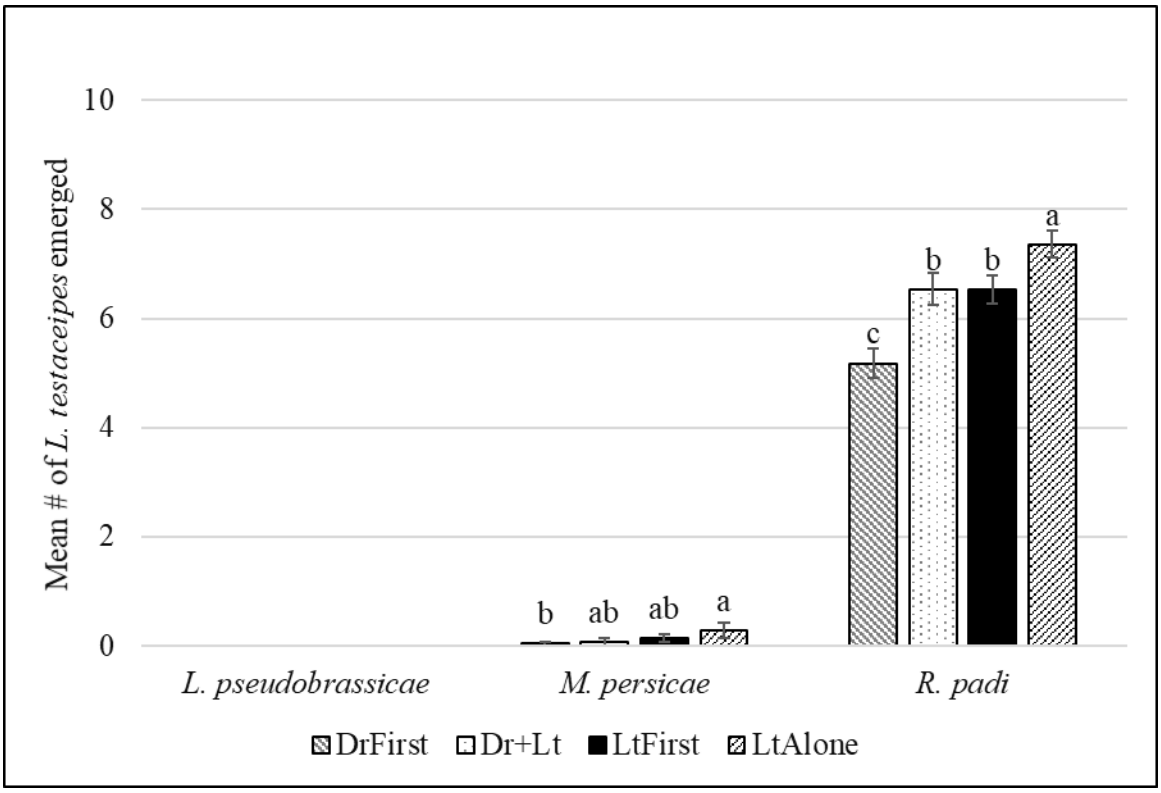

Figure 3.8. Mean number of adult *Lysiphlebus testaceipes* emerging from mummies collected from each competition scenario on *L. pseudobrassicae*, *M. persicae*, and *R. padi* aphid hosts.

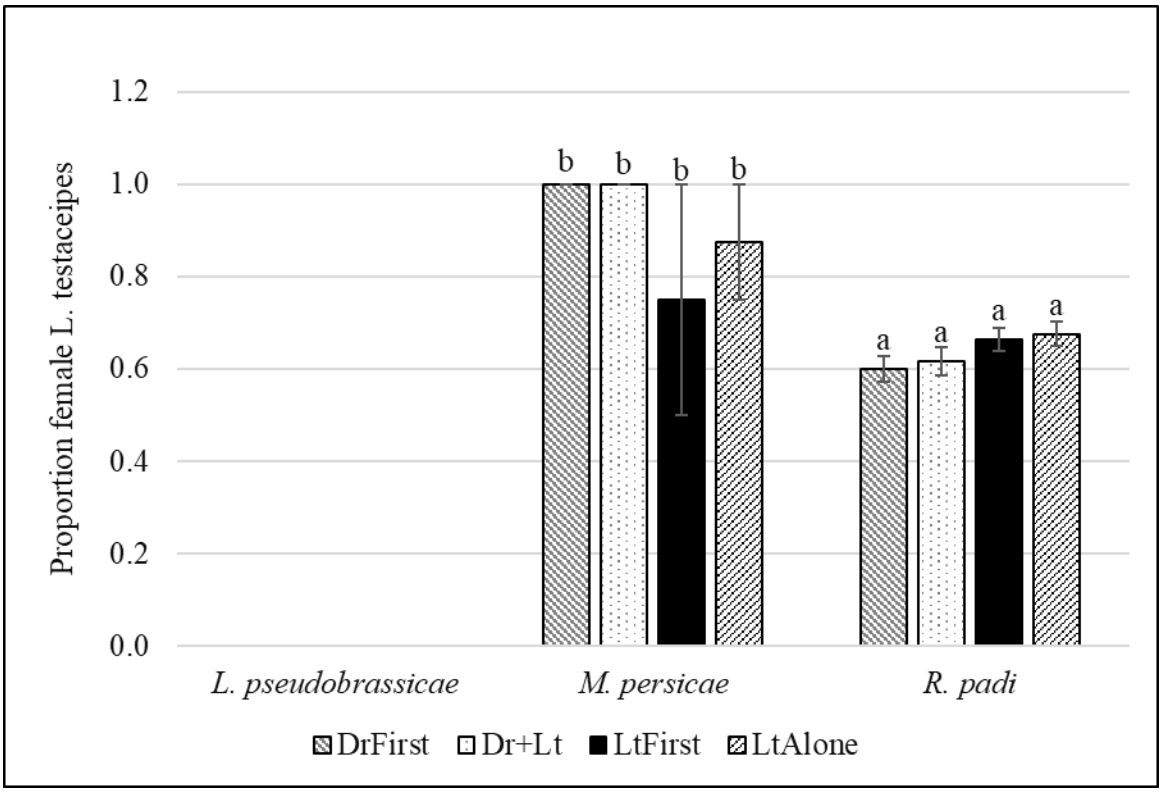

Figure 3.9. Mean proportion of *Lysiphlebus testaceipes* adults that were female within each competition scenario on *L. pseudobrassicae*, *M. persicae*, and *R. padi* aphid hosts.

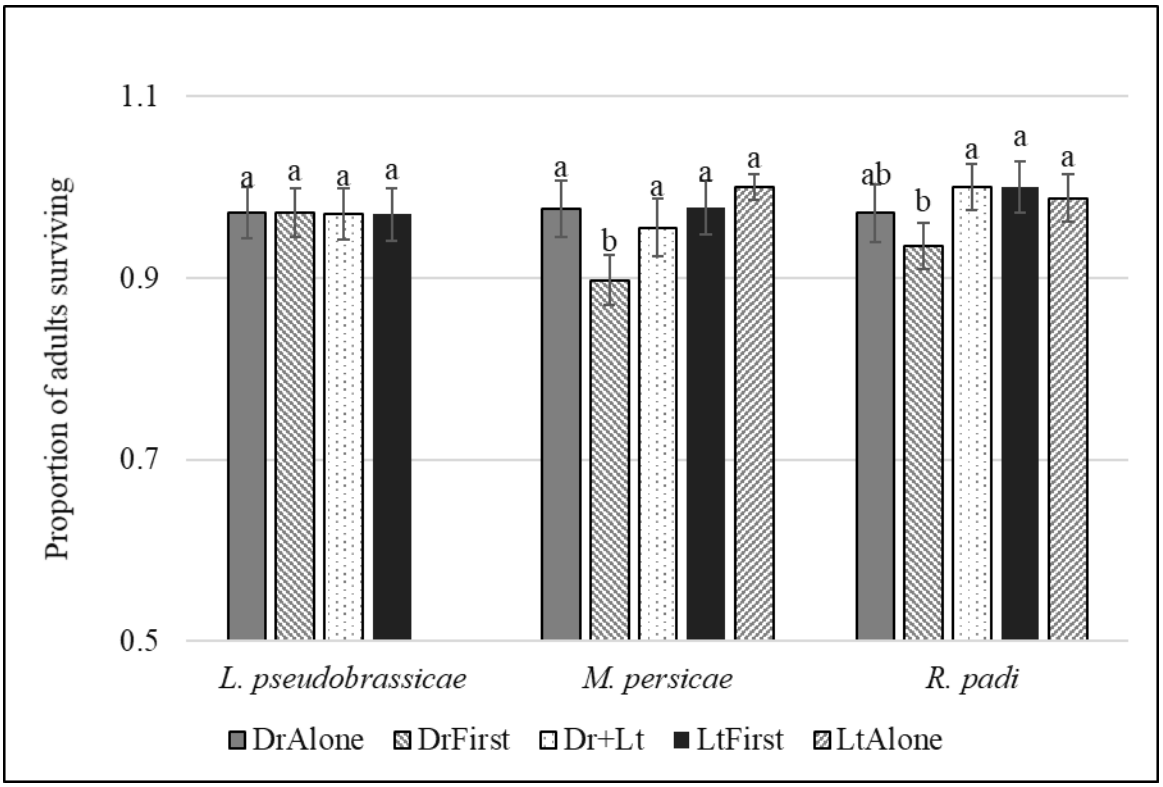

Figure 3.10. Proportion of mummies surviving until adult emergence from each competition scenario on *L. pseudobrassicae*, *M. persicae*, and *R. padi* aphid hosts.

CHAPTER IV

MANUSCRIPT TWO

Effects of flonicamid and sulfoxaflor on *Diaeretiella rapae*

Introduction

Winter canola (*Brassica napus* L.) (Brassicales: Brassicaceae) has experienced frequent and severe aphid outbreaks annually since widespread cultivation in the US Southern Great Plains began in the mid 2000's (Franke et al. 2009, Royer and Giles 2017). Damage has been mitigated by the use of broad-spectrum insecticides as seed and foliar treatments, but reliance on chemical control is rarely sustainable. Resistance to repeatedly applied chemical formulations, often with overlapping modes of action, has resulted in resistance to some insecticides by the most frequent pests of winter canola (Ahmad and Akhtar 2013, Voudris et al. 2017). Continued chemical use is both environmentally and economically expensive, and implementing a reliable integrated pest management (IPM) program for winter canola will involve evaluations of naturally occurring biological control. This has been successful in another commonly grown crop in the Southern Great Plains, winter wheat (*Triticum aestivum* L.) (Poales: Poaceae), where a prominent parasitoid wasp, *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae), is a reliable and consistent natural enemy that has been factored into cereal aphid (Hemiptera: Aphididae) sampling and management plans (Giles et al. 2003, Giles et al. 2017). In winter canola, the primary aphid parasitoid is *Diaeretiella rapae* (McIntosh), which readily parasitizes crucifer-feeding aphids. This species is present in low numbers across the canola growing region, but reliable parasitism by this species

has not been observed (French et al. 2001, Elliott et al. 2014, Jessie 2017).

Although some canola fields support larger *D. rapae* populations than others, the relatively low acreage of canola across the US Southern Great Plains (approximately 65,000 ha) results in a highly fragmented and patchy canola landscape at a scale to which *D. rapae* may be unable to respond (Theis et al. 2005, Brewer et al. 2008). Furthermore, frequent insecticide applications reduce both aphid host and parasitoid populations. By comparison, winter wheat fields treated with insecticides are likely to be colonized rapidly by *L. testaceipes* because reservoir populations exist in high numbers in neighboring untreated wheat fields, summer crops, and/or abundant native grasses (Jessie 2017, N.C. Elliott unpublished data). The primary source habitat of *D. rapae* in the US Southern Great Plains is not fully described. Wild mustard habitats in Oklahoma have not been evaluated for the presence of *D. rapae*, and winter wheat fields harbor extremely low densities of this parasitoid (Jessie 2017). Winter canola is the only source of large cruciferous crop habitat for *D. rapae*; thus, conservation of populations in canola fields is potentially the most effective measure of increasing their efficacy as biological control agents. This may be facilitated by the use of selective insecticides, which have specific activity against a pest and little to no toxicity to beneficial organisms (Ripper et al. 1951). Selective insecticides have been a recent focus of agrochemical companies as they minimize effects on beneficial, non-target organisms such as natural enemies and pollinators. Sulfoxaflor (Transform® WG, Dow AgroSciences LLC, Indianapolis, IN) and flonicamid (Beleaf® WG, FMC Corporation, Philadelphia, PA) are two novel insecticides with narrow-spectrum activity against hemipteran pests that are currently registered for use in winter canola, but specific information on how they affect natural enemies in this crop is lacking.

Sulfoxaflor is classified as a sulfoxamine (IRAC class 4C); although the mode of action is similar to neonicotinoids, the specific mechanisms of its toxicity are distinct (Watson et al. 2011), as evidenced by the lack of cross-resistance (Sparks et al. 2013, Wang et al. 2016). Sulfoxamines act as agonists of post-synaptic acetylcholine receptors, and trigger action potentials along the

neuron causing rapid insect death (Babcock et al. 2011). Sulfoxaflor also shows systemic activity in plants, and may provide an alternative to broad-spectrum seed treatments toxic to natural enemies and pollinators (Stapel et al. 2000, Sabahi et al. 2011, Moscardini et al. 2014 and 2015). Recently, in 2015, registration for this insecticide was revoked amid concerns that toxicity to *Apis mellifera* L. (Hymenoptera: Apidae) was underestimated (EPA Docket # EPA-HQ-OPP-2010-0889). Its registration was re-issued in late 2016 but restricted to usage only after petal-fall in crops such as canola that are attractive to bees. Although not yet thoroughly studied, sulfoxaflor's effects on non-target insects appear to be variable, depending upon the route of exposure and species tested; however, recent studies have confirmed that sulfoxaflor is highly toxic to *A. mellifera* (Zhu et al. 2017). Adult predators appear relatively unaffected by direct contact with sulfoxaflor (Garzón et al. 2015, Tran et al. 2016, Colares et al. 2017), but ingestion of contaminated prey by larvae may result in significant mortality (Robideau 2015, Colares et al. 2017). The effects of sulfoxaflor on parasitoid wasps also appear to be significant, with 94-100% mortality in *Eretmocerus mundus* Howard (Hymenoptera: Aphelinidae), and 100% mortality of *Aphidius ervi* Haliday (Hymenoptera: Braconidae) (Fernández et al. 2015). However, other studies suggest the lethal doses for parasitoids are as much as three times greater than for their hosts (Brar et al. 2017).

Flonicamid belongs to a new group of insecticides, the chordotonal organ modulators (IRAC class 29), with an undefined target site distinct from chordotonal organ modulators in IRAC class 9. Flonicamid does not produce rapid death through neurological activity, but instead inhibits hemipteran feeding by preventing stylet penetration (Morita et al. 2007). Although feeding ceases rapidly, aphids may remain on the host plant for up to 48hr and serve as prey for predators or hosts to parasitoids (Morita et al. 2007). Non-target effects of flonicamid have not been widely studied, but research to date suggests varying degrees of toxicity depending on the life stage exposed and the method of exposure. *Aphidius rhopalosiphi* (DeStefani-Perez) (Hymenoptera: Braconidae) females exposed to flonicamid residues on glass plates experienced a reduction in survival and fecundity; when exposed to residues on field-treated plants, however, no significant effects were

detected (Jansen et al. 2014). *Microplitis mediator* (Haliday) (Hymenoptera: Braconidae) pupae treated with flonicamid had >20% mortality, and although adults treated with flonicamid had good survival, parasitism rate and female longevity were reduced (Moens et al. 2012). When *E. mundus* adults were treated with flonicamid there was an approximately 23% increase in mortality and a 9% decrease in parasitism (Fernández et al. 2015). When treated as mummies, toxicity was slightly lower and resulted in a 5% reduction in adult emergence and parasitism rate. These results are similar to studies on other beneficial insects, which suggest less than 10% mortality in *A. mellifera* (Thomazoni et al. 2009)**,** 5% in *E. mundus* (Moens et al. 2012), and 0% in *Leptomastix dactylopii* (Howard) (Hymenoptera: Encyrtidae) (Cloyd and Dickenson 2006)**.** Though not commonly used in Oklahoma winter crops, sulfoxaflor and flonicamid are potentially compatibility with natural enemies and may be well suited for incorporation into aphid management strategies (Giles et al. 2003, Hallett et al. 2014, Giles et al. 2017).

Although studies of selective insecticides on pupal and adult parasitoids reveal variable and often insignificant effects, most field populations can exist as larvae inside aphid hosts, especially when populations diapause in winter crops (Giles et al. 2003). The effects of pre-imaginal exposure to selective insecticides has largely gone unstudied, primarily because of rapid host death associated with insecticides. Because aphids treated with flonicamid are not subject to immediate death but rather die from starvation, they may still serve as hosts for parasitoids shortly after exposure. *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) treated with flonicamid were found to be suitable for the development of *L. dactylopii* parasitoids even when parasitized 24hr after treatment (Cloyd and Dickenson 2006). However, there is likely a lower threshold for survival of koinobiont parasitoids as these rely on additional nutrients obtained through continued host feeding (Sequeria and Mackauer 1992). If aphids stop feeding, developing parasitoid larvae may not be able to complete development with the limited nutrients available prior to host starvation and death. Larger hosts may therefore continue to serve as hosts if sufficient nutrients are available for complete preimaginal development.

These studies were designed to evaluate the effects of registered selective insecticides on *D. rapae*; first by examining field level effects on parasitism rates and then quantification of lethal and sublethal effects in the laboratory. The objectives for this study were to: 1) determine whether aphid parasitoids will be conserved by the use of selective insecticides (sulfoxaflor and flonicamid) compared to a pyrethroid in winter canola fields; 2) examine stage-specific survival of preimaginal *D. rapae* following exposure to sulfoxaflor and flonicamid in laboratory microcosms; and 3) quantify sublethal effects of these insecticides when applied to aphid hosts containing pre-imaginal parasitoids.

Methods

Field Study - parasitism and insecticide applications

Experiments were initiated in spring 2015 and repeated in spring 2016 in commercial winter canola production fields. Eleven fields were selected across Central and Western Oklahoma in 2015 and 10 in 2016. Winter canola fields in Oklahoma are typically rotated with winter wheat yearly, therefore no field locations were identical between study years. Each field was assigned either a flonicamid, sulfoxaflor, or pyrethroid insecticide treatment, but were otherwise managed (cultivar and production) according to typical agronomic practices used by the landowner. None of the available cultivars expressed significant aphid resistance that might be expected to alter aphid-parasitoid dynamics. Applications of fertilizer and pyrethroid insecticides occurred in late winter 1-2 months prior to the start of experiments.

Because use of sulfoxaflor was restricted to pre-flowering winter canola during both study years, applications of Transform® at the recommended label rate were made in early spring following top-dress applications of fertilizer. A surfactant (Wetcit) was mixed with the insecticide as per product label recommendations. For flonicamid- and pyrethroid-treated fields, growers were contacted as aphid infestations were identified and applications of the designated chemical (at label recommended rates) were made. Method of application was determined by the landowner based on plant stage, weather and soil conditions, and cost of application method. In most cases, flonicamid

and pyrethroid applications were made by aerial spray equipment. In 2015, flonicamid treatments were made using Beleaf®; in 2016, Carbine® was used. Both of these products contain 50% flonicamid by weight and are registered for use in canola.

In the spring, sampling transects (100m) were established 10m from the field edge and parallel with crop rows, and 100 plants were inspected for aphid infestations; samples of canola leaves and racemes were collected every 3-7 days (pre-application). Post-application, aphid samples were collected approximately every two days for two weeks for flonicamid and pyrethroid treated fields. However, due to the systemic activity and early applications of sulfoxaflor, samples were collected from these fields for the rest of the season (up to 42 days after treatment).

All collected plant material was returned to the laboratory and aphids were isolated from samples and placed in groups of up to 100 on individual, 21-day old susceptible winter canola plants topped with 10x15cm mesh-ventilated plastic cylinders. Plants were maintained in the laboratory at 24.4±0.9°C and 43±1.2% RH with 400-watt high-pressure sodium lighting set to 16:8 (L:D). Mummies were isolated as they formed, and apparent parasitism (#mummies / #aphids), effective parasitism (#adult wasps / #aphids), proportional survival (#adults / #mummies), and parasitoid sex ratio were all determined for each field on each sampling day. Fields that remained untreated are included in tables and figures for comparisons but were not included in statistical analyses. The combined means for all samples prior to insecticide application (pre-treatment) were compared to the means of post-treatment samples. This was done because of the variability in number of fields and sample days among insecticide treatments. Means and arcsine-transformed proportions were compared among chemical treatments for pre- and post-application data using generalized mixed models (GLIMMIX) with year and location as random effects and degrees of freedom adjusted using the Kenward-Rodger method. When significant effects were detected at $\alpha = 0.05$, means were separated using least squares means. Statistical analyses were performed with SAS 9.4 software (SAS Institute Inc., Cary, NC).

Laboratory Study - development, survival, and sublethal effects of insecticides

Insect Colonies. Winter canola plants were individually grown in a 1:1 mixture of potting soil and fritted clay absorbent material in either 14cm or 10cm diameter pots (1.8L and 0.5L volume, respectively) and fertilized with a 20:20:20 (N:P:K) water-soluble fertilizer upon planting. Clean plants (uninfested) were kept under both fluorescent lighting (40 watt and 2,000 lumen) and high-pressure sodium lighting (400 watt and 50,000 lumen) to maintain plant vigor. *Lipaphis pseudobrassicae* (Davis) were collected from winter canola fields throughout north-central Oklahoma during the fall of 2014 and placed onto individual winter canola seedlings (cv 'Wichita') in 10cm pots to be screened for the presence of parasitoids. Previously un-attacked *L. pseudobrassicae* were then transferred to 21-day old winter canola plants individually potted in 14cm diameter pots and kept within double-walled mesh cages. Colonies of *L. pseudobrassicae* were reared in the laboratory at 24.4±0.9°C and 43±1.2% RH under fluorescent lighting set at 16:8 (L:D). Fresh canola plants were added to aphid colonies weekly and watered as needed with a dilute 20:20:20 (N:P:K) water-soluble fertilizer.

Separately, parasitized aphids collected in winter canola fields were isolated in the laboratory on seedling winter canola plants; emerging adult wasps were identified as *D. rapae* using morphological keys (van Achterberb 1997) before being released into wasp colony cages. Two colonies of *D. rapae* were established on *L. pseudobrassicae* and maintained at 24.4±0.9°C and 43±1.2% RH. Infested plants from aphid colonies were added to parasitoid colonies bi-weekly and plants within colonies were exchanged weekly to maintain genetic variability. One week prior to the start of laboratory experiments, aphid-infested 10cm pots of winter canola were placed into parasitoid colonies for approximately 12hr. Pots were then removed from the cages, isolated, and cleaned of adult parasitoids to establish mummy cohorts. All mummies forming on these plants were removed and placed into small emergence chambers, which consisted of an opaque 50mL centrifuge tube with the bottom removed, attached to a second, transparent 50mL centrifuge tube containing a cotton ball moistened with a 10% honey solution (Fig. 4.1). Adult wasps remained in

the emergence chamber for 48hr to ensure mating success. Adult females were then transferred to experimental units as needed using a cartridge aspirator (Klittich et al. 2016).

Evaluation of parasitoid development, survival, and reproduction. Experimental units each consisted of a 21-day-old winter canola plant planted in a 10cm pot. Pots contained a 1:1 mixture of potting soil and fritted clay absorbent, followed by fine sand substrate. A white filter paper was then fitted around the plant base to allow for visual inspection of dead aphids and parasitoids. Plants were covered with a clear, 10x15cm plastic cylinder with a mesh-vented top and randomly assigned to a chemical treatment group (flonicamid, sulfoxaflor, or distilled water) and a treatment day (D0, D2, D4, or D8). By applying each chemical across these daily time-frames, the developmental stage at which *D. rapae* are likely to survive a chemical treatment was investigated. Parasitized aphids sprayed at D0 are treated at the parasitoid egg stage, at D2 the first parasitoid stadium, at D4 the third or fourth parasitoid stadium, and by D8 most parasitized aphids were in the early mummy stage (Spencer 1926, WPJ personal observation).

Approximately 100 apterous *L. pseudobrassicae* were transferred from colony plants to experimental units using a fine, camel-hair brush. This aphid density was targeted, but handling mortality and rapid reproduction by adults resulted in slight variation in aphid density on D0 for most experimental units. After 24h, all aphids were counted prior to the introduction of two, mated *D. rapae* females (see above). Wasps were allowed to attack aphids within experimental units for a 24h period before being removed via aspirator. Experimental units within which two living adult *D. rapae* were not found were considered to have incomplete parasitism and were not included in analyses. Plants were then treated on their respective treatment day with their designated chemical.

Spray applications were made within closed fine-mesh cages dedicated to each chemical to prevent cross-contamination of treatments. Insecticide quantities were determined based on the recommended field rates scaled to treat one plant with an 81cm^2 ground surface area (81µg flonicamid [159µg Beleaf®] or 21µg sulfoxaflor [43µg Transform®]). Insecticides were used as a water-dispersible granular formulation, and were mixed with 1mL of distilled water in a medical-

grade atomizer before application to all foliar surfaces (Robideau 2015). An additional 1mL of distilled water was added to the atomizer and applied to ensure complete application of the chemical. After application, experimental units were removed from spray cages and maintained on laboratory benches until mummy development. Mummies were removed from plants on day 10 and isolated into 1.7mL microcentrifuge tubes topped with a cotton ball. For each experimental unit, the apparent parasitism (#mummies / #initial aphids), parasitoid survival (#adults / #mummies), and the proportion of female adults were calculated. Upon adult emergence, wasps were sexed and up to three F1 females from each experimental unit were selected for tests of sublethal insecticide effects.

Sublethal effects of each chemical were examined by releasing a single, unmated 24hr-old female collected from insecticide trials into the center a 5cm petri dish arena containing 20 early instar *L. pseudobrassicae* on a single winter canola leaf. Each *D. rapae* female was observed until the first attack was made, then allowed to forage for 24hr before being removed from the arena. To reduce handling mortality of aphids, infested leaves from each replicate were transferred to a winter canola seedling in a 10cm pot. The time elapsed before the first attack (attack latency), number of probes during the first attack, apparent parasitism, and proportion survival were recorded for each female. To test the effects of insecticide treatments on the ability of *D. rapae* to produce female offspring, an additional 20 females were allowed to mate for 24h prior to their release into petri dish arenas. For both direct and sublethal effect experiments, comparisons of each metric were made among insecticides for each day of treatment using linear mixed-model ANOVA (GLIMMIX) and pair-wise comparisons were made using least-squares means at a significance level of 0.05. Degrees of freedom were estimated with the Kenward-Rodger method. Statistical analyses were performed with SAS software, Version 9.4 (SAS Institute Inc., Cary, NC).

Results

Field Study - parasitism and insecticide applications

No significant differences were found among fields prior to insecticide applications (pre-treatment) for aphid abundance ($F_{2,57} = 0.35$, $p = 0.7088$), apparent parasitism ($F_{2,57} = 0.86$, $p = 0.4278$), *D. rapae* survival ($F_{2,47} = 0.50$, $p = 0.6091$), effective parasitism ($F_{2,57} = 0.98$, $p = 0.3833$), or the proportion of female *D. rapae* emerging from parasitized aphids ($F_{2,47} = 0.48$, $p = 0.6246$; Table 4.1). Because sulfoxaflor was used earlier in the season than other insecticides, mean aphid abundance before treatments was lowest in those fields, but was not significantly different from other treatments ($p \geq 0.6346$).

Significant differences in aphid abundance were detected among treatments post-application ($F_{2,83} = 7.02$, $p = 0.0015$). Flonicamid-treated fields contained more aphids post-treatment than those treated with sulfoxaflor ($p = 0.0004$) or pyrethroids ($p = 0.0404$). However, aphid abundance was similar between sulfoxaflor and pyrethroid treatments ($p = 0.4538$; Fig. 4.2). Levels of apparent parasitism post-treatment also differed among insecticide treatments ($F_{2,77} = 14.30$, $p < 0.0001$), but was not significantly different between pyrethroid and sulfoxaflor treatments ($p = 0.4404$; Fig. 4.3). Flonicamid-treated fields contained more mummified aphids than those treated with either sulfoxaflor or pyrethroid ($p \leq 0.0013$). Furthermore, this treatment resulted in significantly higher survival than either sulfoxaflor ($p \leq 0.0059$) or pyrethroid treatments ($p = 0.0279$; Fig. 4.4). Overall, the effective proportion parasitized was different among post-treatment fields ($F_{2,77} = 17.48$, $p < 0.0001$). A significantly greater proportion of adult parasitoids emerged from mummified aphids collected from flonicamid-treated fields ($p < 0.0001$; Fig 4.5). The proportion surviving was not significantly different between pyrethroid and sulfoxaflor treatments ($p = 0.6077$). No significant differences in the proportion of female *D. rapae* were detected among insecticide treatments ($F_{2,19} = 0.53$, $p = 0.5947$; Fig. 4.6). Mean (±SE) aphid abundance, parasitism levels, survival, and proportion female are reported in appendix Table A9.

Laboratory Study - development, survival, and sublethal effects

No aphid mummies formed in D0 and D2 flonicamid or sulfoxaflor treatments; therefore, only 12 replication were conducted for these treatment days, and only D4 and D8 treatment days with 30 replicates were included in analyses. Mean (±SE) parasitism, survival, and female proportions are reported in Appendix Table A10. Apparent parasitism was significantly affected by insecticide treatments on D4 ($F_{2,87.0} = 212.75$, $p < 0.0001$) and D8 ($F_{2,87.0} = 44.02$, $p < 0.0001$; Table 4.2). Apparent parasitism was lowest for the sulfoxaflor D4 treatment ($p < 0.0001$) followed by sulfoxaflor D8 which was lower than other D8 treatments ($p \leq 0.0052$; Fig. 4.7). Experimental units treated with water had significantly higher parasitism than other treatments regardless of treatment day ($p < 0.0001$). Flonicamid treatments had lower levels of parasitism when compared to the control ($p < 0.0001$), but were significantly higher than sulfoxaflor treatments ($p \leq 0.0052$). Survival was also affected by insecticide treatments for both D4 ($F_{2,76.0} = 67.16$, $p < 0.0001$) and D8 treatments ($F_{2,87.0} = 102.37$, $p < 0.0001$). Fewer adults emerged from mummified aphids in the sulfoxaflor treatment on both D4 and D8 ($p < 0.0001$; Fig 4.8). Flonicamid treatments resulted in approximately 60 and 80% survival on D4 and D8, respectively, values significantly lower than controls ($p < 0.0001$). No differences in sex ratios were detected among D4 ($F_{2,70.0} = 0.03$, $p = 0.7701$) or D8 treatments ($F_{2,87.0} = 1.34$, $p = 0.2675$; Fig. 4.9).

The F1 generation of parasitoids collected from experimental units exhibited significant differences in attack latency for both D4 ($F_{2,125.1} = 8.84$, $p = 0.0003$) and D8 treatments ($F_{2,126.4} = 7.66$, $p = 0.0165$; Table 4.3). When exposed to sulfoxaflor on D4 or D8, emerging females took significantly longer to make the first attack when compared with flonicamid or water ($p \leq 0.0038$; Fig. 4.10). Attack latency was similar between flonicamid and water at D4 ($p = 0.0996$) and D8 ($p = 0.5031$). Insecticide treatment also influenced the number of attacks made on the first encounter regardless of treatment day ($F_{2,\ 126.4} = 7.66$, $p < 0.0007$ for D4; $F_{2,\ 157.8} = 9.05$, $p < 0.0002$ for D8). Female wasps emerging from D4 or D8 sulfoxaflor treatments made fewer probes on their first encounter when compared with water ($p \leq 0.0003$; Fig. 4.11). The number of attacks was similar

between D4 treatments of flonicamid and sulfoxaflor ($p = 0.0653$), but were different between D8 treatments ($p = 0.0020$). Similarly, the number of probes on the first attack was significantly different between flonicamid and water at D4 ($p = 0.0096$) but were similar when treated at D8 ($p = 0.2896$).

Apparent parasitism by F1 females was also affected by the insecticide type ($F_{2,127.6} = 126.00$, $p < 0.0001$ for D4; $F_{2,155.6} = 81.80$, $p < 0.0001$ for D8; Table A11). The proportion of aphids mummified was significantly different between flonicamid and sulfoxaflor treatments at D4 ($p < 0.0001$) and D8 ($p \leq 0.0484$), and these were significantly lower than the controls ($p \leq 0.0484$; Fig. 4.12). Treatment chemical was also found to influence survival of F1 progeny regardless of treatment day ($F_{2,127.0} = 64.01$, $p < 0.0001$ for D4; $F_{2,157.2} = 12.00$, $p < 0.0001$ for D8). Interestingly, the survival of offspring produced by flonicamid-treated females was not different from those treated with water on D4 ($p = 0.2057$) or D8 ($p = 0.4960$), but progeny of females treated with sulfoxaflor at either D4 or D8 had lower proportion surviving compared to all other treatments ($p \leq 0.0001$; Fig. 4.13). Similarly, the proportion of females emerging from mated F1 female experiments was significantly lower for sulfoxaflor-treated females ($p \leq 0.0083$) when compared with control or flonicamid-treated females, regardless of treatment day (Fig. 4.14).

Discussion

Sulfoxaflor and flonicamid represent two new selective chemistries capable of reducing hemipteran pest populations with minimal non-target effects, whereas pyrethroid treatments cause non-specific arthropod mortality. During this study, treatment of fields with pyrethroid insecticides reduced aphid populations but also reduced the proportion parasitized to near zero and mummified aphids collected from these fields had adult survival of less than 50%. Similarly, sulfoxaflor-treated fields maintained very low aphid numbers, apparent parasitism, and parasitoid survival. On the other hand, fields treated with flonicamid had relatively high levels of parasitism and high parasitoid survival post-treatment. The contrast between *D. rapae* survival in flonicamid-treatment

fields versus those treated with sulfoxaflor indicates that this novel insecticide is compatible with conservation biological control in winter canola.

Results from laboratory studies paralleled those in the field. When immature *D. rapae* were exposed to sulfoxaflor shortly after oviposition or as early instars within aphids (D0 or D2 treatment), no wasps successfully completed development. When treated as later instar larvae (D4 treatment), some immature parasitoids were able to survive to adulthood, but exhibited significant reproductive impairment. These results indicate that treated aphids are not viable hosts, and oviposition must occur at least 96hr before treatment in order for wasps to survive. Even then, adult wasps exposed to sulfoxaflor as late-instar larvae (D4) took significantly longer to attack hosts and parasitized fewer of them compared to those exposed to flonicamid or water controls. When attacking aphids, these females appeared sluggish and would attempt to probe from distances too great to reach the host with their ovipositor. Frequently, sulfoxaflor-treated females would hesitate much longer than flonicamid- or water-treated females, often stopping mid-probe for several seconds. The proportion of these females' offspring surviving to adulthood was < 50% and the proportion female was < 30%.

When treated with sulfoxaflor as pupae (D8 treatment), the effects were less severe, with approximately 80% survival and 50% female offspring. These results contrast with those reported on an aphelinid parasitoid by Fernández et al (2015). They reported an almost 95% reduction in adult *E. mundus* emergence and 0% parasitism following application of sulfoxaflor to mummies. However, our assays used a recommended field rate (21µg sulfoxaflor/mL) three times lower than the 60µg/mL used by Fernández et al. (2015). Another study performed on *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) and its parasitoid *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) exposed to sulfoxaflor found the parasitoid's LC_{50} (27.12µg/mL) was three times greater than that of its host (Brar et al. 2017). This concentration is similar to those used in our study (21µg/mL) that followed recommended field rates. Using a sulfoxaflor concentration three times greater than the recommended field rate is likely to overestimate non-target effects.

Parasitized aphids sprayed with flonicamid at D0 and D2 did not form mummies, in contrast with other studies which found that flonicamid-treated hosts were suitable for parasitoid development. Cloyd and Dickenson (2006) found *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) treated with flonicamid at up to four times the recommended field rate were still suitable as hosts for ovipositing *L. dactylopii* up to 24hr after exposure. Likely, the nutritional ecology of mealybugs and their encyrtid parasitoids do not reflect those of aphid-parasitoid systems.

Flonicamid treatments at D4 and D8 resulted in parasitism and survival of *D. rapae* greater than sulfoxaflor, but still lower than water controls. Sublethal effects of flonicamid were observed in both D4 and D8 treatments, but were not as significant as sulfoxaflor treatments. No differences in the number of offspring surviving or sex ratios were detected for either treatment day, and only slightly fewer aphids were parasitized by flonicamid-treated females when compared to controls. These results agree with previous studies on other natural enemies that flonicamid has minimal impact on development and survival of non-target insects. When *M. mediator* were treated with flonicamid as pupae, survival was significantly lower than in water-treated controls (Moens et al. 2012). When treated as adults, attack rate and female longevity were significantly lower, but mortality and proportion parasitized were not different from controls. Fernández et al. (2015) determined flonicamid was harmless to *E. mundus* mummies, increasing mortality < 20% and adult emergence and parasitism by 5%.

When compared with less selective insecticides (i.e., broad-spectrum pyrethroids), both sulfoxaflor and flonicamid present fewer lethal and sublethal effects to natural enemies (Garzon et al. 2015, Brar et al. 2017, Colares et al. 2017). Although lifetime fecundity of parasitoids exposed to these compounds may be significantly lower through reduced parasitism rates and longevity, parasitoids may still persist in the environment (Cloyd and Dickenson 2006, Varenhorst and O'neal 2012, Fernández et al. 2015). The incorporation of flonicamid into pest management strategies may

result in a reduced frequency of aphid outbreaks by conserving their most important natural enemies.

Selective insecticides with unique modes of action that suppress insect pests while conserving beneficial organisms have become a recent focus for agrochemical companies. Documenting the environmental impacts of selective insecticides in agricultural landscapes is a first step towards integrating them into sustainable pest management programs. Winter canola may benefit from such compounds, as aphids have been a limiting factor in economical production of this crop (Franke et al. 2009, Royer and Giles 2017). Predaceous natural enemies commonly occur in Oklahoma winter canola crops (Jessie 2017), and may provide significant contributions to biological control of cruciferous aphids despite the chemical defenses of two common aphid species (Jessie et al. 2015). *Diaeretiella rapae* is the only known parasitoid of *B. brassicae* (Pike et al. 1999), and is a major contributor of aphid suppression in cruciferous crops (Mackauer and Kambhampati 1984, Neuville et al. 2016). It remains unclear if incorporation of flonicamid or sulfoxaflor into canola pest management programs could have beneficial long-term effects on parasitism levels, but use of such compounds is likely to benefit overall IPM goals.

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Tables and Figures

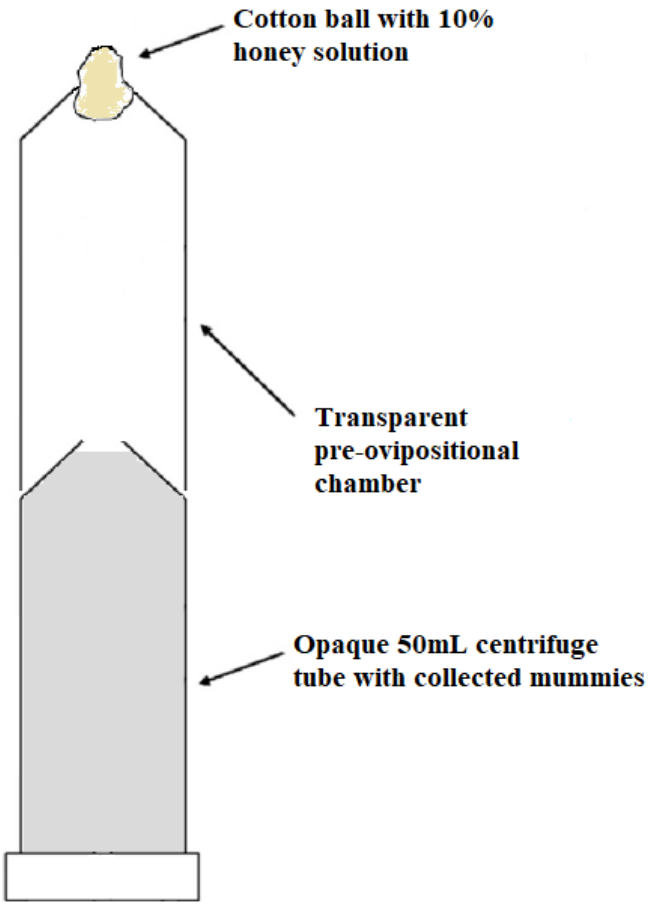

Figure 4.1. Emergence and pre-ovipositional chambers for parasitoid wasps.

Table 4.1. Generalized mixed model analysis results from before and after insecticidal treatment in winter canola fields.

Response Variable	Field condition	df	F	*p*
Aphid abundance	Pre-treatment	2, 57	0.35	0.7088
	Post-treatment	2, 83	7.02	0.0015
Proportion parasitized (Apparent)	Pre-treatment	2, 57	0.86	0.4278
	Post-treatment	2, 77	14.30	<.0001
Survival	Pre-treatment	2, 47	0.50	0.6091
	Post-treatment	2, 23	6.54	0.0056
Proportion parasitized (Effective)	Pre-treatment	2, 57	0.98	0.3833
	Post-treatment	2, 77	17.48	<.0001
Proportion female	Pre-treatment	2, 47	0.48	0.6246
	Post-treatment	2, 19	0.53	0.5947

Table 4.2. Generalized mixed model analysis results of insecticide treatment effects on *Diaeretiella rapae* at 4 or 8 days after oviposition.

Preimaginal Survival[a]	**Response variable**	**df**	**F**	***p***
Day 4 treatment	Proportion parasitized	2, 87.0	204.68	<.0001
	Survival	2, 77.0	67.42	<.0001
	Proportion female	2, 71.0	0.30	0.7424
Day 8 treatment	Proportion parasitized	2, 87.0	44.02	<.0001
	Survival	2, 87.0	102.37	<.0001
	Proportion female	2, 87.0	1.34	0.2675

[a]Experimental units in the Day 0 and Day 2 treatments did not result in mummy formation and were not included in statistical analyses.

Table 4.3. Generalized mixed model analysis results of insecticide treatment effects on reproductive abilities of F1 *Diaeretiella rapae* at 4 or 8 days after oviposition.

Sublethal Effects	Response variable	df	F	*p*
Day 4 treatment	Attack latency	2, 125.1	8.84	0.0003
	Number of attacks	2, 126.4	7.66	0.0007
	Proportion parasitized	2, 127.6	126.00	<.0001
	Survival	2, 127.0	64.01	<.0001
	Proportion female	2, 35.0	9.36	0.0006
Day 8 treatment	Attack latency	2, 167.0	4.20	0.0165
	Number of attacks	2, 157.8	9.05	0.0002
	Proportion parasitized	2, 155.6	81.80	<.0001
	Survival	2, 157.2	12.00	<.0001
	Proportion female	2, 38.0	8.09	0.0012

Experimental units in the Day 0 and Day 2 treatments did not result in mummy formation and were not included in statistical analyses.

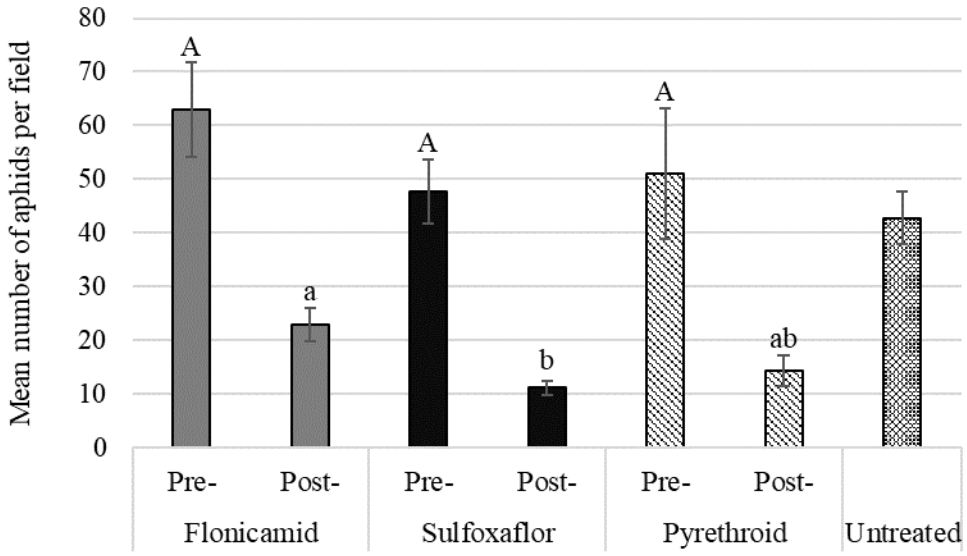

Figure 4.2. Mean (±SE) aphid abundance for all pre- and post-treatment samples. Fields that were untreated were not included in statistical comparisons.

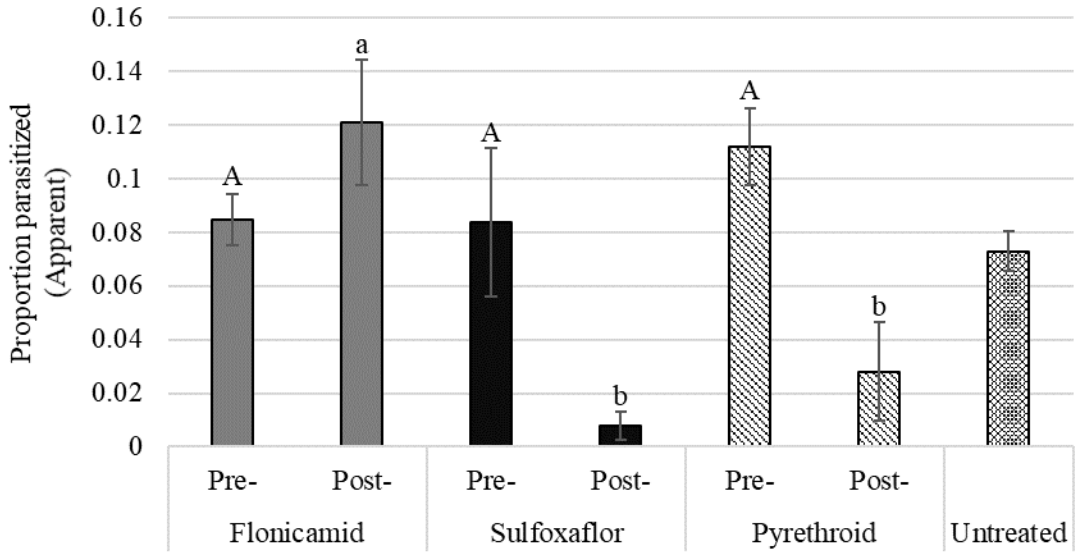

Figure 4.3. Apparent parasitism levels (±SE) for all pre- and post-treatment samples. Fields that were untreated were not included in statistical comparisons.

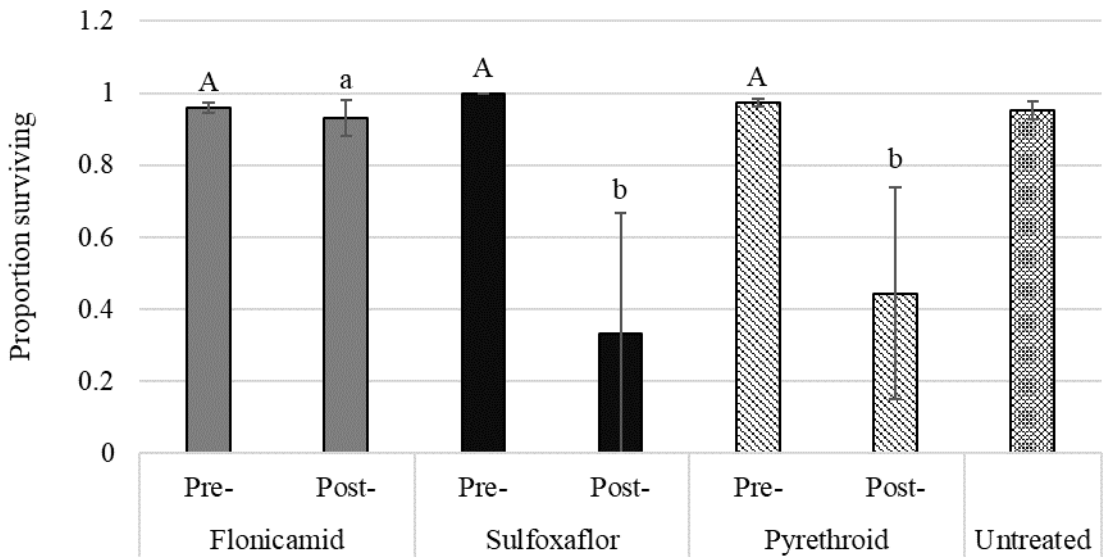

Figure 4.4. Proportion of mummified aphids that successfully produced adult wasps for all pre- and post-treatment samples. Fields that were untreated were not included in statistical comparisons.

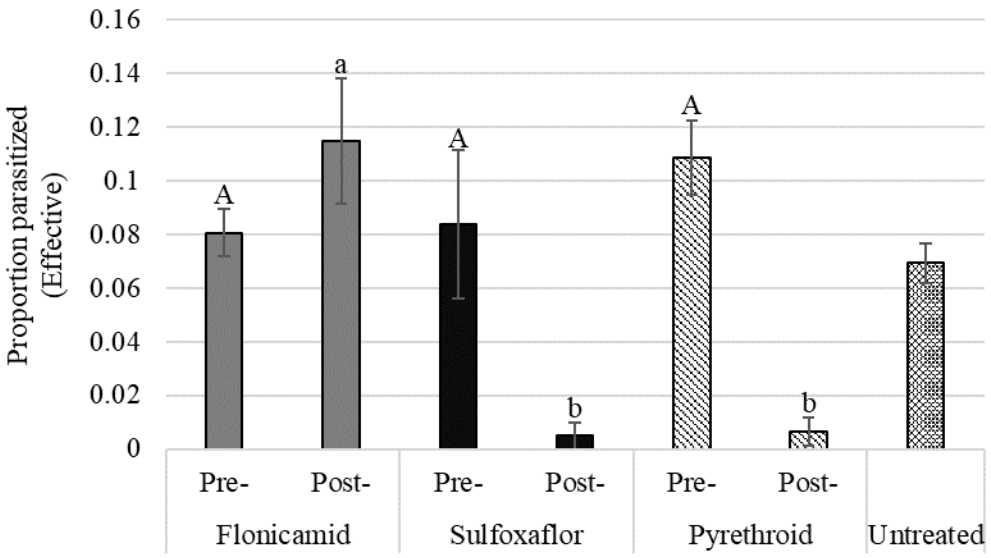

Figure 4.5. Effective parasitism of aphids for all pre- and post-treatment samples. Fields that were untreated were not included in statistical comparisons.

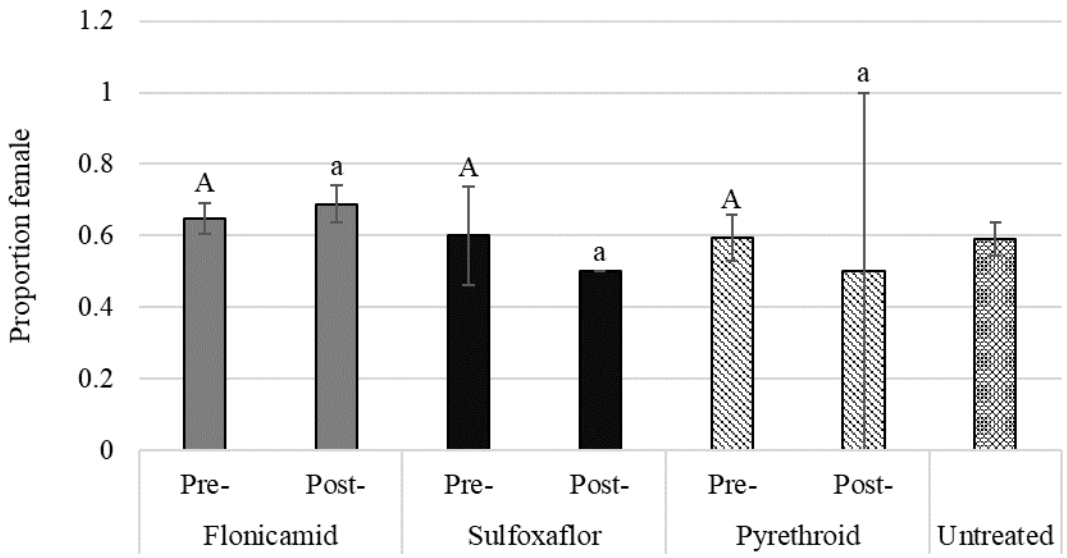

Figure 4.6. Proportion of adult female parasitoids emerging from aphids for all pre- and post-treatment samples. Fields that were untreated were not included in statistical comparisons.

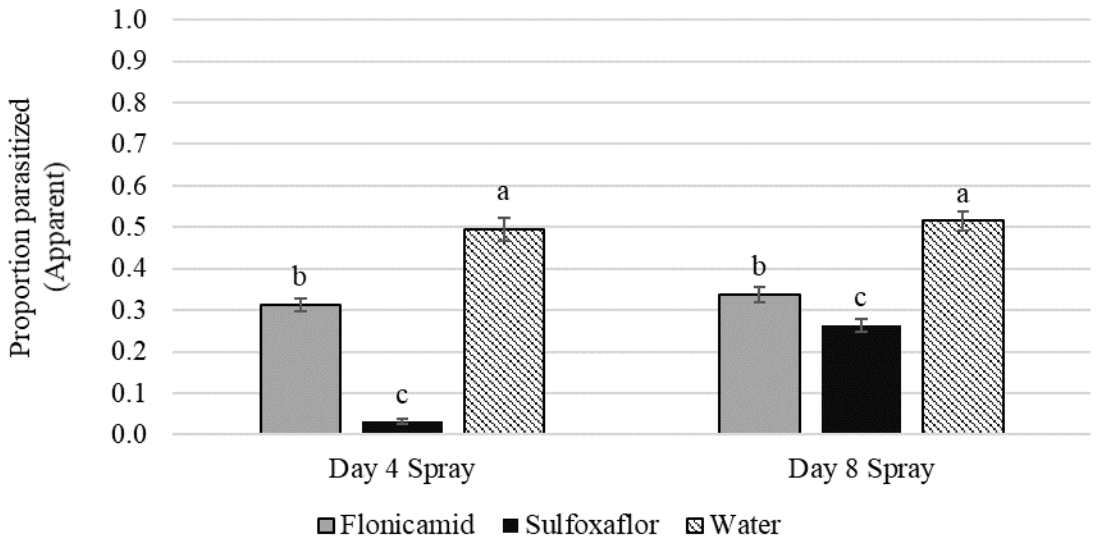

Figure 4.7. Apparent parasitism of aphids exposed to chemical treatments four or eight days after parasitism.

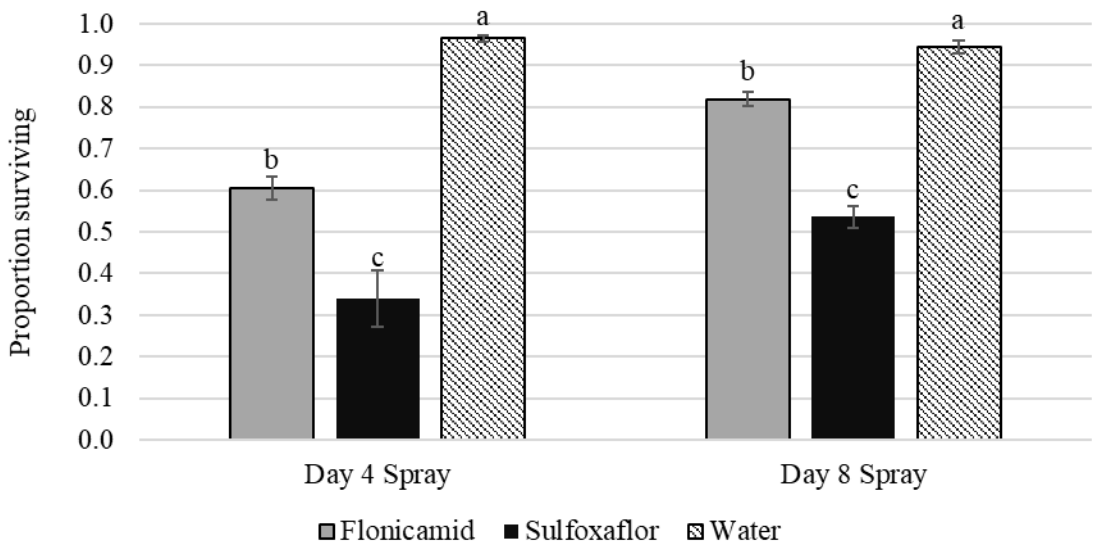

Figure 4.8. Proportion of mummified aphids surviving to adulthood after exposure to chemical treatments at four or eight days after parasitism.

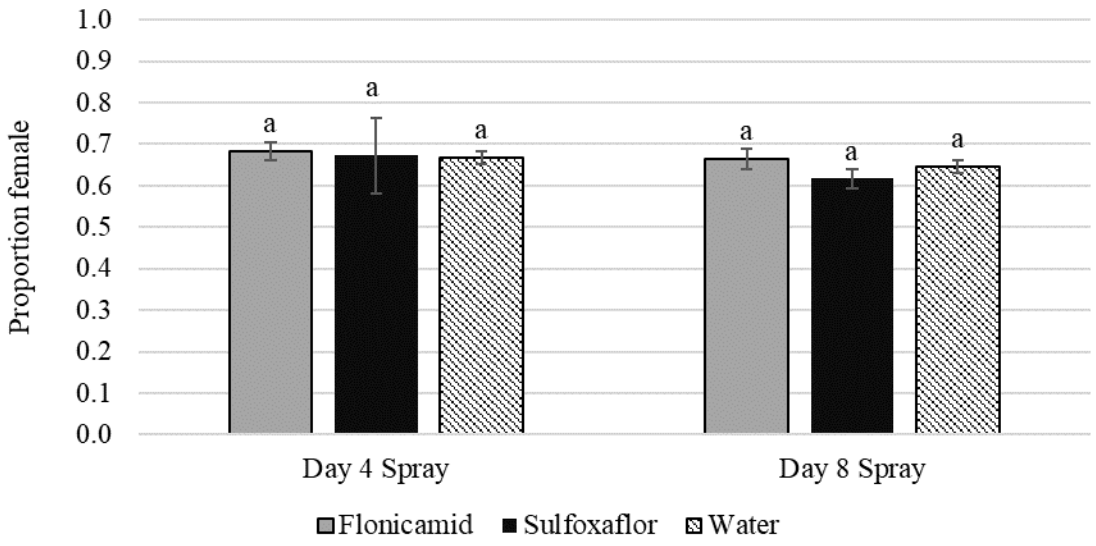

Figure 4.9. Proportion of *D. rapae* females emerging from aphids exposed to chemical treatments at four or eight days after oviposition.

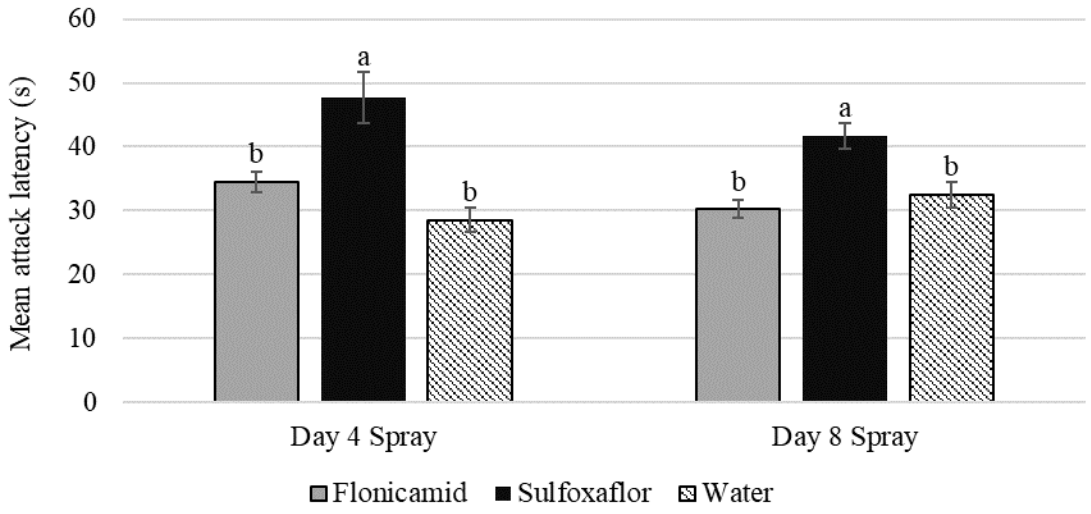

Figure 4.10. Time until first attack of F1 *D. rapae* females emerging from treated aphids.

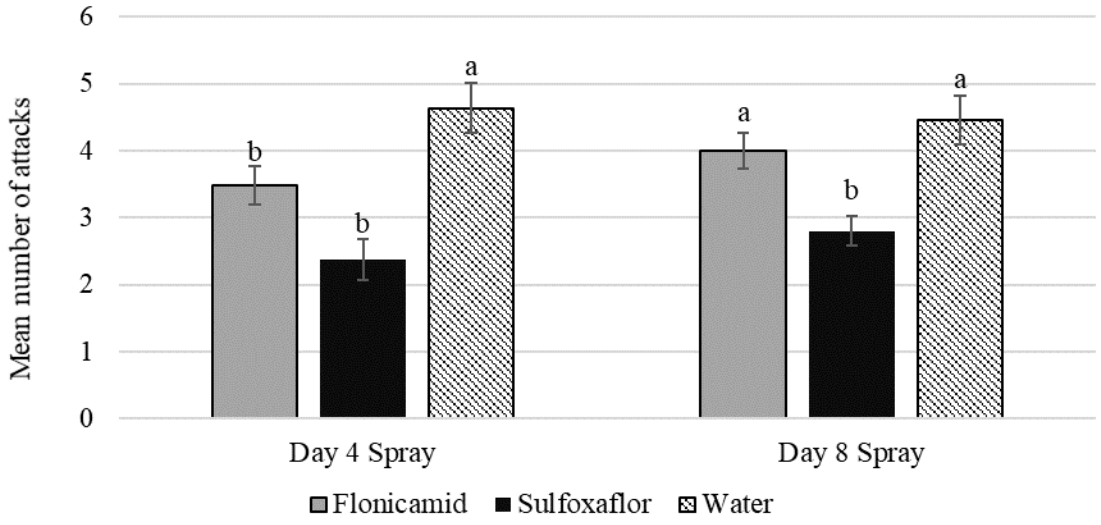

Figure 4.11. Number of ovipositor contacts made by F1 *D. rapae* females emerging from treated aphids.

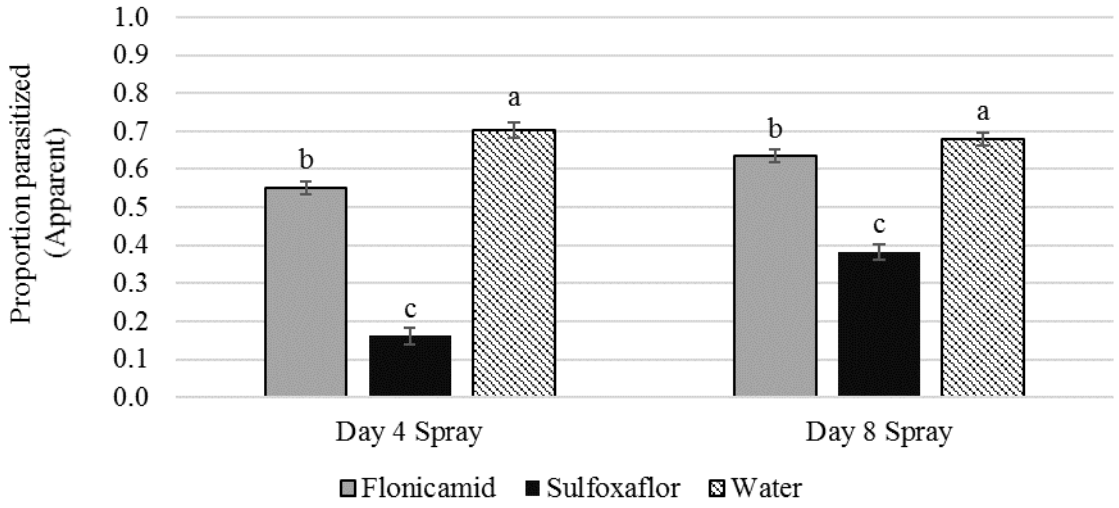

Figure 4.12. Proportion of aphids parasitized by F1 *D. rapae* females emerging from treated aphids.

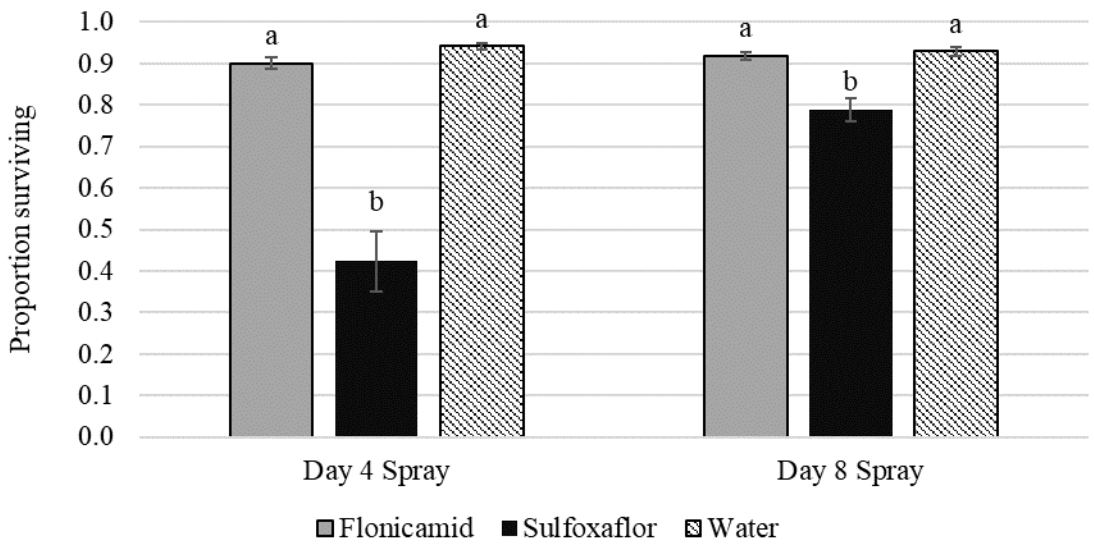

Figure 4.13. Survival of offspring produced by F1 *D. rapae* females emerging from treated aphids.

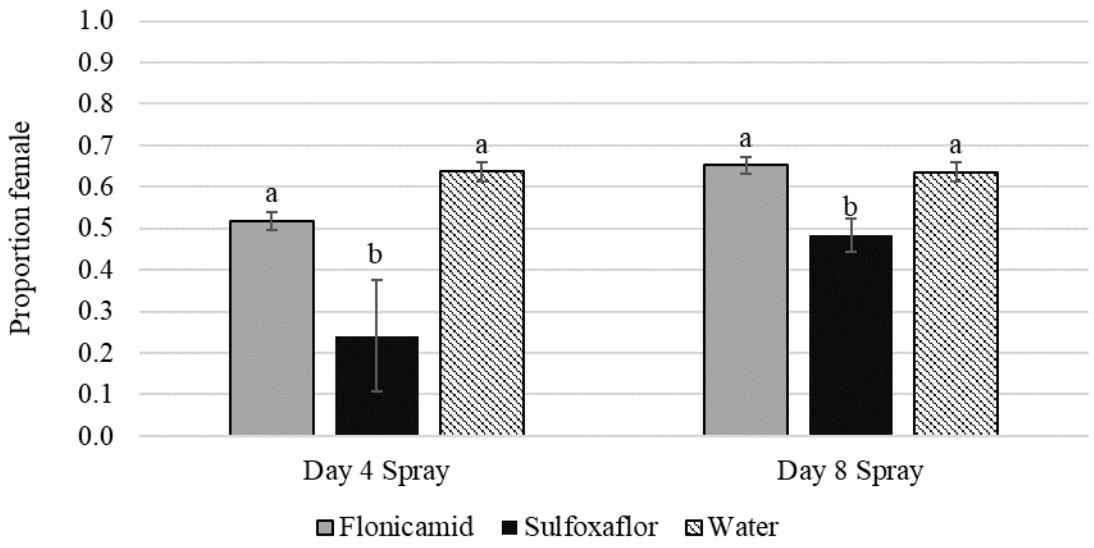

Figure 4.14. Proportion female offspring produced by F1 *D. rapae* females emerging from treated aphids.

CHAPTER V

GENERAL CONCLUSIONS

Winter canola (*Brassica napus* L.) (Brassicales: Brassicaceae) has been widely grown in the United States Southern Great Plains since 2001, and production has increased steadily despite difficulties in production (Franke et al. 2009, Royer and Giles 2017, USDA NASS 2017). Prices for canola oilseed occasionally surpass those for winter wheat (*Triticum aestivum* L.) (Poales: Poaceae), providing an alternative income source when grain prices are low. Rotations of winter canola with wheat can also reduce disease and weed pressure, providing as much as 22% increase in subsequent wheat yield (Bushong et al. 2012). However, management of insect pests has been the primary concern of canola growers in Oklahoma, as severe annual outbreaks of hemipteran and lepidopteran pests can frequently limit production (Franke et al. 2009, Royer and Giles 2017). Neighboring wheat crops benefit from predictable suppression of aphid (Hemiptera: Aphididae) pests by the parasitoid *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae); however, natural pest suppression in canola does not occur reliably, despite the occurrence of *Diaeretiella rapae* (McIntosh), a parasitoid specializing on crucifer-feeding aphids.

The overall purpose of this research was to evaluate how *D. rapae* responds to two of the apparent hurdles it faces in Oklahoma. Firstly, *L. testaceipes* is by far the most abundant insect found in this crop (Jessie 2017), where it attacks aphids, although with little success (WPJ, pers. obs.). This may result in competition that can be either extrinsic (adult interference) or intrinsic (larval combat) if oviposition by both parasitoids commonly occurs on canola aphids. Secondly, frequent annual outbreaks of aphids in canola have resulted in widespread use of broad-spectrum

insecticides, which can quickly reduce parasitoid populations. Applications of selective insecticides may conserve populations of *D. rapae* and facilitate this species' numerical response to subsequent aphid infestations.

In the first study, competition between *D. rapae* and *L. testaceipes* was evaluated in individual, no-choice experiments, and on host plants containing varying densities of either *Myzus persicae* (Sulzer), *Lipaphis pseudobrassicae* (Davis), or *Rhopalosiphum padi* (L.) hosts. The parasitoid *L. testaceipes* attacked and successfully developed in *M. persicae*, but not in *L. pseudobrassicae*. The proportion of canola aphids parasitized by *D. rapae* was significantly lower when foraging after, or simultaneously with, *L. testaceipes*. When individual canola aphids were presented to both parasitoid species in quick succession (< 24hr), only *D. rapae* emerged, suggesting that *D. rapae* is an intrinsically superior competitor in canola aphids, and also that discrimination occurs when heterospecific oviposition events are separated by longer periods.

When competing for *R. padi* hosts on wheat, multiparasitism increased the likelihood that *L. testaceipes* would emerge and suggested an intrinsic advantage for *L. testaceipes* in this host. Discrimination of *L. testaceipes*-parasitized hosts by *D. rapae* after 24hr cannot be completely ruled out, but no differences in *D. rapae* parasitism were detected between staggered and simultaneous competition scenarios, suggesting that the timing of oviposition events was not a factor in these experiments. Because large *L. testaceipes* populations in winter wheat frequently spill over into neighboring canola fields (Jessie 2017), disruption of *D. rapae* parasitism by *L. testaceipes* may be relatively common. However, quantification of within-field levels of multiparasitism and other competitive interactions would be needed to confirm this.

The second study revealed that treatments of sulfoxaflor in winter canola fields resulted in parasitism levels similar to broad-spectrum pyrethroid treatments. Sulfoxaflor treatments resulted in very low aphid abundance, and parasitoids may have been negatively affected by an absence of hosts. Aphids in these fields were primarily alate or early instar aphids, and the systemic nature of sulfoxaflor may have resulted in short-lived colonies that were not persistent enough for *D. rapae*

development. However, the similarity in parasitoid mummy survival between sulfoxaflor and pyrethroids suggests sulfoxaflor can be lethal to *D. rapae*. Laboratory studies revealed significant lethal and sublethal effects of this insecticide, but approximately 25% of F1 *D. rapae* were able to survive and reproduce following exposure.

Treatments of flonicamid in winter canola fields did not significantly reduce parasitism. Aphid numbers were higher in these fields post-treatment, mostly in the first sampling period, as aphids are not immediately killed by flonicamid. In laboratory experiments, *D. rapae* successfully emerged from aphids treated four days after parasitization, but exhibited some sublethal effects. Effects of flonicamid treatment eight days after *D. rapae* oviposition were less severe, as survival of F1 progeny was not significantly different from water-treated controls. These results indicate that flonicamid is highly compatible with *D. rapae*. This chemical may support conservation of parasitoids of hemipteran pests across many agricultural systems, ultimately reducing reliance on chemical control.

If adoption of winter canola continues across the Southern Great Plains, increased acreage may provide more suitable habitats and hosts for *D. rapae*. Larger populations of *D. rapae* may not be as susceptible to the negative effects of competition with *L. testaceipes*, and if broad-spectrum insecticides can be reduced, more reliable natural suppression of canola aphids may be achieved.

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APPENDICES

Appendices start on the next page.

Table A1. Mean (±SE) number of probes, larvae, and adult *Diaeretiella rapae* for each aphid species and parasitoid exposure at 24.4±0.9°C, 43±1.2% RH..

Aphid Species	Probes	Larvae	Adults
Unparasitized aphids			
Lipaphis pseudobrassicae	3.84 ± 0.2770 a	0.93 ± 0.0667 a	0.93 ± 0.0463 a
Myzus persicae	3.67 ± 0.3226 ab	1.00 ± 0.0000 a	0.97 ± 0.0333 a
Rhopalosiphum padi	3.09 ± 0.2031 b	0.93 ± 0.0667 a	0.93 ± 0.0463 a
Previously exposed to *L. testaceipes*			
Lipaphis pseudobrassicae	3.40 ± 0.1970 ab	1.00 ± 0.0000 a	0.90 ± 0.0557 a
Myzus persicae	3.56 ± 0.2237 ab	1.20 ± 0.1069 a	0.93 ± 0.0463 a
Rhopalosiphum padi	2.36 ± 0.2228 c	1.53 ± 0.1333 b	0.23 ± 0.0785 b

Statistical analyses are reported within each daily competition scenario. Values in each column group followed by the same letter are not significantly different ($p > 0.05$).

Table A2. Mean (±SE) number of probes, larvae, and adult *Lysiphlebus testaceipes* for each aphid species and parasitoid exposure at 24.4±0.9°C, 43±1.2% RH.

Aphid Species	Probes	Larvae	Adults
Unparasitized aphids			
Lipaphis pseudobrassicae	1.33 ± 0.0899 b	0.00 ± 0.0000 b	0.00 ± 0.0000 b
Myzus persicae	1.60 ± 0.1570 b	0.13 ± 0.0909 b	0.10 ± 0.0557 b
Rhopalosiphum padi	4.27 ± 0.3055 a	1.13 ± 0.0909 a	0.90 ± 0.0557 a
Previously exposed to *D. rapae*			
Lipaphis pseudobrassicae	1.36 ± 0.0908 b	0.93 ± 0.0667 b	0.00 ± 0.0000 b
Myzus persicae	1.51 ± 0.1173 b	1.27 ± 0.1182 b	0.00 ± 0.0000 b
Rhopalosiphum padi	4.33 ± 0.3065 a	1.60 ± 0.1309 a	0.87 ± 0.0631 a

Statistical analyses are reported within each daily competition scenario. Values in each column group followed by the same letter are not significantly different ($p > 0.05$).

Table A3. Mean (±SE) proportion parasitized in response to different competition scenarios on three aphid species at 24.1 ± 0.08 °C and 36.4 ± 0.62 %RH.

		Proportion Parasitized		
First Species	**Second Species**	***L. erysimi***	***M. persicae***	***R. padi***
D. rapae	-	0.75 ± 0.0284 a	0.72 ± 0.0309 a	0.64 ± 0.0319 b
D. rapae	*L. testaceipes*	0.73 ± 0.0263 a	0.64 ± 0.0274 b	0.70 ± 0.0254 ab
Simultaneous	-	0.50 ± 0.0284 b	0.56 ± 0.0319 c	0.77 ± 0.0256 a
L. testaceipes	*D. rapae*	0.56 ± 0.0288 b	0.61 ± 0.0297 bc	0.73 ± 0.0287 a
L. testaceipes	-	0.00 ± 0.0000 c	0.03 ± 0.0144 d	0.75 ± 0.0260 a

Statistical analyses are reported within each daily competition scenario. Values in each column followed by the same letter are not significantly different ($p > 0.05$).

Table A4. Mean (±SE) number of adult *Diaeretiella rapae* in response to different competition scenarios on three aphid species at 24.1 ± 0.08 °C and 36.4 ± 0.62 %RH.

		Adult *D. rapae*		
First Species	**Second Species**	***L. erysimi***	***M. persicae***	***R. padi***
D. rapae	-	7.25 ± 0.2846 a	7.00 ± 0.3043 a	6.25 ± 0.3155 a
D. rapae	*L. testaceipes*	7.11 ± 0.2589 a	5.64 ± 0.2633 b	1.29 ± 0.1695 b
Simultaneous	-	4.79 ± 0.2590 b	5.25 ± 0.3027 b	1.18 ± 0.1460 b
L. testaceipes	*D. rapae*	5.50 ± 0.3191 b	5.86 ± 0.3031 b	0.79 ± 0.1655 b

Statistical analyses are reported within each daily competition scenario. Values in each column followed by the same letter are not significantly different ($p > 0.05$).

Table A5. Mean (±SE) proportion female *Diaeretiella rapae* successfully emerging from competition scenarios on three aphid species at 24.1 ± 0.08 °C and 36.4 ± 0.62 %RH.

		Proportion Female *D. rapae*		
First Species	**Second Species**	***L. erysimi***	***M. persicae***	***R. padi***
D. rapae	-	0.67 ± 0.0225 a	0.66 ± 0.0269 a	0.68 ± 0.0251 a
D. rapae	*L. testaceipes*	0.69 ± 0.0194 a	0.67 ± 0.0306 a	0.78 ± 0.0674 a
Simultaneous	-	0.63 ± 0.0305 a	0.65 ± 0.0303 a	0.79 ± 0.0681 a
L. testaceipes	*D. rapae*	0.64 ± 0.0257 a	0.51 ± 0.0285 b	0.68 ± 0.1047 a

Statistical analyses are reported within each daily competition scenario. Values in each column followed by the same letter are not significantly different ($p > 0.05$).

Table A6. Mean (±SE) number of adult *Lysiphlebus testaceipes* in response to different competition scenarios on three aphid species at 24.1 ± 0.08 °C and 36.4 ± 0.62 %RH.

		Adult *L testaceipes*		
First Species	**Second Species**	***L. erysimi***	***M. persicae***	***R. padi***
D. rapae	*L. testaceipes*	-	0.04 ± 0.0357 b	5.18 ± 0.2675 c
Simultaneous	-	-	0.07 ± 0.0714 ab	6.54 ± 0.2976 b
L. testaceipes	*D. rapae*	-	0.14 ± 0.0673 ab	6.54 ± 0.2545 b
L. testaceipes	-	-	0.29 ± 0.1442 a	7.36 ± 0.2424 a

Statistical analyses are reported within each daily competition scenario. Values in each column followed by the same letter are not significantly different ($p > 0.05$).

Table A7. Mean (±SE) proportion female *Lysiphlebus testaceipes* in response to different competition scenarios on three aphid species at 24.1 ± 0.08 °C and 36.4 ± 0.62 %RH.

		Proportion Female *L testaceipes*		
First Species	**Second Species**	***L. erysimi***	***M. persicae***	***R. padi***
D. rapae	*L. testaceipes*	-	1.00 ± 0.0000 a	0.60 ± 0.0286 a
Simultaneous	-	-	1.00 ± 0.0000 a	0.62 ± 0.0295 a
L. testaceipes	*D. rapae*	-	0.75 ± 0.2500 a	0.66 ± 0.0256 a
L. testaceipes	-	-	0.88 ± 0.1250 a	0.68 ± 0.0269 a

Statistical analyses are reported within each daily competition scenario. Values in each column followed by the same letter are not significantly different ($p > 0.05$).

Table A8. Mean (±SE) parasitoid survival in response to different competition scenarios on three aphid species at 24.1 ± 0.08 °C and 36.4 ± 0.62 %RH.

		Proportion Surviving		
First Species	**Second Species**	***L. erysimi***	***M. persicae***	***R. padi***
D. rapae	-	0.97 ± 0.0104 a	0.98 ± 0.0098 a	0.97 ± 0.0126 ab
D. rapae	*L. testaceipes*	0.97 ± 0.0105 a	0.90 ± 0.0252 b	0.94 ± 0.0293 b
Simultaneous	-	0.97 ± 0.0127 a	0.96 ± 0.0163 a	1.00 ± 0.0000 a
L. testaceipes	*D. rapae*	0.97 ± 0.0167 a	0.98 ± 0.0178 a	1.00 ± 0.0000 a
L. testaceipes	-	-	1.00 ± 0.0000 a	0.99 ± 0.0067 a

Statistical analyses are reported within each daily competition scenario. Values in each column followed by the same letter are not significantly different ($p > 0.05$).

Table A9. Means (±SE) for field studies of *Diaeretiella rapae* collected from parasitized aphids in winter canola fields treated with flonicamid, sulfoxaflor, or pyrethroid insecticides.

Insecticide	Aphid Abundance	Apparent Parasitism	Survival	Effective Parasitism	Proportion Female
Pre-					
Flonicamid	62.98 ± 8.7755 A	0.08 ± 0.0094 A	0.96 ± 0.0140 A	0.08 ± 0.0090 A	0.65 ± 0.0436 A
Sulfoxaflor	47.67 ± 5.8973 A	0.08 ± 0.0276 A	1.00 ± 0.0000 A	0.08 ± 0.0276 A	0.60 ± 0.1388 A
Pyrethroid	51.07 ± 12.152 A	0.11 ± 0.0143 A	0.97 ± 0.0122 A	0.11 ± 0.0139 A	0.59 ± 0.0649 A
Post-					
Flonicamid	22.86 ± 3.0895 a	0.12 ± 0.0235 a	0.93 ± 0.0498 a	0.11 ± 0.0233 a	0.69 ± 0.0530 a
Sulfoxaflor	11.08 ± 1.2544 b	0.01 ± 0.0052 b	0.33 ± 0.3333 b	0.00 ± 0.0050 b	0.50 ± 0.0000 a
Pyrethroid	14.20 ± 2.8705 ab	0.03 ± 0.0182 b	0.44 ± 0.2940 b	0.01 ± 0.0053 b	0.50 ± 0.5000 a
Untreated	42.74 ± 4.9388	0.07 ± 0.0074	0.95 ± 0.0243	0.07 ± 0.0075	0.59 ± 0.0464

Statistical analyses are reported within pre-or post-treatments of insecticide for each response variable. Values followed by the same letter are not significantly different ($p > 0.05$).

Table A10. Means (±SE) for studies of *Diaeretiella rapae* preimaginal survival when exposed to flonicamid, sulfoxaflor, or distilled water at either 4 or 8 days after oviposition.

Insecticide	Parasitism	Survival	Proportion Female
Day 4			
Flonicamid	0.31 ± 0.0163 b	0.60 ± 0.0268 b	0.68 ± 0.0205 a
Sulfoxaflor	0.03 ± 0.0061 c	0.33 ± 0.0712 c	0.67 ± 0.0981 a
Water	0.49 ± 0.0275 a	0.96 ± 0.0084 a	0.67 ± 0.0165 a
Day 8			
Flonicamid	0.34 ± 0.0183 b	0.82 ± 0.0175 b	0.66 ± 0.0250 a
Sulfoxaflor	0.26 ± 0.0163 c	0.54 ± 0.0261 c	0.62 ± 0.0232 a
Water	0.51 ± 0.0226 a	0.94 ± 0.0154 a	0.65 ± 0.0146 a

Statistical analyses are reported within each treatment day. Values in each column group followed by the same letter are not significantly different ($p > 0.05$).

Table A11. Means (±SE) for studies of *Diaeretiella rapae* sublethal effects when exposed to flonicamid, sulfoxaflor, or distilled water at either 4 or 8 days after oviposition.

Insecticide	Attack Latency	# Attacks	Parasitism	Survival	Proportion Female
Day 4					
Flonicamid	39.28 ± 2.8958 b	3.48 ± 0.2837 b	0.55 ± 0.0170 b	0.90 ± 0.0149 a	0.52 ± 0.0214 a
Sulfoxaflor	57.24 ± 7.7346 a	2.38 ± 0.3045 b	0.16 ± 0.0215 c	0.42 ± 0.0736 b	0.24 ± 0.1339 b
Water	32.46 ± 3.1399 b	4.64 ± 0.3690 a	0.70 ± 0.0190 a	0.94 ± 0.0085 a	0.64 ± 0.0236 a
Day 8					
Flonicamid	35.22 ± 3.1952 b	4.00 ± 0.2700 a	0.63 ± 0.0173 b	0.92 ± 0.0097 a	0.65 ± 0.0207 a
Sulfoxaflor	49.55 ± 3.6955 a	2.80 ± 0.2176 b	0.38 ± 0.0192 c	0.79 ± 0.0280 b	0.48 ± 0.0394 b
Water	38.82 ± 4.3708 b	4.46 ± 0.3717 a	0.68 ± 0.0176 a	0.93 ± 0.0097 a	0.64 ± 0.0226 a

Statistical analyses are reported within each treatment day. Values in each column group followed by the same letter are not significantly different ($p > 0.05$).

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VITA

William Paul Jessie

Candidate for the Degree of

Doctor of Philosophy

Thesis: EFFECTS OF INTERSPECIFIC COMPETITION AND NARROW-SPECTRUM INSECTICIDES ON THE SURVIVAL AND DEVELOPMENT OF *DIAERETIELLA RAPAE* IN OKLAHOMA WINTER CANOLA

Major Field: Entomology

Biographical:

Education:

Completed the requirements for the Doctor of Philosophy in Entomology at Oklahoma State University, Stillwater, Oklahoma in December 2017.

Completed the requirements for the Master of Science in Entomology and Plant Pathology at Oklahoma State University, Stillwater, Oklahoma in December 2013.

Completed the requirements for the Bachelor of Science in Zoology at Oklahoma State University, Stillwater, Oklahoma in May 2009

Completed the requirements for the Bachelor of Science in Natural Resource Ecology and Management at Oklahoma State University, Stillwater, Oklahoma in May 2008.

Experience:

Graduate Research Associate: January 2014 – December 2017

Graduate Research Assistant: August 2009 – December 2013

Graduate Teaching Assistant: Introduction to Entomology (Fall 2012, 2013, 2014, 2015, 2017); Introductory Biology (Fall 2009, Spring 2010, 2011); Mammalian Physiology (Fall 2010).

Professional Memberships:
Entomological Society of America, 2012-current
International Organisation for Biological Control, 2016-current