ECOLOGICAL STUDIES OF PARASITOID HYMENOPTERANS (SUBCLADE PARASITOIDA) IN SOUTHERN ALBERTA AGRICULTURAL SYSTEMS

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For Katie, even though you never liked bugs.

ABSTRACT

Parasitoid hymenopterans are important biocontrol agents of agricultural pests. Despite their relevance to sustainable agriculture and an integrated pest management strategy, this subclade of insects is understudied. Here, we conducted two studies related to the biology of parasitoid hymenopterans in southern Alberta: 1) A survey of parasitism rates by a native genus of parasitoid, *Peristenus*, on a significant agricultural pest, *Lygus*, across 17 cultivated and uncultivated host plants; 2) A survey of parasitoid genera in canola fields as part of a study on the influence of type of edge boundaries and distance from the edge on parasitoid populations and diversity. Mean *Peristenus-Lygus* parasitism rates ranged from 0 – 45% across various host plant species, with a significant interaction between instar age and parasitism rates in late-stage canola and hemp. Type of edge boundary and distance from the edge did not significantly affect Hymenoptera diversity, and *Diolcogaster* is reported as the dominant genus of parasitoid hymenopterans in canola crops. Both studies contribute to enhanced integrated pest management studies, and more generally, to the understanding of southern Alberta insects.

USE OF GENERATIVE AI

OpenAI’s generative artificial intelligence (AI) model, ChatGPT-4 (https://chatgpt.com/), was used to troubleshoot some of the R script used in the statistical analyses of chapters 2 and 3 of this thesis.

PREFACE

Please note that chapter 2 of this thesis, *Parasitism rates of Peristenus spp. (Hymenoptera: Braconidae) on Lygus plant bugs (Hemiptera: Miridae) in cultivated and uncultivated plant hosts in southern Alberta*, was intentionally written with plural pronouns to acknowledge the contributions of the collaborators.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

°C	degree Celsius
BBCH	Biologische Bundesanstalt, Bundessortenamt and CHemische Industrie
CAD	Canadian dollar
cm	centimetre
EtOH	ethanol
GLMM	generalized linear mixed model
IPM	integrated pest management
km	kilometre
m	metre
mL	millilitre
mm	millimetre
NMDS	non-metric multidimensional scaling
SDI	Shannon diversity index
USD	United States dollar

1. GENERAL INTRODUCTION

1.1 Overview

Wasps are a poorly understood group of insects, often maligned and disliked by the general public. However, these invertebrates, belonging to the order Hymenoptera, are crucial in agriculture, particularly as part of integrated pest management strategies through their use as biocontrol agents. This thesis focuses on a specific class of wasps known as parasitoid hymenopterans and examines their role in agricultural ecosystems in southern Alberta. I address topics related to the basic biology and phenology of parasitoid wasps, as well as methods for their conservation, through two studies presented in chapters 2 and 3.

Chapter 2 of this thesis is a survey of the parasitism rates of the native parasitoid Hymenopteran genus *Peristenus* across a variety of cultivated and uncultivated plants in southern Alberta. Chapter 3 is a survey of the different genera of parasitoid hymenopterans found in southern Albertan canola fields and investigates how surrounding edge boundaries may influence parasitoid diversity and abundance. Here, I define edge boundaries as the type of habitat bordering a field (i.e., coulees, shelterbelts, road verges).

This general introduction addresses the following topics: 1) Agricultural intensification as well as sustainable methods to reduce the harm of intensive practices; 2) Integrated pest management strategies and the role of parasitoid hymenopterans as biocontrol agents; 3) A general summary of Hymenopteran biology, and the current phylogenetic status of parasitoid hymenopterans; 4) A review of the parasitoid wasp genus, *Peristenus*, and its role as a biocontrol agent in southern Alberta.

With this literature review, I intend to provide the reader with the necessary background information needed for understanding the studies expanded upon in chapters 2 and 3 of this thesis, and perhaps even elicit some curiosity about the strange and wonderful group of insects known as parasitoid hymenopterans along the way.

1.2 Agricultural intensification & its contemporary impacts

The United Nations predicts that the global population will increase from 8.2 billion in 2024 to over 10.3 billion people within the next 50 to 60 years (United Nations Department of Economic and Social Affairs, Population Division, 2024). Consequently, agricultural expansion will be required to feed this growing population, even though agroecosystems already occupy 30% to 40% of terrestrial surface area and are one of the largest biomes on the planet (Campbell *et al.*, 2017; Ramankutty *et al.*, 2018). While agriculture is a crucial global industry, it is also one of the leading drivers of climate change (Tilman *et al.*, 2001; Campbell *et al.*, 2017; Ramankutty *et al.*, 2018). Specifically, many contemporary practices associated with agricultural intensification have been driving harmful effects on the environment, human health, and the planet. Agricultural intensification is defined by Ramankutty *et al.* (2018) as "increasing agricultural output per unit land area with increased inputs" (p. 806); in other words, increasing the yield of crop or livestock per unit of farmland by increasing the input of resources such as water, fertilizer, and pesticides. Other practices are associated with agricultural intensification such as decreasing crop diversity, shortening crop rotation cycles, and deep field plowing (Tscharntke *et al.*, 2005). Hypothetically, this would seem like a good idea as producers would be able to increase their productivity on a smaller land area; however, agricultural intensification generally results in the expansion of farmland as more producers adopt the practice to increase their output (Ramankutty *et al.*, 2018). The resulting conversion of natural landscapes into cropland releases a significant amount of CO_2 into the atmosphere, fragments landscapes, and decreases biodiversity (Tscharntke *et al.*, 2005; Campbell *et al.*, 2017) and leads to ecosystem simplification and loss of ecosystem function (Tilman *et al.*, 2001). Ecosystem simplification harms the health of the agricultural environment through a dramatic loss of pollinators (González-Varo *et al.*, 2013), the emergence of new plant viruses (Roossinck & García-Arenal, 2015), and decreased crop yield and natural pest control (Grab *et al.*, 2018).

By properly managing non-crop habitats, rather than converting them to arable land, we improve the surrounding agroecosystem through increased biodiversity, which in turn promotes ecosystem resilience and functionality and reduces ecosystem simplification (Benton *et al.*, 2003). In particular, the more complex or heterogenous the habitat is (e.g., different vegetation types, landscape features, geographic size, etc.), the more biotic and abiotic resources are available to support niches for organisms to occupy. This is the core concept of the Habitat Heterogeneity Hypothesis in ecological theory. Thies *et al.* (2003) found that complex and structurally rich non-crop landscapes greatly influenced the pest-management of rape pollen beetles (*Meligethes aeneus* syn. *Brassicogethes aeneus*) via parasitoid wasps. Their 2003 study highlighted a strong positive correlation between mean parasitism rates and percentage of non-crop area (i.e., landscape complexity), and a negative correlation between pest damage and percentage of non-crop area, meaning they were able to maintain crop quality without sacrificing yield.

Maintaining non-crop habitat not only has environmental benefits, but economic ones as well. Kulshreshtha and Kort (2008) evaluated the benefits of shelterbelts (i.e., rows of trees and/or shrubs) planted on or near arable land in the Canadian Prairies from 1981 to 2001. Social goods or benefits measured from shelterbelts were quantified and expressed in monetary terms, leading to an estimated $140,000,000 CAD (approximately $239,000,000 CAD in 2024) worth of benefits acquired within the sampled 20-year span. The majority of the benefits were due to reduced greenhouse gas emissions, either through carbon sequestration ($56,000,000 CAD in 2001 or $92,000,000 CAD in 2024) or by reducing the total area farmed (i.e., lower agricultural intensification; worth approximately $17,000,000 CAD in 2001 or $27,000,000 CAD in 2024). Other benefits of these shelterbelts included improved water and air quality, reduced soil erosion, increased biodiversity, and potentially reduced pesticide drift (Kulshreshtha & Kort; 2008).

Another major concern in agricultural intensification is the increased use of pesticides (insecticides, herbicides, fungicides), which is directly linked to much of the environmental degradation observed today (Ukhurebor *et al.*, 2020); some researchers even contend that the negative effects of pesticides outweigh the positive (Mahmood *et al.*, 2016). For example, pesticides induce non-target effects on beneficial organisms, decrease biodiversity, and pollute the environment (Turusov *et al.*, 2002; Bourguet & Guillemaud, 2016; Ukhurebor *et al.*, 2020). Excessive pesticide use may lead to the evolution of resistance in target pests, producing strains of pests that become increasingly difficult and costly to manage (Metcalf & Luckmann, 1994). Human exposure to pesticides–either chronic or acute, and through direct means such as inhalation during application or indirect means such as through contaminated drinking water–is well documented to have harmful effects on health. These effects include, but are not limited to, skin and eye disorders, neurotoxicity, cognitive dysfunction, birth defects, and reproductive issues (Sanborn *et al.*, 2007). Furthermore, certain classes of pesticides such as organochlorides, organophosphates, and carbamates, as well as exposure to multiple types of pesticides, may be carcinogenic to humans and cause diseases such as Hodgkin’s and non-Hodgkin’s lymphoma (Alavanja *et al.*, 2004; Navaranjan et al, 2013). The first synthetic insecticide, Paris green (cupric acetoarsenite), was first developed as a colour pigment in 1814, before its insecticidal properties were discovered (Metcalf & Luckmann, 1994). By 1867 it became a popular form of pest control and stayed that way until the 1940's (Metcalf & Luckmann, 1994), despite being linked to chronic arsenic poisoning as it leached into drinking water (Mackay *et al.*, 1934). Paris green was eventually replaced by the now mostly banned DDT (dichlorodiphenyltrichloroethane) which, as Metcalf and Luckmann (1994) described, had "unprecedented effectiveness" (p. 257) at controlling pest populations. The introduction, effectiveness, and subsequent uncontrolled use of DDT has led to contemporary agriculture's now overreliance on pesticides resulting in many of the environmental and health issues we see today (Turusov *et al.*, 2002; den Hond *et al.*, 2003).

I would like to take a moment to note that I am not outlining these negative effects to demonize the agricultural industry or the hardworking producers that put food on the table for billions of people worldwide. However, unchecked agricultural intensification resulting in harmful effects on environmental and human health should be discussed, as alternative practices and sustainable methods exist. It is impossible not to acknowledge that pesticides (despite their potential harm) are still highly effective at maintaining agriculture productivity (Metcalf & Luckmann, 1994; den Hond *et al.*, 2003). But, as both Metcalf & Luckmann (1994) and den Hond *et al.* (2003) outline, the key is to use strategic applications of these chemicals (e.g., only applying them when pests reach a certain threshold, timing application based on pest phenology, or preventatively reducing the need for pesticides through land use and cropping systems), rather than excessive, non-targeted pesticide application. Strategic, sustainable agriculture management such as this decreases overall reliance on chemical controls, which in turn lowers the external costs associated with it. Furthermore, practices such as choosing to preserve and restore natural and semi-natural habitats not only improves ecosystem health, but benefits agricultural production through improved crop, water, and soil quality, increased insect services such as pollination and pest control, decreased herbivore damage, and more (Benton et al, 2003; Scherr & McNeely, 2008; Radcliffe *et al.*, 2009). These alternative and strategic agricultural practices fall under the umbrella of integrated pest management, which is outlined below.

1.3 Integrated pest management & hymenopterans as biocontrol agents

Integrated pest management (IPM) is an alternative pest-management strategy that relies on both chemical and non-chemical intervention while also considering environmental, social, and economic factors

(Metcalf & Luckmann, 1994; Radcliffe *et al.*, 2009). While certain IPM methods may still strategically use pesticides (or use less harmful chemical variations), emphasis is placed on reducing reliance on pesticides to promote physical and environmental health. The term ‘pest’ in IPM is typically only associated with insects; however, the definition can include pathogens, weeds, and invertebrate and vertebrate animals alike (Kogan, 1998). Generally, integrated pest management strategies have been developed for agricultural use; however, other fields such as medicine, silviculture, and urban development have adopted IPM with the shared goal of reducing reliance on pesticides (Olkowski & Olkowski, 1978). However, this thesis intends to focus on IPM methods within agriculture.

There are a variety of different IPM approaches, many of which can be combined to create a more holistic pest-control program. Some strategies in agriculture include: trap cropping (attracting pests to a separate, undesired plant rather than the primary target crop), crop rotation (alternating crops each season to disrupt pest life cycles), weed control (cutting, pulling, or mowing weeds to reduce pest populations), education (educating producers on monitoring techniques, pest biology, cost/benefits of pesticides, and effective control methods), timed pesticide application (strategically applying pesticides based on economic threshold levels and periods of activity of predatory insects), and more (Metcalf & Luckmann, 1994; Radcliffe et al, 2009).

One of the most common IPM techniques is the use of natural enemies. Natural enemies are native or exotic beneficial arthropods that cull pests through predation and parasitism, reducing the need for pesticides while maintaining crop quality and yield (Thies *et al.*, 2003; Naranjo *et al.*, 2019). These insects may reside within crops or in adjacent non-crop habitats, where they attack pests from the field boundaries (Thies *et al.*, 2003). Some examples of natural enemies include beetles (Coleoptera; particularly those in the family of Carabidae), spiders (Araneae), and predatory mites (Acari); however, of the natural enemies, those within the order Hymenoptera are among the most successful pest suppressors (LaSalle & Gauld, 1991; Narendran, 2001). While Hymenoptera is a large and diverse order of insects, the focus of this thesis is on parasitoid hymenopterans within the subclade Parasitoida (discussed further in section 1.4), also known as parasitoid wasps or occasionally referred to as only “parasitoids”.

Various examples of successful IPM case studies in which a pest is either controlled or nearly eradicated have been attributed to the intervention of parasitoid hymenopterans. A classic case study was reported by Dean *et al.* in 1979 in the United States where, in the early 1940s, the Asian rhodegrass mealybug, *Antonina graminis* Maskell (Hemiptera: Pseudococcidae) became a major invasive pest in Texas. *Antonina graminis* fed on forage grasses which in turn caused major economic loss for the cattle and turf industries. In response, the small parasitoid *Neodusmetia sangwani* Rao (Encyrtidae) was introduced to combat the invasive pest and did so successfully by the 1960s, nearly eliminating *A. graminis*. The cost of introducing and establishing *N. sangwani* was approximately $200,000 USD, while Dean *et al.* (1979) estimated that the parasitoid was able to save approximately $17,000,000 USD worth of turf grass and $177,000,000 USD worth of cattle production yearly. In 2024, that would amount to saving a total of nearly $840,000,000 USD or $1,161,000,000 CAD worth of products per year.

Another, more recent case study from the Indian states of West Bengal and Bihar reported by Pal *et al.* (2024) found that ten naturally occurring parasitoid species were effective at controlling the invasive fall armyworm (*Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae)). *Spodoptera frugiperda* is a major agricultural pest and is linked to food insecurity as the larvae feed on maize, a major crop in Africa and Asia (Bannor *et al.*, 2022; Pal *et al.*, 2024). Pal *et al.* (2024) reported in their study that maize crops with high rates of insecticide use showed greater *S. frugiperda* damage compared to sites where no insecticides were used, suggesting the insecticides were not controlling *S. frugiperda* as effectively as the naturally occurring parasitoids and potentially even suppressing natural enemies. Parasitoid management of the pest was more effective in maize crops with lower insecticide use and parasitism peaked at sites with no insecticide use.

Both studies highlight how parasitoid hymenopterans are not only effective pest control agents, but also lessen costs for producers–either by reducing pesticide use or by salvaging products that may be lost to pests. The fact that integrated pest management–specifically the use of parasitoids as biocontrol agents–is both economically and environmentally favourable is well researched and generally agreed upon in the scientific community (LaSalle & Gauld, 1991; Narendran, 2001; Redcliffe *et al.*, 2009; Wang *et al.*, 2019). Despite this, hymenopterans are a surprisingly poorly understood and severely understudied group of insects (LaSalle & Gauld, 1991; Goulet & Huber, 1993; Sharkey, 2007). There are large gaps in knowledge regarding the basic biology and life cycles of many hymenopterans, particularly parasitoids, as they are often small in body size making them difficult to study (Goulet & Huber, 1993; Gibson *et al.*, 2007).

1.4 Hymenoptera biology & Parasitoida phylogeny

Hymenoptera is one of the largest orders of insects, consisting of an estimated 100,000 to 150,000 described species (Goulet & Huber, 1993; Sharkey, 2007; Peters *et al.*, 2017; Polaszek & Vilhemsen, 2023) distributed across all continents, excluding Antarctica (Johnson, 2013). In Canada, approximately 25% of our entire described insect population consists of hymenopterans (Goulet & Huber, 1993), and perhaps 10% of all species on earth are of the order Hymenoptera (Sharkey, 2007). Hymenoptera may also be the largest order of insects, exceeding coleopterans in abundance (Forbes *et al.*, 2018) as well as richness, with some researchers estimating the number of undescribed extant species to be in the millions (Sharkey, 2007; Peters *et al.*, 2017).

These insects are generally characterized by the presence of two pairs of membranous wings, mandibular mouthparts, complete metamorphosis, and compound eyes (Johnson, 2013). Females have an ovipositor, which may be modified (e.g., a stinger; common in the clade Aculeata), and all hymenopterans exhibit haplodiploid sex determination. In this system of sex determination, males are haploid and arise from parthenogenesis (unfertilized eggs), whereas females are diploid and develop from fertilized eggs. Hymenopterans vary immensely in size and include some of the smallest insects in the world, such as individuals from the genus *Megaphragma* Timberlake (Trichogrammatidae) whose adults on average measure 0.3 mm in length (Polilov, 2017), or the astonishing *Dicopomorpha echmepterygis* Mockford (Mymaridae), whose adult males measure a mere 0.139 mm (Chown & Gaston, 2010). Conversely, the “mammoth” wasp, *Megascolia procer* Illiger (Scoliidae), grows upwards of 50 mm in length with a wingspan of 116 mm (Sarrazin *et al.*, 2008).

The majority of hymenopterans are parasitoids, although they may also be predatory or, less commonly, phytophagous (Goulet & Huber, 1993). Parasitoids differ from parasites as they kill their host upon maturation; this life strategy can be further categorized as either endo- or ectoparasitism. Endoparasitoids are those that live inside of their host, maturing within the organism before ultimately killing it. The majority of endoparasitoids are koinobionts, meaning their host develops somewhat normally throughout the parasitism process. This is beneficial for the endoparasitoid, as they are able to feed and grow on the maturing host while still being protected inside of it. The other category of parasitoid, ectoparasitoids, are those that deposit their eggs on or near their host, developing outside of the organism. Ectoparasitoids are generally idiobionts, meaning they attack hosts who have halted their development process (e.g., a paralyzed host) and mature quickly to avoid being dislodged. Parasitoids may be solitary, meaning a female generally lays a single egg that develops in or on a single host at one time, or gregarious, meaning one or more females lays multiple eggs in or on a single host, thus resulting in multiple larvae developing in or on the host organism at one time. These lifestyles can be further categorized, such as in the case of hyperparasitoids—a parasitoid whose primary host is another parasitoid—or autoparasitoids—a parasitoid that attacks and parasitizes members of its own species. Hymenopterans exhibit all these lifestyles and more, which has assisted them in being such highly diverse and successful pest suppressors (LaSalle & Gauld, 1991; Narendran, 2001).

The order Hymenoptera can be divided into two suborders: Symphyta and Apocrita. Symphyta is the ancestral suborder consisting of woodwasps, horntails, and sawflies, and is relatively small with fewer species compared to Apocrita (Goulet & Huber, 1993). Conversely, Apocrita is a much larger suborder and evolved from Symphyta (Johnson, 2013) and consists of many easily identifiable insects such as ants (Formicidae), yellowjackets (Vespidae), and honeybees (Apidae). Members of the Apocrita suborder are typically distinguished from Symphyta by their iconic pinched abdomen, or "wasp-waist", known as the petiole. It is important to note that the use of the Symphyta-Apocrita suborder to characterize hymenopterans is debatable due to Symphyta being paraphyletic and Apocrita being monophyletic (Johnson, 2013; Polaszek & Vilhemsen, 2023); however, most research still distinguishes this division in Hymenoptera phylogeny.

Apocrita has been historically further divided into the subclades Parasitoida (e.g., parasitoid wasps; also referred to as the subclade 'Parasitica' in some literature) and Aculeata (e.g., stinging wasps, bees, and ants). Like the Symphyta-Apocrita suborder division, there is dispute in the taxonomic community about the use of these subclades as Parasitoida is a paraphyletic group, whereas Aculeata is generally thought of as a monophyletic group (Peters *et al.*, 2017; Polaszek & Vilhemsen, 2023). This nomenclature dispute is further compounded by the fact that many aculeates are parasitoids; however, as Polaszek and

Vilhemsen (2018) point out, many summaries of, and studies on, Hymenopteran phylogeny continue to use the Parasitoida-Aculeata subclades for convenience and simplicity.

The most recent summaries of parasitoid hymenopterans suggest there are three major lineages within Parasitoida: Ichneumonoidea, Ceraphronoidea, and Proctotrupomorpha (Peters *et al.*, 2017; Polaszek & Vilhemsen, 2023).

The superfamily Ichneumonoidea comprises two of the largest families of hymenopterans, Ichneumonidae and Braconidae, of which there are between 41,000 and 48,000 described species (Sharkey & Wahl, 1992; Goulet & Huber, 1993; Huber, 2017). This superfamily is one of the most prevalent and successful of the parasitoids (Lotfalizadeh & Mohammadi-Khoramabadi, 2021), and famously helped inspire Charles Darwin's Theory of Evolution, leading to them being given the colloquial name of "Darwin wasps" (Klopfstein *et al.*, 2019). Wasps in this superfamily consist of both endo- and ectoparasitoids, as well as some hyperparasitoids; individuals of the family Ichneumonidae generally attack holometabolous insects (especially Lepidopterans) and spiders, whereas members of Braconidae commonly parasitize hemimetabolous insects (Goulet & Huber, 1993; Polaszek & Vilhemsen, 2023).

The superfamily of Ceraphronoidea is poorly understood, with little known about its hosts and habits despite being considered "common" (Goulet & Huber, 1993; Johnson & Musetti, 2004; Polaszek & Vilhemsen, 2023). Ceraphronoidea includes the two families Ceraphronidae and Megaspilidae, of which there are over 800 described species (Goulet & Huber, 1993); these wasps are likely endoparasitoids and/or hyperparasitoids (Goulet & Huber, 1993; Johnson & Musetti, 2004). Although few studies exist on this superfamily, Walker and Cameron (1981) reared wasps from the genus *Dendrocerus* Ratzeburg (Megaspilidae) from various species of the aphid parasitoid, *Aphidius* Esenbeck (Braconidae). This suggests that at least some individuals in this superfamily are hyperparasitoids of other hymenopterans.

Finally, the monophyletic clade Proctotrupomorpha is the third major lineage within Parasitoida. According to the most recent phylogenetic analyses on parasitoid hymenopterans, the superfamilies of Chalcidoidea, Cynipoidea, Diaprioidea, Mymarommatoidea, Platygastroidea, and Proctotrupoidea likely belong to this subclade (Blaimer *et al.*, 2023; Polaszek & Vilhemsen, 2023). Specifically, Cynipoidea and Platygastroidea probably form two lineages within Proctotrupomorpha, while the other four superfamilies belong to a clade (Peters *et al.*, 2017).

Chalcidoidea is the second-largest superfamily within Parasitoida, with approximately 23,000 described species spread across 19 families (Gibson *et al.*, 2011; Huber, 2017). Like Ichneumonoidea, chalcidoids are commonly used, and highly successful, pest control agents (Gibson *et al.*, 2011; Lotfalizadeh & Mohammadi-Khoramabadi, 2021). However, despite this superfamily being speciose with a cosmopolitan distribution, and providing significant economic/agricultural benefits, they are still considered understudied (Gibson *et al.*, 2011). Thus, although many species have been described, many of the specific biologies and lifestyles of these insects have yet to be uncovered. In terms of parasitism, chalcidoids attack species in over 13 orders of holo- and hemimetabolous insects (including other hymenopterans), as well as various other invertebrates such as ticks (Acari), spiders, pseudoscorpions (Pseudoscorpiones), and nematodes (Nematoda) (Gibson *et al.*, 2011). Some families of Chalcidoidea are also phytophagous, such as the 'fig wasps' in the family Agaonidae which have a mutualistic relationship with fig trees (*Ficus* spp.) (Kjellberg *et al.*, 2005). Other families, such as Aphelinidae, are hyperparasitoids and even autoparasitoids, in which males of certain species develop as parasites within larval females (Williams & Polaszek, 1996).

Cynipoidea is a superfamily of parasitoid wasps with approximately 3,000 described species (Liu *et al.*, 2007). There are six families within Cynipoidea; however, they are best known for the gall-forming family of Cynipidae–one of the few other examples of phytophagous hymenopterans (Goulet & Huber, 1993). Other members of this superfamily are endoparasitoids of holometabolous insects and hyperparasitoids of braconid and chalcidoid wasps (Liuet *et al.*, 2007; Buffington *et al.*, 2020). Many cynipoids are pests as they destroy agricultural crops through gall formation (Borowiec *et al.*, 2014) and negatively impact the efficacy of certain biocontrol agents through hyperparasitism (Ferrer-Suay *et al.*, 2021). Other cynipoids, however, have been a potential source of biocontrol for weeds. For example, the gall-forming *Aulacidea subterminalis* Nisbett (Cynipidae) has been used to successfully combat the invasive mouse-ear hawkweed (*Pilosella officinarum* L.) in New Zealand (Klöppel *et al.*, 2003).

Diaprioidea is a small, newly designated superfamily within Proctotrupomorpha with approximately 2,100 described species (Polaszek & Vilhemsen, 2023). Past literature (e.g., Goulet & Huber, 1993; Loiácono *et al.*, 2013; Huber, 2017) designated this superfamily as part of Proctotrupoidea; however, recent genetic studies suggest it is its own independent lineage (Blaimer *et al.*, 2023; Polaszek & Vilhemsen, 2023). Diaprioid wasps are generally endoparasites of dipterans, although some families also attack coleopterans and formicids (Goulet & Huber, 1993; Polaszek & Vilhemsen, 2023). Interestingly, some species in this family have a close association with ant colonies and have evolved unique morphologies to live inside of nests and parasitize broods (Loiácono *et al.*, 2013).

The superfamily of Mymarommatoidea is tiny, both in body size and number of described species. Mymarommatoidea contains only one extant family–Mymarommatidae–of which there are 10 described species (Gibson *et al.*, 2007; Huber *et al.*, 2008). These insects range between 0.3 mm and 0.8 mm in size and, consequently, are rarely collected and thus poorly understood (Gibson *et al.*, 2007). Almost nothing is known about their biology, although they may be egg parasitoids as other microscopic hymenopterans (Goulet & Huber, 1993; Gibson *et al.*, 2007).

Platygastroidea is a moderately sized superfamily with over 4,000 described species (Goulet & Huber, 1993; Huber, 2017). Previous literature divided this superfamily into two families (Platygastridae and Scelionidae; Goulet & Huber, 1993); however, recent phylogenetic research suggests that there are seven extant families: Geoscelionidae, Janzenellidae, Neuroscelionidae, Nixoniidae, Platygastridae, Scelionidae, and Sparasionidae (Chen *et al.*, 2021). Platygastroid wasps are almost exclusively egg parasitoids, attacking various insect groups (e.g., Diptera, Coleoptera, Hemiptera, Neuroptera, Lepidoptera, etc.) as well as spiders (Chen *et al.*, 2021).

Finally, Proctotrupoidea is the last subfamily within Proctotrupomorpha to be discussed and is one of the least speciose since Diaprioidea (Diapriidae) was declared its own superfamily. This superfamily has 10 extant families and approximately 400 described species, almost all of which originate from the family Proctotrupidae (Huber, 2017). These wasps generally parasitize larval coleopterans, although some may parasitize dipterans and, rarely, neuropterans (Huber, 2017).

1.5 *Peristenus* spp. as biocontrol agents

Peristenus Förster is a genus of solitary wasp belonging to the superfamily Ichneumonoidea, family Braconidae, subfamily Euphorinae. These wasps are endoparasitoids of Hemipterans in the family Miridae (plant bugs). Female *Peristenus* wasps attack nymphal mirids, laying their eggs inside of the host insect. From there, the larval *Peristenus* eventually mature and rupture from their host, killing them (Loan, 1974). This trait makes them a desirable biocontrol agent and, indeed, *Peristenus* spp. are best known for their status as biocontrol agents of the phytophagous pests, *Lygus* Hahn (Miridae); however, it should be noted that certain species of *Peristenus* parasitize other mirid pests such as *Adelphocoris* spp. Reuter (Day *et al.*, 1992; Goulet & Mason, 2006).

Peristenus spp. have a near-global distribution, found in Nearctic, Palearctic, Indo-Malayan, and Afrotropical regions (GBIF Backbone Taxonomy, 2023). Goulet and Mason (2006) described 29 species of *Peristenus* found in North America, twelve of which were associated with parasitizing *Lygus*. Of these twelve, nine species were native to North America (*P. braunae* Goulet, *P. dayi* Goulet, *P. carcamoi* Goulet, *P. gillespiei* Goulet, *P. mellipes* Cresson, *P. otaniae* Goulet*, P. pseudopallipes* Loan, *P. broadbenti* Goulet, and *P. howardi* Shaw) and three species were introduced from Europe (*P. digoneutis* Loan, *P. relictus* Ruthe, and *P. rubricollis* Thomson). A recent molecular taxonomic revision by Zhang *et al.*, (2017) concluded that only three species of native *Lygus*-parasitizing *Peristenus* exist in the Nearctic: *P. dayi, P. mellipes*, and *P. howardi*. This resulted in six of the previously described species by Goulet and Mason (2006) to be reclassified as these three species: *P. braunae* syn. *P. dayi*; *P. carcamoi, P. otaniae,* and *P. pseudopallipes* syn. *P. mellipes*; *P. broadbenti* and *P. gillespiei* syn. *P. howardi* (Zhang *et al.*, 2017).

All three native species of *Peristenus* occur in southern Alberta and are active at staggered times during the growing season, beginning with *P. dayi*, followed by *P. mellipes* and then *P. howardi* (Fernández *et al.*, 2018). These wasps attack *Lygus* spp. in various crop types (i.e., alfalfa (*Medicago sativa* L.), canola (*Brassica napus* L.), mustard (*Sinapis arvensis* L.), etc.); however, mean parasitism levels of *Peristenus* spp. recorded across North America are generally considered to be low, ranging between approximately 0% to 20% (e.g., Lim & Stewart, 1976; Braun *et al.*, 2001; Broadbent *et al.*, 2006; Carignan *et al.*, 2007; Fernández *et al.*, 2018). While there are some exceptions, such as Braun *et al.* (2001) and Fernández *et al.* (2018) reporting peak parasitism levels in alfalfa of 70% and 49%, respectively, in the Canadian Prairies, generally parasitism rates are not considered high enough to control *Lygus* populations below certain economic thresholds, such as in canola (Cárcamo *et al.*, 2024). Thus, attention has shifted towards establishing European species of *Peristenus* as biocontrol agents in North America.

One species of *Peristenus*, *P. digoneutis*, was first released in New Jersey, United States in 1979, where it nearly tripled the parasitism rates of native *Lygus* species pests in alfalfa (Day *et al.*, 1990). Subsequent sampling in alfalfa showed that the mean parasitism rate of *Lygus* rose to approximately 40%

to 50% after the establishment of *P. digoneutis* by the 1980's, resulting in a nearly 75% reduction in *Lygus* numbers (Day, 1996). *Peristenus digoneutis* has since migrated into Québec, Canada (Broadbent *et al.*, 1999), and there has been interest in relocating it to the Canadian Prairies due to its success as a biocontrol agent in the northeast USA. However, prior to doing so, further baseline data must be established on the parasitism rates of *Lygus* by native *Peristenus* across a multitude of crop types. Previous studies in the Canadian Prairies (e.g., Braun *et al.*, 2001; Fernández *et al.*, 2018) have primarily focused on *Peristenus-Lygus* parasitism rates in canola and alfalfa, without extensive sampling of other, less common crops such as flax (*Linum usitatissimum* L.), hemp (*Cannabis sativa* L.), and lentils (*Vicia lens* (L.) Coss. & Germ.). Similarly, little data are available on parasitism rates in uncultivated plant species (i.e., "weeds"), leading to a gap in knowledge that should be addressed before the potential relocation of the exotic *P. digoneutis*.

1.6 Proposed studies & predictions

Understanding the life cycles, phenology, and natural history of beneficial insects is essential to enhancing integrated pest management strategies, which in turn allows us to better balance sustainability and conservation with agricultural productivity. The focus of my thesis is to expand our knowledge on the biology and composition of parasitoid hymenopterans in southern Alberta by 1) evaluating the parasitism rates of *Peristenus* spp. across various plant species and 2) surveying the genera of Parasitoida found in canola crops and how their diversity may change in response to surrounding edge types. These studies are presented in chapters 2 and 3 of this thesis, respectively.

In chapter 2 of this thesis, a two-year study beginning in 2022 was conducted to survey the *Peristenus-Lygus* parasitism rates across various cultivated and uncultivated plants throughout the summer growing seasons. The complete list of sampled plant species is outlined in chapter 2's methods section; however, of the sampled cultivated crops I predict that parasitism rates will be highest in alfalfa and lowest in brassica crops such as canola and mustard, as shown in previous surveys done in the Canadian Prairies (Braun *et al.*, 2001; Fernández *et al.*, 2018). Regarding uncultivated plants, few studies have evaluated *Peristenus-Lygus* parasitism rates in the specific species that were sampled in this study. The majority of previous literature simply refers to uncultivated plants as "weeds" or an unspecified species (e.g., Broadbent *et al.*, 2006), or the sampled plant species were not of the same variety (e.g., Lim & Stewart, 1976; Carignan *et al.*, 2007). However, I predict that parasitism rates may be higher in uncultivated plants (weeds) compared to cultivated crops, as it is uncommon agricultural practice to spray uncultivated plants with insecticides.

In chapter 3, I surveyed the genera of parasitoid hymenopterans in canola fields and evaluated whether different non-crop edge boundaries affected the biodiversity of said parasitoids. In this thesis, I quantify using biodiversity indices (Morris *et al.*, 2014); thus, I measured biodiversity via the Shannon Diversity Index based on richness (the total number of unique individuals) and evenness (the relative abundance of individuals). I also used richness and abundance (the total number of individuals) as additional, individual measurements of diversity. As the survey in chapter 3 only identified specimens to genus, I used richness of genera rather than richness of species. Three different types of surrounding edge boundaries were evaluated: road verges, shelterbelts, and coulees. I speculate that these three edge boundaries range from low to medium to high community complexity. Thus, I predict that biodiversity will be highest in fields bordering coulees based on the habitat heterogeneity hypothesis, which posits that species diversity increases when more resources (and therefore niches) are available within a habitat, including agroecosystems (Benton *et al.*, 2003). I also expect to collect specimens from nearly all of the superfamilies of Parasitoida, excluding Mymarommatoidea as there are only 10 species that are very difficult to observe (Gibson *et al.*, 2007). In particular, I expect the majority of my samples to come from Ichneumonoidea and Chalcidoidea due to the sheer abundance and speciosity of both subfamilies (Sharkey & Wahl, 1992).

1.7 Summary of main results

My findings from chapter 2 conclude that: 1) *Lygus-Peristenus* parasitism levels were higher across all sampled crops compared to previous studies, and 2) An interaction between host plant and *Lygus* instar stage effected parasitism levels in two different plant types (canola and hemp). This observed effect may be linked to the flowering times of both plants, which coincides with parasitoid and/or pest phenology, parasitoid and/or pest preference for specific crops, or other contributing factors. In chapter 3, I report that: 1) Edge types are not the major driver of parasitoid biodiversity in canola fields in southern Alberta; rather parasitoid diversity and community composition differed greatly between farms, and 2) A genus of braconid wasp, *Diolcogaster*, is the dominant group of parasitoids found in sampled canola fields, far outweighing other parasitoids in abundance.

Both studies not only provide new insight into the biology of the lesser-known parasitoid hymenopterans but, as is with most research, raise new questions that can inspire future research.

1.8 References

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2. PARASITISM RATES OF *PERISTENUS* SPP. (HYMENOPTERA: BRACONIDAE) ON *LYGUS* PLANT BUGS (HEMIPTERA: MIRIDAE) IN CULTIVATED AND UNCULTIVATED PLANT HOSTS IN SOUTHERN ALBERTA.

2.1 Abstract

The plant bugs, *Lygus* Hahn (Hemiptera: Miridae), are serious agricultural pests that feed on a variety of plant species, causing significant damage and loss of quality to crop products. These plant bugs can be managed by the biocontrol agent, *Peristenus* Förster (Hymenoptera: Braconidae), a genus of parasitoid wasp. In southern Alberta, three species of native *Peristenus* attack *Lygus* at staggered times throughout the growing season; however, previous surveys on *Peristenus-Lygus* parasitism rates in North America have suggested that native *Peristenus* may be ineffective at keeping *Lygus* populations below economic threshold levels, leading to a lack of adequate crop protection. This study aimed to evaluate *Peristenus-Lygus* parasitism rates in southern Alberta by surveying a varied selection of both cultivated and uncultivated host plants. Seventeen types of host plants were sampled, twelve of which were cultivated crops and five of which were uncultivated species. We found no significant difference in parasitism rates across all sampled host plants and observed higher overall parasitism rates across most plant types compared to previous studies. In late-stage canola and hemp, we report significantly different parasitism rates among *Lygus* instar ages. This interaction is likely associated with the flowering stages of these plants, either through 1) temporal synchronization of the seasonal occurrence of both *Lygus* and *Peristenus* spp., or 2) plant preference of either parasitoid or pest. This survey provides baseline data on *Peristenus-Lygus* parasitism rates in the Canadian Prairies, and emphasizes the importance of considering crop, parasitoid, and pest phenology in integrated pest management strategies to suppress pest populations.

2.2 Introduction

In North America, *Lygus* Hahn (Hemiptera: Miridae) plant bugs are polyphagous insects that feed on a wide variety of cultivated and uncultivated plants (e.g., Kelton, 1975; Lim & Stewart, 1976; Schwartz & Foottit, 1998; Broadbent *et al.*, 2006; Carignan *et al.*, 2007; Braun *et al.*, 2012). *Lygus* are significant agricultural pests as they use their proboscides to pierce and feed on plant tissue, causing buds and flowers to shed and seeds to collapse, thereby lowering crop quality and yield (Kelton, 1975; Butts & Lamb, 1990). Historically, these pests have been controlled via chemical intervention; however, they can evolve pesticide resistance (Snodgrass, 1996), necessitating the use of alternative techniques, including biocontrol.

Forty-three species of *Lygus* exist in North America (Schwartz & Foottit, 1998), three of which are common in southern Alberta agroecosystems (*Lygus borealis* Kelton, *L. keltoni* Schwartz & Foottit, and *L. elisus* Van Duzee), and one of which is rare (*L. lineolaris* Palisot de Beauvois; Cárcamo *et al.*, 2012). These Canadian prairie *Lygus* species undergo two or three generations during the growing season (Cárcamo *et al.*, 2006). The first generation arises in the spring from overwintering adults while the second generation arises later in the growing season, with the potential for a third generation, depending on temperature fluctuations.

One group of natural enemies of *Lygus* plant bugs comprises species of parasitoid wasp in the genus *Peristenus* Förster (Hymenoptera: Braconidae). In Alberta, three species of *Peristenus* occur at staggered times during the growing season, i.e., the spring species, *Peristenus dayi* Goulet, the early summer species, *P. mellipes* Cresson, and the late summer species, *P. howardi* Shaw (Fernández *et al.*, 2018). *Peristenus* wasps are endoparasitoids that oviposit into young *Lygus* bug nymphs (2nd - 3rd instars). When an egg hatches, the resulting wasp larva then matures inside of its host, killing the plant bug.

The use of parasitoid wasps is a popular and effective pest management strategy in agroecosystems (Wang *et al.*, 2019); however, native North American *Peristenus* may be ineffective at crop protection due to low parasitism rates (e.g., Lim & Stewart, 1976; Wise *et al.*, 2000; Braun *et al.*, 2001; Carignan *et al.*, 2007). For example, in canola and other Brassicaceae crops, mean parasitism rates are approximately 0 - 5% (Braun *et al.*, 2001; Fernández *et al.*, 2018). Other species such as alfalfa can support slightly higher levels of *Peristenus* parasitism of *Lygus*: in Quebec, Lim & Stewart (1976) reported an average parasitism rate of 5% in surveyed alfalfa, while in Ontario, Broadbent *et al.* (2006) found the mean parasitism in weedy alfalfa sites to be less than 11%. Similar results were reported in a mixed-alfalfa site by Carignan *et al.* (2007), with a mean percent parasitism of ~10%. Fernández *et al.* (2018) found typical parasitism rates between 8 - 17% in alfalfa in southern Alberta, although they reported peak rates as high as 49% during the season. Similarly, Braun *et al.* (2001) reported a mean percent parasitism of <10%, although peak parasitism rates reached as high as 70% in Saskatchewan alfalfa for single dates.

While the above studies do not designate a difference, alfalfa crops can be classified as hay (forage) alfalfa or seed alfalfa due to differing crop-management practices. Hay alfalfa is grown for animal feed and/or bedding and is cut throughout the growing season, while seed alfalfa is left to flower and produce seeds for future replanting. Mayer *et al.* (1998) surveyed *Peristenus-Lygus* parasitism rates in both seed and hay alfalfa in Washington state and found the mean percent parasitism of hay alfalfa to be 16%, and seed alfalfa to be 23%. Seymour *et al.* conducted a similar survey in 2005 and found hay alfalfa parasitism rates to be <6% while seed alfalfa recorded parasitism rates between 15 - 26%.

Lygus bugs are generally less prevalent in cereal crops in Canada compared to Europe. Wise *et al.* (2000) observed that juvenile *L. lineolaris* (an uncommon species in southern Alberta) collected from Manitoba do not feed on wheat and that adults will only feed briefly before overwintering. Similarly, European species composition of *Lygus* is different than those in North America (Bilewicz-Pawinska, 1982), which could contribute to the lower occurrence of *Lygus* spp. observed in cereal crops in Canada. Thus, sampling was not conducted in any cereal crop for this study.

Studies on uncultivated host plants have found varying levels of *Peristenus-Lygus* parasitism and are generally underrepresented in the literature. Lim & Stewart (1976) found mixed-weed sites (*Erigeron philadelphicus* L., *Brassica hirta* Moench, *Chrysanthemum leucanthemum* L., etc.) in Quebec to have a mean rate of parasitism of 8%. Carignan *et al.* (2007) reported parasitism levels between 3 and 14% in dandelion (*Taraxacum officinale* L.), 2 - 8% in yellow rocket (*Barbarea vulgaris* L.), and 1 - 3% in fleabane (*Erigeron* sp.). Fernández *et al.* (2018) sampled various weeds (ball mustard, flixweed, wild mustard, etc.) in southern Alberta and found parasitism ranging from 7 - 42%. In the northwestern United States, Seymour *et al.* (2005) found parasitism to peak at 11% in kochia (*Bassia scoparia* L.), and Mayer *et al.* (1998) found parasitism ranging between 3 - 33% in uncultivated plants (white clover (*Trifolium repens* L.), pepper weed

(*Lepidium campestre* (L.) R.Br.), and hoary cress (*Cardaria draba* (L.) Desv.)), although the survey's sample size was small.

The current study was conducted to evaluate the levels of *Peristenus*-*Lygus* parasitism in both cultivated and uncultivated host plants in southern Alberta. This survey will provide baseline data on native species of *Peristenus* parasitism rates before the potential relocation of an exotic biocontrol agent, *Peristenus digoneutis*, into the Canadian Prairies. This parasitoid, a native of Europe, was released in 1979 in alfalfa sites in the northeastern USA (Day *et al.*, 1990); it has since moved adventively to Quebec and Ontario, where it is now well established and dominant (Goulet and Mason 2006). *Peristenus digoneutis* is a highly effective pest control agent of *Lygus* bugs, with parasitism levels exceeding 40% in New Jersey alfalfa sites, compared to native braconid species whose *Lygus* parasitism levels only reached 15% (Day, 1996). This has raised interest in relocating the parasitoid to Western Canada, where native *Peristenus* species have been unable to control pests below economic threshold levels in crops such as canola (Cárcamo *et al.*, 2024).

2.3 Material & Methods

2.3.1 Study sites

Lygus bugs were sampled from May to August in 2022 and 2023 in a mix of cultivated and uncultivated plants across 61 sites in 23 different locations. Sampling occurred at each site one to three times throughout the growing season, corresponding with early (May to early-June), mid (early-June to mid-July), and late (mid-July to August) growing season stages. All sampling sites were within a 200 km radius of Lethbridge, Alberta (49°41′59″ N, 112°49′06″ W). Lethbridge is located within the Moist-Mixed Grass Ecoregion of the Canadian Prairies Ecozone. This ecoregion is a semiarid, mixed grass biome with a mean annual temperature of 5 °C and annual precipitation ranging from 250 to 350 mm (Shorthouse, 2010). Surrounding ecoregions are the Fescue Grassland and the Mixed Grassland, whose annual rainfall ranges between 400 - 500 mm and 250 - 350 mm respectively, with average temperatures of 3.5°C and 5°C, respectively (Shorthouse, 2010). Of the 61 sampled sites, 42 were in the Moist-Mixed Grass Ecoregion, four were in Fescue Grassland, and 15 were located in Mixed Grassland.

2.3.2 Host plants

Sampling occurred in nine cultivated host plant species, five uncultivated species, and five mixed-crops. The cultivated crops were alfalfa (*Medicago sativa* L. (Fabaceae)), canola (*Brassica napus* L. (Brassicaceae)), faba beans (*Vicia faba* L. (Fabaceae)), flax (*Linum usitatissimum* L. (Linaceae)), hemp (*Cannabis sativa* L. (Cannabaceae)), lentils (*Vicia lens* (L.) Coss. & Germ. (Fabaceae)), mustard (*Sinapis arvensis* L. (Brassicaceae)), sainfoin (*Onobrychis viciifolia* Scop. (Fabaceae)), and sunflower (*Helianthus annuus* L. (Asteraceae)). Alfalfa was separated into two separate study crops: hay alfalfa and seed alfalfa.

Wild host plants that were sampled were ball mustard (*Neslia paniculate* (L.) Desv. (Brassicaceae)), flixweed (*Descurainia sophia* (L.) Webb ex Prantl (Brassicaceae)), kochia (*Bassia scoparia* L. (Amaranthaceae), shepherd's purse (*Capsella bursa-pastoris* (L.) Medik. (Brassicaceae)), and wild mustard (*Sinapis arvensis* L. (Brassicaceae)). Finally, two types of alfalfa-dominant and three types of hemp-dominant mixed-crops were sampled: alfalfa-clover (*M. sativa-Trifolium* sp. (Fabaceae)), alfalfa-sainfoin (*M. sativa-O. viciifolia*), hemp-peas (*C. sativa-Pisum* sp. (Fabaceae)), hemp-sunflower (*C. sativa-H. annuus*), and hemp-lamb's quarters (*C. sativa-Chenopodium album* L. (Amaranthaceae)). We have combined alfalfa-dominant and hemp-dominant mixed-sites and renamed them alfalfa-mix and hemp-mix, respectively.

2.3.3 Lygus collection

Lygus nymphs were collected with a standard 38 cm diameter sweep net at increments of 10 sweeps in 180° arcs, and then aspirated into separate plastic containers. A minimum of 10 nymphs per third, fourth, and fifth instar were collected at each sampling site. Second instar nymphs were also collected from the field when available and were included in total parasitism rates when applicable. First instars were excluded from collection due to their size (<1 mm), making them difficult to dissect and detect parasitoids (Day, 2007). Nymphs were either dissected the day of collection or placed in a cold room for up to three days. The cold room was kept at 12 °C with a 16:8 light:dark cycle.

2.3.4 Dissection protocol

Lygus instars were identified morphologically (Schwartz & Foottit, 1992) using a dissection microscope. Live nymphs were euthanized in a large petri dish with 20% ethanol before dissecting them (ventral side up) using fine forceps or #0 size pins. If no *Peristenus* larvae immediately emerged from the perforated host, the nymph's abdomen was briefly explored with the insect pin to confirm a lack of parasitism. Any *Peristenus* larvae recovered from dissections were preserved in 95% ethanol.

2.3.5 DNA barcoding

Sixty-one parasitoid larvae across all host plants (25 from 2022 samples and 36 from 2023 samples) were sent to the Canadian Centre for DNA Barcoding in Guelph, Ontario, for DNA barcoding to identify the parasitoid species. While we expected that the majority, if not all the larvae that were found parasitizing *Lygus* nymphs would be *Peristenus*, molecular-level identification was needed to confirm this supposition, as other taxa, such as dipterans from the family Tachinidae, also parasitize *Lygus* bugs (Clancy & Pierce, 1966). DNA barcoding was also used to confirm the seasonal activity pattern of *Peristenus* species in southern Alberta, as reported by Fernández *et al.* (2018).

2.3.6 Analyses

The data were analyzed by separating the early and mid-crop growth stages into one data set, and the late crop growth stage into another. This division was chosen based on *Lygus* seasonal occurrence (Cárcamo *et al.*, 2006). The first generation of Lygus appears during the early- and mid-crop growth stages, while the second-generation spawns during the late-crop growth stage. Thus, we expect a bimodal peak in parasitism throughout the entire growing season, as parasitism rates would be driven by Lygus seasonality rather than that of *Peristenus*.

The percent parasitism was calculated by dividing the total number of parasitized nymphs by the total number of dissected nymphs. A generalized linear model with binomial distribution was used to analyze the percent parasitism across different crop types as well as instar ages, and least-square method was used to estimate the mean percent parasitism within the model. A Type III ANOVA was used to test if interactions between instar stage and crop type significantly affected parasitism levels. For post-hoc analysis, we used the Scheffe method to adjust for multiple comparisons. Finally, to analyze variables nested within any observed interactions, we used both effects slicing and pairwise comparisons.

All analyses were completed using R (version 4.3.1; R Core Team, 2024) or SAS/STAT (version 9.4; SAS Institute Inc., 2013) with the GLIMMIX procedure for generalized linear model fitting.

2.4 Results

2.4.1 Cultivated and uncultivated host plant parasitism survey

A total of 3,692 *Lygus* nymphs were dissected across all sampled host plants from 2022 to 2023, from which we found the total mean percent parasitism to be 30.60 ± 1.49% (mean ± SE); 95% CI [27.65%, 33.55%]. Figure 1 shows the mean percent parasitism (± SE) across all crop types sampled in 2022 and 2023. These data are also displayed in Table 1, which includes the total number of nymphs collected for each crop type and the peak parasitism levels recorded in each crop throughout our sampling timeline. Most notably, Table 1 shows sampled hemp crops peaking at 94.12% parasitism, followed by hay alfalfa at 88.00%, and canola at 73.33%.

There was no significant difference between parasitism levels across all sampled host plants, both in early/mid- ($F_{20, 101} = 1.37$, $p = 0.1531$) and late- ($F_{10, 122} = 0.98$, $p = 0.4656$) stage crop data. Similarly, there was no significant difference in parasitism levels across instar ages, where $F_{3, 101} = 0.00$, $p = 1.0000$ and $F_{3, 122} = 0.00$, $p = 1.0000$ for early/mid and late crop stage data, respectively. However, there was a significant interaction between instar age and crop type in late growth stage analysis ($F_{21, 122} = 2.09$, $p = 0.0070$). This interaction was not observed in early/mid-crop stages ($F_{39, 101} = 1.16$, $p = 0.2702$).

As there was a significant interaction between instar age and plant type in the late-stage crop data, we tested the least squares means with Sheffe-adjustment across all host plants and instar ages but did not find any samples with significantly differing means ($p \simeq 1.0000$). We then analyzed the individual effects of crop type and instar age nested within the interaction via effect slicing and found that canola ($p = 0.0035$) and hemp ($p < 0.0001$) significantly affected parasitism levels in third ($p = 0.0034$) and fifth ($p = 0.0285$) instars and had a marginally significant effect on fourth instars ($p = 0.0795$). There was no significant effect on second instar *Lygus* ($p = 0.5258$).

Using a simple effect comparison with Scheffe-adjustment we found that canola and hemp were driving the significant interaction between instar stage and plant type, which can be observed in Figure 2. In late-stage canola, the mean percent parasitism of second instars was 19.44 ± 4.12% (mean ± SE), which was significantly lower than the observed parasitism rate of 37.30 ± 3.21% (mean ± SE) in fourth instars (p = 0.0459). Similarly, third instars also had a lower parasitism rate of 24.44 ± 2.94% (mean ± SE) compared to fourth instars (p = 0.0345). In late-stage hemp, third instars had a significantly higher parasitism rate of 48.00 ± 4.81% (mean ± SE) compared to both fourth (17.02 ± 5.51%; mean ± SE) (p = 0.0035) and fifth instars (12.77 ± 4.89%; mean ± SE) (p = 0.001). No other significant interactions were detected across all crop types, instar ages, and growth stages.

2.4.2 Dissected larvae DNA sequencing

A sample of 61 larvae dissected from *Lygus* nymphs between 2022 and 2023 were sent for DNA barcoding. Four samples failed to be sequenced; the remaining 57 sequenced larvae were all identified to be in the genus *Peristenus*. For the early crop stage larvae, six out of six samples were identified as *P. dayi*. For the mid-stage sample of 13 larvae, two were *P. dayi*, seven were *P. mellipes*, and four were *P. howardi*. For late-stage larvae, three out of 38 samples were identified as *P. mellipes*, while the remaining 35 samples were all recorded as *P. howardi*. Thus, eight larvae were identified as *P. dayi*, 10 were identified as *P. mellipes*, and 39 were identified as *P. howardi*.

2.5 Discussion

Generally, we observed a higher parasitism rate across all crop types than previously reported in the literature. DNA barcoding confirmed that these parasitism levels were driven exclusively by *Peristenus* wasps, rather than other parasitoids of *Lygus* bugs.

In canola, parasitism levels averaged 32% and peaked at 73%, which was notably higher than previous recorded levels by both Fernández *et al.* (2018) and Braun *et al.* (2005), who reported <5% parasitism in Canadian Prairie canola crops. Similarly, sampled alfalfa sites exceeded previously reported parasitism levels (e.g., Lim & Stewart, 1976; Braun *et al.*, 2001; Broadbent *et al.*, 2006; Carignan *et al.*, 2007; Fernández *et al.*, 2018). Braun *et al.* (2001) also reported peak parasitism in alfalfa to be 70% in Saskatchewan, whereas we report a peak of 88%.

Interestingly, Seymour *et al.* (2005) reported Lygus parasitism rates to be between 15 - 26% in seed alfalfa, and a mean parasitism rate of <6% in hay alfalfa with one hay alfalfa site reaching peak parasitism of 81%. While we report a similar peak parasitism rate of 88% in hay alfalfa, Seymour *et al.*'s (2005) findings contrast our results as we report sampled hay alfalfa to have a mean percent parasitism of 35%, and seed alfalfa to be 7%. Similarly, Mayer *et al.* (1998) reported parasitism levels of 16% in hay alfalfa and 23% in seed alfalfa. Based on these previous surveys, we expected seed alfalfa to host a higher mean parasitism rate than hay alfalfa. This assumption was bolstered by the fact that seed alfalfa is not mowed throughout the growing season, unlike hay alfalfa, which is cut upwards of three times throughout the year to produce feed and bedding for livestock. Mowing is associated with a significant reduction in insect populations (Stiedle *et al.*, 2022), which could in turn affect parasitism levels. This discrepancy in lower observed parasitism rates in seed alfalfa could be due to seed alfalfa being sprayed with pesticides against alfalfa weevil throughout the growing season to preserve the seed-bearing crop, whereas hay alfalfa may be left untouched by pesticides to serve as animal feed. Other explanations of this contrast between previous surveys and ours could simply be due to seasonal differences, inter-annual differences, or geographic location, as both Mayer *et al.* (1998) and Seymour *et al.*'s (2005) surveys were conducted in the pacific northwest United States compared to the arid climate of southern Alberta.

The overall trend observed in this study of higher observed parasitism rates across all sampled crop types compared to previous studies could be attributed to several factors, such as typical seasonal variation, or possibly warming climate. As climate change continues to drive global temperatures upward, the Canadian Prairies have been notably susceptible to increasing temperatures (Mapfumo et al, 2023). Zhou *et al.* (2022) predict that the prairie provinces–particularly areas situated close to the Rocky Mountain region, where our sampling took place–are expected to reach historically high temperatures in the upcoming decades. Higher temperatures may promote *Lygus* growth and reproduction (Cárcamo *et al.*, 2006), which could in turn provide more hosts for *Peristenus* leading to increased populations and, consequently, increased parasitism rates.

We report a significant interaction between *Lygus* instar age and plant type on parasitism rates in late growth stages. This interaction was driven by sampled fourth instars in canola, whose mean parasitism rate of 37% was significantly higher than that observed in second and third instars (19% and 24%, respectively). Similarly, in hemp, third instars had a significantly higher parasitism rate (48%) than fourth and fifth instars (17% and 13%, respectively).

This observed interaction and differences in parasitism rates may be rooted in both ecological and phenological factors. One possible explanation could be the late-season flowering stages of both hemp and canola corresponding with *Peristenus* phenology. The timing of crop growth stages plays an important role in the population structure of *Lygus* bugs; Fleischer and Gaylor (1987) found that *L. lineolaris* are particularly attracted to crops at the start of their reproductive stage, specifically during the budding and flowering phases. Andow (2023) also found that *L. lineolaris* prefer to feed on meristematic tissue, apical buds, terminal flowers, and young seeds of host plants, and that females will lay their eggs on these tissues as they develop to provide important nutrition for the developing insects. Thus, we expect *Lygus* nymphs to be abundant on host plants during their flowering phases. Both canola and hemp flower later in the growing season compared to other sampled plants, and this late-season flowering corresponds with the activity period of the late-summer species, *P. howardi* (Fernández *et al.*, 2018). Our larvae sent for DNA barcoding corroborated the appearance of *P. howardi* during this seasonal timeframe, as 92% of dissected late-stage nymphs were parasitized by this species. Furthermore, this later flowering stage in both hemp and canola correlates with the seasonal occurrence of the second generation of *Lygus* bugs (Cárcamo *et al.*, 2006), leading to a likelihood of more nymphs being present on the plants; this would be further suitable for *Peristenus* spp., as they typically attack younger instar stages (Loan, 1974; Day, 2007). Finally, this explanation would support why we observed a significant peak in parasitism for third instars in hemp and fourth instars in canola: canola likely bloomed earlier than hemp, hence older instars having a higher parasitism rate. Thus, this temporal synchronization between the availability of suitable instar stages of *Lygus* bugs and the peak activity of *Peristenus* wasps, particularly the species *P. howardi*, may have led to the significant interaction between instar stage and plant type observed in our study.

Another possible explanation for the interaction between instar age and plant type could be due to plant species preference of either pest or parasitoid. Both *Lygus* and *Peristenus* spp. have been documented to show preference for certain plants; for example, Shahjahn (1974) found that *Peristenus pseudopallipes* (syn. *P. mellipes*) had a preferential reaction to species of *Erigeron* L. (Asteraceae) when compared to other plants tested in an olfactometer. Female wasps preferred to feed on species of *Erigeron* compared to the control (no plants) and other plant species approximately 65 – 70% of the time, and preferred feeding on flowering *Erigeron* spp. as opposed to ones without flowers approximately 70% of the time. Similarly, Halloran *et al.* (2013) documented strong preference in both *Lygus rubrosignatus* Knight and *Peristenus relictus* Ruthe for *Erigeron* plants when compared to cotton. Interestingly, when given the choice of cotton that had been damaged by feeding *Lygus* nymphs and undamaged *Erigeron*, female *P. relictus* wasps chose *Erigeron* ~50% of the time, yet chose *Erigeron* damaged by feeding nymphs compared to undamaged cotton nearly 100% of the time. *Lygus* routinely preferred *Erigeron* over cotton, regardless of nymphal damage on either plant. However, *Lygus* spp. have also been shown to have a strong preference to alfalfa (Sevacherian & Stern, 1974; Barman *et al.*, 2010; Shrestha & Rondon, 2024), a species we sampled but did not find the same significant interaction in, as we did canola and hemp. Should the hypothesis that plant preference be driving the interaction we see in this study, then it is likely due to *Peristenus* plant preferences rather than that of *Lygus* bugs. In other words, *Peristenus* prefer to parasitize *Lygus* on these plants because they are attracted to the plants themselves, not the relative abundance of *Lygus* nymphs. Future research examining this interaction between instar stage and parasitism rates in canola and hemp crops should be pursued, as the potential causal relationship of this phenomenon could be driven by a combination of ecological, phenological, or biological factors, or by something else entirely.

To conclude, few studies have collected *Peristenus-Lygus* parasitism data on such a wide range of cultivated and uncultivated host plants, and thus this survey provides valuable information on *Peristenus* biology and future direction of *Lygus* pest control. As there is growing intent to relocate *P. digoneutis* into the Canadian Prairies, these data will also provide baseline parasitism trends of native *Peristenus* before the potential introduction of the exotic biocontrol agent. Future research by the Cárcamo Lab to assess the feasibility and potential impact of integrating *P. digoneutis* into southern Alberta will include a competition study between the exotic and native *Peristenus* species, as well as determining the relative abundance of native *Peristenus* spp. Finally, this survey provides insight into the role of crop phenology, as well as the lifecycles of both pests and their natural enemies, when considering pest management strategies. The interactions between ecological and phenological factors observed in this study underscore the need for more research on these topics to create more effective integrated pest management programs.

2.6 Figures

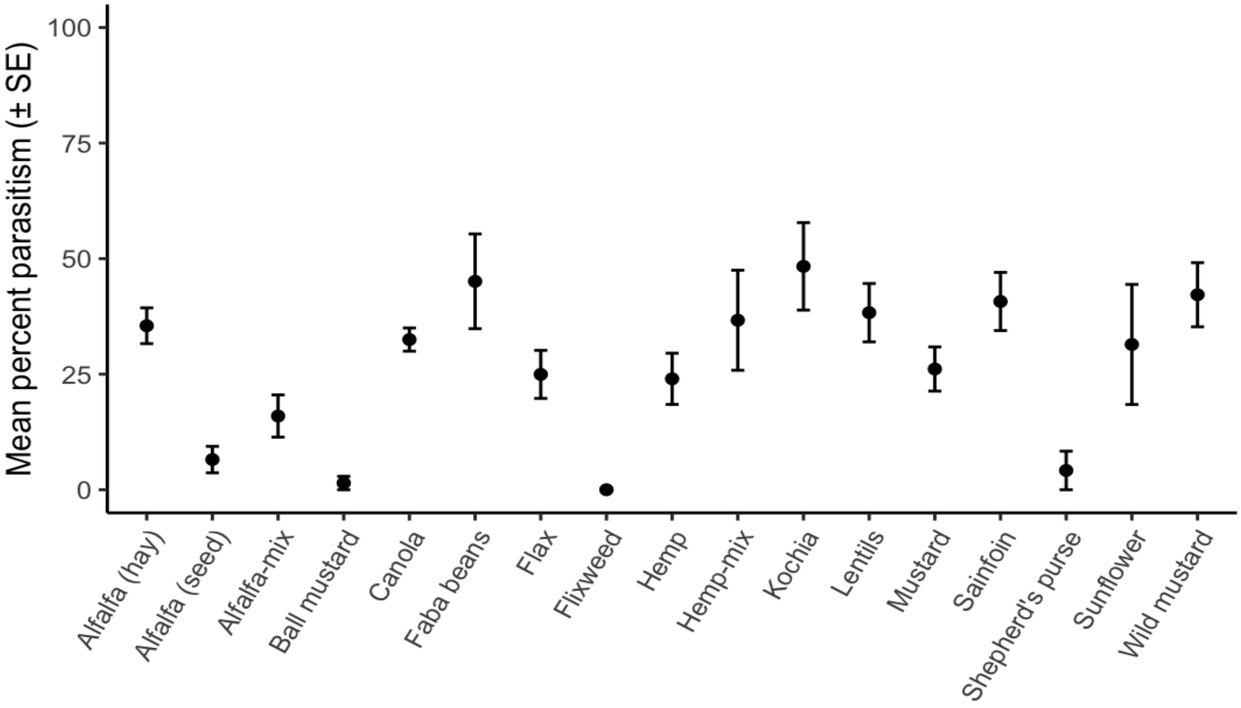

Figure 1. A scatter plot of the mean percent parasitism (± standard error) across all sampled host plants from 2022 to 2023.

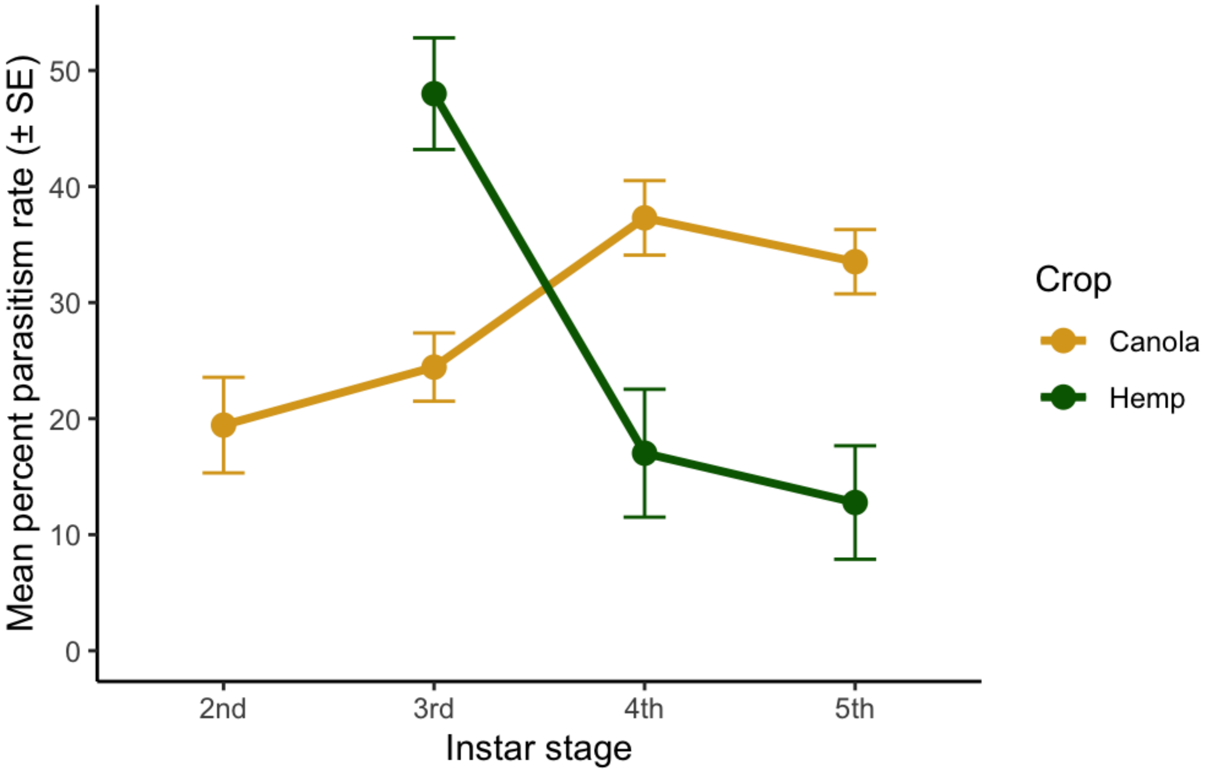

Figure 2. The interaction between instar age and plant type in late-stage canola and hemp.

2.7 Tables

Table 1. The total number of collected Lygus nymphs, the mean percentage (± standard error), and the upper and lower confidence intervals (CI) of the means in all sampled host plants from 2022 to 2023. Highest and lowest parasitism rates were those recorded in a single site at one time.

	Host plant	Total nymphs collected	Average parasitism rate (± SE)	Lower CL	Upper CL	Lowest parasitism rate	Highest parasitism rate
Cultivated	**Alfalfa** (hay) *Medicago sativa*	729	35.48 ± 3.87%	27.75%	43.22%	0.00%	88.00%
	Alfalfa (seed) *M. sativa*	142	6.53 ± 2.68%	0.47%	12.58%	0.00%	11.76%
	Canola *Brassica napus*	1080	32.49 ± 2.51%	27.51%	37.46%	1.67%	73.33%
	Faba beans *Vicia faba*	51	45.09 ± 10.25%	16.64%	73.54%	36.67%	57.14%
	Flax *Linum usitatissimum*	192	24.96 ± 5.20%	13.98%	35.94%	0.00%	47.50%
	Hemp *Cannabis sativa*	258	24.00 ± 5.54%	12.59%	35.40%	0.00%	94.12%
	Lentils *Vicia lens*	114	38.31 ± 6.31%	23.38%	53.24%	23.53%	46.39%
	Mustard *Sinapis arvensis*	373	26.12 ± 4.78%	16.07%	36.12%	10.00%	48.33%
	Sainfoin *Onobrychis viciifolia*	200	40.73 ± 6.29%	27.58%	53.89%	10.00%	68.42%
	Sunflower *Helianthus annuus*	70	31.43 ± 12.99%	-0.35%	63.21%	0.00%	55.00%
	Alfalfa-mix *M. sativa-Trifolium sp.* *M. sativa-O. viciifolia*	133	15.95 ± 4.57%	5.98%	25.91%	0.00%	43.75%
	Hemp-mix *C. sativa-Pisum sp.* *C. sativa-H. annuus* *C. sativa-Chenopodium album*	76	36.67 ± 10.82%	11.08%	62.25%	20.00%	69.23%
Uncultivated	**Ball mustard** *Neslia paniculate*	67	1.45 ± 1.45%	-4.79%	7.69%	1.45%	1.45%
	Flixweed *Descurainia sophia*	48	0.00 ± 0.00%	0.00%	0.00%	0.00%	0.00%
	Kochia *Bassia scoparia*	55	48.33 ± 9.46%	24.02%	72.64%	28.00%	66.67%
	Shepherd’s purse *Capsella bursa-pastoris*	24	4.17 ± 4.17%	48.78%	57.11%	4.17%	4.17%
	Wild mustard *Sinapis arvensis*	80	42.19 ± 6.94%	20.10%	64.28%	42.19%	42.19%

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3. EDGE EFFECTS ON PARASITOID HYMENOPTERAN BIODIVERSITY AND A SURVEY OF SUBCLADE PARASITOIDA IN CANOLA FIELDS.

3.1 Abstract

In the agriculture industry, parasitoid hymenopterans (subclade Parasitoida) are a highly effective form of pest control. These beneficial insects provide multiple services including the ability to target insecticide-resistant pests, host-specific suppression, and secondary pollination. Utilizing these beneficial insects also reduces the need for pesticides, which in turn provides both economic and environmental benefits. Non-crop habitats adjacent to arable land provide critical ecological services for these insects and can act as a corridor from uncultivated land to farmland; this interaction is a form of edge effect.

This study was conducted to evaluate how three types of non-crop field edges (road verges, tree shelters, and coulees) affected the diversity, richness, and abundance of parasitoid hymenopterans in canola fields, as well as how this diversity varied from distance from the edge. Sampled genera were then compiled into a survey of Parasitoida to better understand the composition of parasitoid hymenopterans found in southern Alberta fields. I conclude that edge type and distance from the edge appear to have little influence on the biodiversity of parasitoid hymenopterans; instead, it appears that individual farms have the greater influence on parasitoid biodiversity, implying that variables such as farm management practices or landscape-scale factors may be a stronger driver of parasitoid richness, abundance, and overall biodiversity, rather than non-crop edge boundaries. I also report that *Diolcogaster* spp. (Ichneumonoidea: Braconidae: Microgastrinae)—likely *Diolcogaster claritibia* Papp, first reported in Canada in 2014—to be the dominant group of parasitoids in southern Alberta canola fields, comprising >70% of collected specimens. This research is intended to both increase our understanding of integrated pest management strategies, as well as provide one of the few contemporary surveys of Parasitoida in southern Alberta.

3.2 Introduction

Contemporary agricultural practices have been extensively documented to have a widespread, negative impact on global biodiversity and environmental health (Campbell *et al.*, 2017). Loss of biodiversity, particularly insects, is a major concern globally (Wagner, 2020), and arthropods within arable land have been particularly susceptible to decline (Seibold *et al.*, 2019). Subsequently, a loss of arthropods leads to a loss of critical ecosystem and agriculture services such as biocontrol and pollination, which in turn has dramatic environmental and economic effects (Losey & Vaughan, 2006; Vandergen, 2013; Wagner, 2020).

Arthropods that prey on crop pests are referred to as natural enemies. Of the natural enemies, parasitoid hymenopterans (hereafter referred to as “parasitoids” or Parasitoida; Peters *et al.*, 2017; Polaszek & Vilhemsen, 2023) are particularly effective for biocontrol and have been suggested to be one of nature's most successful pest-suppressors (LaSalle & Gauld, 1992; Narendran, 2001). In addition to controlling pests, parasitoids also provide pollination (Zemenick, 2018) and may serve as bioindicators in agricultural settings due to their trophic interactions and close ecological relationships with other invertebrate groups (Anderson *et al.*, 2010). These qualities suggest that parasitoid hymenopterans may be keystone species (LaSalle & Gauld, 1992; Narendran, 2001), meaning their absence in the ecosystem would cause dramatic, cascading effects, and potential collapse and loss of function. Thus, the need to preserve and promote these valuable insects is evident as invertebrate populations decline globally, and hymenopterans are one of the most threatened groups (Sánchez-Bayo & Wyckhuys, 2019).

In agroecosystems, one driver of biodiversity decline is the disappearance of non-crop spaces (Benton *et al.*, 2003). Non-crop areas provide key resources for beneficial insects including shelter and mating sites (Thomson & Hoffmann, 2010), protection for overwintering (Geuger *et al.*, 2009), and food sources such as pollen and nectar (Zemenick *et al.*, 2018). Killewald *et al.* (2023) found that the number of beneficial insects increased when more non-crop resources were included around arable land, regardless of how the land was managed. The presence of these non-crop environments facilitates access to farmland, enabling natural enemies, including parasitoids, to contribute their pest-control services (Thies *et al.*, 2003).

More complex environments (including those within or around agroecosystems) are associated with increased biotic and abiotic resources, which in turn promotes biodiversity through niche availability (Pianka, 1966; Benton *et al.*, 2003). This is a fundamental hypothesis within ecological theory, termed the habitat heterogeneity hypothesis. Thus, my study set out to compare whether different types of non-crop edges increasing in complexity affected the biodiversity of parasitoid hymenopterans in adjacent canola fields. I sampled and identified hymenopterans belonging to the subclade of Parasitoida, which includes the three major lineages of Ichneumonoidea, Ceraphronoidea, and Proctotrupomorpha (Polaszek & Vilhemsen, 2023). I placed emphasis on this paraphyletic subclade because many groups within Parasitoida are understudied (LaSalle & Gauld, 1992)–with few surveys documenting the population composition of parasitoid hymenopterans in southern Alberta–and a vast majority of biocontrol agents reside within the superfamilies of Ichneumonoidea and Chalcidoidea (LaSalle & Gauld, 1992; Goulet & Huber, 1993; Gibson *et al.*, 1997; Polaszek & Vilhemsen, 2023).

I evaluated three different types of non-crop edges found within southern Alberta: road verges, shelterbelts, and coulees. These sites range from low, moderate, to high habitat complexity, respectively. Road verges are strips of groundcover, typically grasses and wildflowers, located along roadside edges. Verges provide basic ecosystem services in urban and agricultural landscapes such as habitats for pollinators, natural enemies, and wildlife (Hopwood *et al.*, 2015). They may also facilitate the dispersal of species or act as connectivity corridors to better quality habitats (Phillips *et al.*, 2019). Mowing road verges is a common form of weed management in agriculture. A review by Phillips *et al.* (2020) suggests that reducing mowing frequency of zero to two cuts per year may help conserve insect pollinator populations (namely lepidopterans); however, I recorded up to four mowings of verges during the summer of 2023 at my road verge sample sites. This may result in fewer resources available for other insects, including hymenopterans, leading to road verges being an overall lower quality, unstable habitat.

Shelterbelts are rows of shrubs or trees traditionally planted to act as windbreaks and to minimize soil erosion. This form of semi-natural habitat is a type of field boundary that was once popular across the Canadian Prairies but has since fallen out of favour in recent years due to lack of financial incentive to replant aging trees (Amichev *et al.*, 2020). Shelterbelts play a positive role in ecosystems through carbon sequestration (Amadi *et al.*, 2016), and by providing habitat sites for beneficial insects (Smith *et al.*, 2015), which in turn decreases greenhouse gas emission and promotes biodiversity, respectively. *Caragana arborescens* Lam. is a common shrub used for windbreaks in the Canadian prairies. As a flowering plant, it provides a source of nectar for parasitoids (Zemenick *et al.*, 2018) that is rarely disturbed, unlike the wildflowers that are frequently mowed in verges. While generally left undisturbed, shelterbelts are only small areas of habitat, usually consisting of one to three rows of trees that span anywhere from 50 m to >200 m. This small area, coupled with limited water availability, is why I chose to describe shelterbelts as mid-quality habitat.

Finally, coulees are a naturally occurring habitat within southern Albertan topography. Coulees are spatially complex basins or ravines that have been eroded by waterways with a variety of plant species within them; this includes trees, shrubs, wildflowers, and wild grasses. These traits and high resource availability suggest that coulees are a highly heterogeneous habitat, which in turn may lead to a high level of species diversity (Pianka, 1966).

This study also collected parasitoid hymenopterans at varying distances from field edges to determine how far the parasitoids migrate into cropland from surrounding habitat and if diversity measurements changed. Various studies have demonstrated conflicting results when investigating this variable. For example, Whitehouse *et al.* (2017) found that the abundance of natural enemies was significantly higher along the border of non-crop habitats comparted to 25 m into blueberry orchards. Furthermore, Rand *et al.*'s 2006 synthesis of literature on this topic generally found that spillover effects between non-crop and arable land does occur, but to varying degrees and distances. However, Bosem Baillod *et al.* (2017) surveyed cereal aphids and their predatory arthropods at field depths of 0 m, 10 m, and 30 m, and did not find a change in the predator density at any of the sampling points. Likewise, Marino & Landis (1996) found that parasitoid species richness was higher in fields surrounding more non-crop habitat, but that parasitoid activity (i.e., parasitism of host insects) did not differ significantly between distances of 5 m and 90 m from non-crop habitats. Thus, I collected samples at 10 m, 50 m, 100 m, and 200 m to determine if parasitoids were found at further distances in arable land bordering non-crop habitats, and if diversity and/or abundance of parasitoids differed the further they were from non-crop boundaries.

3.3 Materials & methods

3.3.1 Study sites

Fifteen study sites, seen in Figure 3, were selected from seven farms; to maintain producer anonymity, each farm was designated with a single letter identifier. One study site was located on Farm

“C”, four study sites were located on Farm “G”, and farms “A”, “E”, “K”, “L”, and “W” each had two study sites.

All sites were located within a 75 km radius of Lethbridge, Alberta (49°41′59″ N, 112°49′06″ W) (Figure 3). Lethbridge is located within the Moist-mixed Grass Ecoregion of the Canadian Prairie Ecozone; this region experiences annual precipitation rates between 350 and 450 mm and a mean annual temperature of 5°C, with the summer averaging 15.5°C (Ecological Stratification Working Group, 1996). Soil types within this ecoregion are Dark Brown Chernozemic or Solonetzic. Surrounding ecoregions include Fescue Grassland to the south and Mixed Grassland to the east. The Fescue Grassland Ecoregion has an annual precipitation rate of 400 to 500 mm, a mean annual temperature of 3.5°C, and a mean summer temperature of 14°C (Ecological Stratification Working Group, 1996). Soil types in this type of grassland are primarily Dark Brown and Black Chernozemic. Finally, Mixed Grasslands exhibit an annual precipitation rate of 250 to 350 mm, a mean summer temperature of 16°C, and an average annual temperature of 5°C (Ecological Stratification Working Group, 1996). This ecoregion has a primary soil type of Brown Chernozemic, with significant sections of Solonetzic soil.

Eleven out of 15 study sites were in Moist-mixed Grasslands; three of which bordered Fescue Grasslands and one of which bordered a Mixed Grassland. One site was in Fescue Grassland that bordered Moist-Mixed Grasslands, and the three remaining sites were located within the Mixed Grassland Ecoregion.

Each of the three sampled edge treatments (i.e., road verges, shelterbelts, and coulees) were represented in five fields, all geographically separated by at least 2 km. All sites were cultivated with canola (*Brassica napus* L.) located either on irrigated land (n = 7), or dryland (n = 8). Common plant species found in each edge type are documented in Table 2.

3.3.2 Sample collection

Each study site had two transects placed 50 m apart, running perpendicular to its designated edge treatment. To observe any changes in parasitoid diversity based on distance from the edge, sampling points were placed along each transect at 10 m, 50 m, 100 m, and 200 m, as illustrated in Figure 4. Collections occurred between May and August of 2023. Each field was sampled four times throughout the growing season, corresponding with key crop development stages to ensure hymenopterans with differing phenologies were included during collection. Utilizing the canola phenological scale outlined by the Biologische Bundesanstalt, Bundessortenamt and CHemische Industrie (BBCH) (Lancashire *et al.*, 1991) as a guideline, crops were sampled at the leaf germination and development stage (BBCH 10 - 29), the stem elongation stage (BBCH 30 - 53), the flowering stage (BBCH 55 - 69), and finally at the pod development and ripening stage (BBCH 71 - 89). Two sampling methods were used: pan traps during the early crop development stages (i.e., leaf development and stem elongation; Figure 4a), and sweep net sampling in later stages (i.e., flowering and pod development; Figure 4b).

I used yellow pan traps measuring 22 cm by 15 cm, with a depth of 6 cm, which I filled with 40% propylene glycol. Three pan traps, 25 m apart, were spread across each of the sampling points between both transects, and insects collected in all three of these pan traps were then counted as a single sample for that specific field depth (e.g., 10 m). As there were four sampling points across the transects (10 m, 50 m, 100 m, and 200 m), there was a total of 12 pan traps, or four samples, collected per field.

In later crop stages, I transitioned to sweep net sampling which consisted of 200 sweeps at 180° arcs between both transects at each sampling point. Effectively, this meant I collected 200 sweeps at each of 10 m, 50 m, 100 m, and 200 m, leading to a total of 800 sweeps per field. This protocol was replicated in each field twice throughout the remainder of the growing season. Samples were then transferred to emergence boxes to selectively capture any parasitoid hymenopterans. Emergence boxes used in this study were 30 cm by 25 cm by 30 cm (length x width x height) cardboard boxes with sealed edges to deprive the inside of light. One face of the box was covered with opaque black fabric to allow the deposit of samples. A mesh screen with 3.5 mm openings was inserted into each box to separate large insects from smaller, flying insects (i.e., parasitoid hymenopterans). A small opening was cut into the box on the other side of the mesh filter, attracting the flying insects to light, where they were subsequently captured in a 50 mL falcon tube filled with 95% EtOH. Emergence boxes were left for a minimum of 4 days to allow for samples to sort themselves; afterwards the boxes were searched manually to retrieve any missing specimens.

3.3.3 Comparison of edge types

Each of the three edge treatments bordered five fields (n = 15), and each field was sampled four times throughout the growing season. During each sampling period, specimens were collected at four distances from the edge; thus, a total of 240 samples were collected (excluding the additional 20 samples that were used to compare collection methods between pan traps and sweep net sampling; see section 2.7). Of the 240 samples collected, seven were excluded due to mislabelling (n = 3) or loss of specimens (n = 4). Examples of specimen loss included pan traps that were overturned in the field, or holes in emergence boxes resulting in the escape of collected samples.

3.3.4 Comparison of sampling methods

Four sites were selected at random to compare collection methods between pan traps and sweep net sampling. Pan traps in these five fields were left for an extra two weeks to collect specimens during the flowering phase, whereas the remaining 10 fields had their pan traps removed prior to flowering. Upon removal of the comparison pan traps, the fields were then swept as per the outlined methodology. Of the 32 samples used in this comparison, three were excluded: one pan trap sample and one sweep net sample at the same distance from the same site, due to mislabelling, and one pan trap sample due to loss of specimens.

Pan trap samples used in this comparison were excluded from the final biodiversity analyses; however, they were included in the overall survey of parasitoid genera.

3.3.5 Identification of genera

Specimens were identified to the level of genus; if genera could not be identified, specimens were identified to the nearest taxonomic level and listed as a morphotype (e.g., “Pteromalinae, Morphotype A”). Identification was done primarily through taxonomic keys (e.g., Goulet & Huber, 1993; Gibson *et al.*, 1997), as well as through DNA barcoding via the Canadian Centre for DNA Barcoding (CCDB, University of Guelph). Barcoding was used to both identify unique specimens, as well as confirm the identity of previously keyed parasitoids.

A level of uncertainty regarding specimens within the Proctotrupomorpha clade and lower-level classification (subfamilies and genera) of Pteromalidae (Chalcidoidea) should be noted. No lower-level taxonomic keys exist for Nearctic Cynipoidea, for example, and thus sample identification relied heavily on DNA barcoding and could not be confirmed morphologically. Few keys exist for other superfamilies in

Proctotrupomorpha. Likewise, pteromalids are highly varied and notoriously difficult to identify via taxonomic key (Gibson *et al.*, 1997), and not all specimens could be identified via DNA barcoding.

3.3.6 Identification of Diolcogaster spp.

Sixteen *Diolcogaster* specimens were selected at random from various sites and crop growth stages to be identified to species. Fifteen specimens were identified morphologically (Fernández-Triana *et al.*, 2014; Moghaddan *et al.*, 2019; Fernández-Triana *et al.*, 2020; J. Fernández-Triana, personal communication, August 8, 2023), and one specimen was sent for DNA barcoding to the Canadian Centre for DNA Barcoding in Guelph, Ontario.

3.3.7 Analyses

Statistical analyses were done in R version 4.3.1 (R Core Team, 2024). Diversity was measured using the Shannon Diversity Index (SDI), genera richness, and abundance of individuals. Analysis of variance (ANOVA) was used to compare the difference between pan trap and sweep net sampling methods. As this study included both fixed effects (i.e., distance from the edge, edge type, crop stage, and farm) and random effects (i.e., repeated sampling of both study site and distance from the edge), a Generalized Linear Mixed Model (GLMM) was used to analyze the data. Specifically, a GLMM using the Template Model Builder in R (package 'glmmTMB'; Brooks *et al.*, 2017) with a negative binomial distribution was used to model genera richness and abundance, as the data were over-dispersed and right-skewed. A gaussian distribution was used to model SDIs as the data were continuous and normally distributed. Likelihood ratio tests (LRT) (package 'car'; Fox & Weisberg, 2019) were used to test the significance of fixed effects in each model. Post-hoc analysis of the data was done using least squares means, with the Tukey method to adjust for multiple comparisons. Since the data were not normally distributed, a Spearman correlation analysis was used to estimate if the presence of *Diolcogaster* impacted other genera as well as community evenness. To estimate the relative frequency and relative abundance that genera are found in relation to edge habitats, an indicator species (i.e., genus) analysis was conducted using package 'labdsv' in R (Roberts, 2023); results are not shown in this chapter (see Appendix A). The Bray-Curtis dissimilarity metric and non-metric multidimensional scaling (NMDS) ordination were performed using the ‘vegan’ package in R (Oksanen *et al.*, 2024) to visualize community composition.

3.4 Results

3.4.1 Comparison of sampling methods

The Shannon Diversity Index, richness of genera, and abundance were used to compare between pan trap and sweep net sampling. The sampling methods were nearly significant when estimating diversity via the SDI ($F_{1, 27} = 3.21$, $p = 0.0843$), with pan trap sampling having a higher SDI than sweep net sampling. Richness of genera did not significantly differ between sampling methods ($F_{1, 27} = 2.85$, $p = 0.1030$). Abundance did significantly differ between the two sampling methods ($F_{1, 27} = 10.35$, $p = 0.0034$), with sweep net sampling resulting in a greater collection of individuals than pan traps.

3.4.2 Effect of fixed effects

There was no significant difference in the SDIs of parasitoid hymenopterans among any of the edge treatments (GLMM, LRT: $\chi^2 = 2.35$, df = 2, $p = 0.3086$), nor was there any significant difference between the richness of genera among any edge treatment (GLMM, LRT: $\chi^2 = 0.89$, df = 2, $p = 0.6392$). Modelling suggested that edge boundaries could have a marginal effect on the abundance of parasitoids (GLMM, LRT: $\chi^2 = 4.51$, df = 2, $p = 0.1048$), particularly between coulee and road verge sites (GLMM: $\beta = -0.35$, SE = 0.20, $z = -1.72$, $p = 0.0842$); however, after adjusting for multiple comparisons, the post-hoc analysis did not find any significant difference between these two treatments (LS-means: $p = 0.1952$).

Parasitoid richness was significantly affected by distance from the field edge (GLMM, LRT: $\chi^2 = 17.91$, df = 3, $p = 0.0005$). After adjusting for multiple comparisons, the post-hoc analysis found that richness was highest closest to the edge at 10 m compared to 50 m (LS-means: $\beta = -0.28$, SE = 0.09, $z = -2.95$, $p = 0.0166$), 100 m ($\beta = -0.34$, SE = 0.09, $z = -3.61$, $p = 0.018$), and 200 m ($\beta = -0.31$, SE = 0.09, $z = -3.35$, $p = 0.0045$) across all treatments (Figure 5a). While the GLMM LRT suggested a nearly significant effect of distance from the edge on SDI ($\chi^2 = 6.45$, df = 3, $p = 0.0914$), there were no significant differences in the Shannon Indices at any distance after adjusting for multiple comparisons (LS-means: $p > 0.05$). Despite this, diversity still tended to be highest at the edge and decreasing further into the field (Figure 5b).

Abundance followed a similar pattern to the other biodiversity measurements, being highest at the edge and lower further into the field (Figure 5c), although there was no significant difference in abundance between distances from the edge (GLMM LRT: $\chi^2 = 6.08$, df = 3, $p = 0.1077$). Figure 5c also shows an outlier at 10 m in the shelterbelt treatment exceeding 800 individuals; this was driven by a high number of *Diolcogaster* spp. (Ichneumonoidea: Braconidae) collected at this site, discussed further in section 4.4.3.

The overall similarities within the community composition can be further visualized through NMDS ordination plots (Figure 6). The 95% confidence intervals of parasitoid hymenopteran community composition for the three different edge types (Figure 6a) and the four different distances from the edge are represented as ellipses and show a high level of overlap of community composition across the levels of both factors.

While edge treatment and distance from the edge had little to no effect on both diversity measurements, GLMM models showed significant differences in all biodiversity measurements between farms (GLMM LRT, SDI: $\chi^2 = 30.65$, df = 6, $p < 0.0001$; richness: $\chi^2 = 39.74$, df = 6, $p < 0.0001$; abundance: $\chi^2 = 43.94$, df = 6, $p < 0.0001$). Farm "A" had significantly higher SDI than Farm "G" ($\beta = 0.50$, SE = 0.10, $z = 4.93$, $p < 0.0001$). Likewise, Farm "C" also had a significantly higher SDI than Farm "G" ($\beta = 0.59$, SE = 0.17, $z = 4.04$, $p = 0.0014$), as did farm "K" ($\beta = 0.40$, SE = 0.12, $z = 3.21$, $p = 0.0254$). Farm "L" had significantly higher genera richness than farms "E" ($\beta = 0.55$, SE = 0.14, $z = 3.90$, $p = 0.0018$), "G" ($\beta = 0.38$, SE = 0.11, $z = 3.40$, $p = 0.0119$), and "W" ($\beta = 0.62$, SE = 0.18, $z = 1.91$, $p = 0.0001$). Farm "C" had significantly higher genera richness than Farms "E" ($\beta = 0.61$, SE = 0.21, $z = 2.92$, $p = 0.0542$), and "W" ($\beta = 0.69$, SE = 0.20, $z = 3.48$, $p = 0.0090$). Furthermore, farm "A" had a higher richness of genera than farm "W" ($\beta = 0.51$, SE = 0.16, $z = 3.28$, $p = 0.0181$). Finally, farm "L" also had higher abundance than farms "E" ($\beta = 0.90$, SE = 0.21, $z = 4.40$, $p = 0.0002$), "K" ($\beta = 1.08$, SE = 0.29, $z = 3.80$, $p = 0.0028$), and "W" ($\beta = 1.01$, SE = 0.20, $z = 5.07$, $p < 0.0001$). Farm "G" had higher abundance than farms "K" ($\beta = 0.76$, SE = 0.25, $z = 3.09$, $p = 0.0327$) and "W" ($\beta = 0.69$, SE = 0.19, $z = 3.61$, $p = 0.0056$).

The differences in community composition between farms can be further visualized in an NMDS ordination plot (Figure 7). In comparison to Figure 6, there is clearly a more distinct clustering of points for each farm, and less overlap between the 95% confidence interval ellipses of individual farms (e.g., farm "G" and farm "W").

3.4.3 Survey of Parasitoida

A total of 15,056 specimens was collected and recorded, of which 12,781 belonged to the superfamily Ichneumonoidea, 682 belonged to the superfamily Chalcidoidea, and the remaining 1,593 were spread between the superfamilies in Proctotrupomorpha: Ceraphronoidea (59), Cynipoidea (1,147), Diaproidea (20), Platygastroidea (362), and Proctotrupoidea (5) (Figure 8; Table 3).

Two-hundred and six individuals were discarded, either due to decomposition (occurring in some pan trap samples), important features such as heads or wings missing, failure of DNA sequencing (due to specimen contamination or failure to process), or mislabelling. The majority of specimens were identified to genus, either morphologically through keys or via DNA barcoding. The remaining, unidentified specimens were listed as morphotypes in their respective subfamilies or families, which accounted for 142 specimens (see Table 3).

Figure 9 illustrates the composition of commonly collected genera, with less common specimens (<100 individuals collected) being grouped as "other". The majority of the samples were identified as *Diolcogaster* spp., consisting of 70 - 80% of all specimens collected. Of the 16 *Diolcogaster* specimens that were identified to species, all were classified as *Diolcogaster claritibia* Papp.

While *Diolcogaster* was extremely abundant, a Spearman's correlation test found that the genus had a weak negative correlation on the abundance of other genera, with correlation coefficients ranging between -0.0833 and -0.0009. One genus, *Torymus* Dalman (Cynipoidea: Torymidae), had the highest negative correlation of $r_s = -0.1361$. Finally, *Diolcogaster* abundance did have a strong negative correlation on community evenness ($r_s = -0.8597$), which is expected due to its dominance across all sites sampled.

3.5 Discussion

Edge type did not appear to have a significant impact on parasitoid biodiversity, based on calculated Shannon diversity indices, richness of genera, and abundance. Similarly, distance from the edge did not significantly impact diversity measurements, other than richness of genera at the edge compared to further into the field. While it is expected that richness and abundance would be higher at the edge compared to further into the field due to edge effects (Nguyen & Nansen, 2018), there was no significant difference in parasitoid diversity after 10 m. This observation of higher genera richness but lower abundance at edge borders many be explained by a variety of factors. For example, Beketov *et al.* (2010) found that manipulating environmental variables such as aquatic vegetation, water quality, and terrestrial habitat composition may influence mosquito communities by increasing richness through resource availability but decreasing abundance through activities such as predation. Similar effects could be occurring in this study, where sampled non-crop habitats provide enough resources or niches for multiple unique parasitoid genera, but factors such as pesticide use, predation from birds, or disturbances from farm machinery may be lowering the overall abundance of individuals within sampled fields. This may also explain why abundance, while not significantly different across sampled distances, was still observed to be higher closest to the edge compared to further distances (Figure 5c). If some variable decreased parasitoid abundance within a field, then non-crop habitats could provide a level of protection for the insects, resulting in spillover effects from non-crop habitat into arable land and therefore more insects along the edge boundaries. These trends of higher diversity and richness closer to the edge parallel other literatures (e.g., Rand *et al.*, 2006; Whitehouse *et al.*, 2017; Nguyen & Nansen, 2018) that suggest components of insect biodiversity are peak at the edge of non-crop habitats and decline sharply with increasing distance into cropland.

There are three possible explanations as to why edge type did not differ in diversity measurements: 1) Coulees, shelterbelts, and road verges were not high, mid, and low-complexity habitats, as originally posited, but instead had equal amounts of resources such as food, shelter, and hosts available; 2) Large-scale landscape factors such as topography, water bodies, and microclimates, have greater influence on parasitoid communities compared to habitats immediately adjacent to sampled fields; 3) External factors, such as surrounding crops, crop rotation, or farming practices, play a stronger role in parasitoid biodiversity than edge types. The underlying cause may be a combination of all three; however, the fact that farms had significantly differing levels of SDIs and genera richness implies that the individual farming practices of producers likely play some sort of role in community diversity. Agricultural intensification—such as heavy tillage, excessive pesticide use, and shortened crop cycles—is well-known to decrease biodiversity (Tscharntke *et al.*, 2005; Campbell et al, 2017). Each producer that took part in the study practices their own farming philosophy, and thus likely engages in varying relative levels of agricultural intensification from high to low. For example, farms “A” and “L” do not till their fields, which may have contributed to their higher biodiversity markers as certain species of parasitoid hymenopterans (and their hosts) overwinter in the field soil (Nilsson 2010). While pesticide use could not be accurately documented or applied to the results of this study due to timing differences and lack of communication from some producers, spraying frequency likely also played a role in diversity measurements. Farms "E", "G", "W", and "K" were all documented to be sprayed at least twice throughout the growing season to combat various pest insects, which would have likely had non-target effects on beneficial parasitoids. These four farms, particularly "G" and "W", generally had lower overall SDIs, abundance of individuals, and richness of genera compared to the other farms sampled. Again, while farming practices and land management could be contributing to the observed low levels of parasitoid biodiversity, other factors such as surrounding crops and landscape-scale effects should be investigated.

The parasitoid survey conducted in conjunction with the edge effect study found that Ichneumonoidea was the most abundant superfamily. Ichneumonoidea is well-studied for its biocontrol agents (Goulet & Huber, 1993; Lotfalizadeh & Mohammadi-Khoramabadi, 2021), and indeed many of the collected genera included natural enemies such as: *Peristenus* Förster, parasitoids of the *Lygus* bug (Loan, 1974); *Bracon* Gahan*,* parasitoids of the Wheat Stem Sawfly, *Cephus cinctus* Nort. (Nelson & Farstad, 1953); *Campoplex* Gravenhorst, parasitoids of lepidopterans (Leong & Oatman, 1968; Eikenbary & Fox, 1968). Most sampled individuals arose from the superfamily Ichneumonoidea, subfamily Braconidae, genus *Diolcogaster*.

It was expected that Ichneumonoidea and Chalcidoidea would be the most common superfamilies, as they are the most populous and speciose (Goulet & Huber, 1993; Huber, 2017); however, the poorly understood superfamily, Cynipoidea, was the second most abundant group of parasitoids after Ichneumonoidea. Cynipoids include Cynipidae, a family of gall-forming wasps that include many known natural enemies, including some of the few examples of phytophagous hymenopterans (Goulet & Huber, 1993). The commonly collected genera in this family were *Hexacola* Förster, *Ganaspis* Förster, and *Trybliographa* Förster, all of which belong to the family Figitidae and include species of known biocontrol agents of dipterans (Wishart & Monteith, 1954; Diamond *et al.*, 2001; Kasuya *et al.*, 2013). *Trybliographa* *rapae* Westwood, for example, attacks the cabbage root fly, *Hylemya brassicae* L. (syn. *Delia radicum*), whose larvae attack the roots of Brassicaceae plants such as canola (Wishart & Monteith, 1954).

Following Ichneumonoidea and Cynipoidea, Chalcidoidea was the third most collected superfamily. Chalcids in this survey included the most morphotypes collected, primarily due to their small size causing difficulty when identifying features morphologically (e.g., Gibson *et al.*, 1997). Chalcidoidea was much less frequently collected in this survey compared to previous ones (e.g. Finnamore, 1994), perhaps again due to their small size making them difficult to detect when hand-sorting through pan trap and sweep-net samples. Indeed, fewer chalcidoids were caught during the early growing season, implying some samples may have been missed during pan trap collection and sorting. Chalcidoidea includes a myriad of well-known natural enemies (Goulet & Huber, 1993; Lotfalizadeh & Mohammadi-Khoramabadi, 2021), including: *Copidosoma* Ratzeburg, parasitoids of lepidopteran larvae (Guerrier & Noyes, 2005); *Macroglenes* Westwood, parasitoids of dipterans (Doane *et al.*, 1989); *Trichomalus* Thomson, parasitoids of coleopterans such as the cabbage seedpod weevil, *Ceutorhynchus obstrictus* Marsham (Haye *et al.*, 2014). One of the most captured genera from Chalcidoidea was *Perilampus* Latreille, a hyperparasitoid that may be considered a pest as it is known to attack other parasitoid Hymenoptera and tachinid flies (Tripp, 1962; Subbiah, 1987; Darling *et al.*, 2021). Considering *Perilampus* spp. have been documented to attack braconids in the subfamily Microgastrinae (Subbiah 1987), it is possible that they are parasitizing *Diolcogaster* spp., although there was no statistical correlation between the two genera.

Finally, the other superfamilies of Parasitoida were collected sparsely in this survey. Only 20 specimens were collected from Diaprioidea, five specimens from Proctotrupoidea, and ~360 specimens from Platygastroidea. No specimens were collected from Mymarommatoidea; however, this is expected, as only 10 species in the superfamily have ever been classified (Gibson *et al.*, 2007).

Here, I also report the emergence of *Diolcogaster* Ashmead (Ichneumonoidea: Braconidae: Microgastrinae) as a dominant genus of parasitoids in all my sampled canola sites across southern Alberta. Over 100 species of *Diolcogaster* have been identified and it is considered one of the most common genera in the subfamily Microgastrinae (Moghaddam *et al.*, 2019), although New World *Diolcogaster* are poorly understood with a complicated history of taxonomic classification (Choi, 2005). These wasps are endoparasitoids of various Lepidopterans larvae and have a global distribution (Fernández-Triana, 2010). In particular, *Diolcogaster claritibia* Papp is likely the dominant species that was collected in my samples; however, only 16 of >11,000 specimens were identified to species and confirmed to be (in the case of DNA barcoding) or likely to be (in the case of morphological identification) *D. claritibia*. Thus, there is a probability that other species of *Diolcogaster* (e.g., *D. facetosa* Weed; Fernández-Triana, 2010) exist in the samples. *Diolcogaster claritibia* was first officially reported in North America in the provinces of Alberta, Manitoba, and Ontario by Fernández-Triana *et al.* in 2014. It may have existed in Alberta as far back as 1994 (e.g. Finnamore, 1994); however, no concrete evidence exists to confirm this. Since Fernández-Triana *et al.*'s 2014 publication, only two news articles documenting *D. claritibia* in the Canadian Prairies have been recorded, both noting high numbers of the parasitoid being collected in Alberta canola fields in 2018 (Canola Council of Canada 2018; Whetter, 2019). This survey is the first empirical report of these specimens in Alberta since 2014. Furthermore, despite *Diolcogaster* being the dominant genus of parasitoid hymenopterans collected in this study, its presence did not have a strong negative correlation on the abundance of other parasitoid hymenopterans, suggesting that it is not out-competing other species. One genus, *Torymus*, could be mildly impacted by the presence of *Diolcogaster*; however, only three specimens from this genus were collected and these wasps are known to parasitize gall-forming insects (such as other hymenopterans in the superfamily Cynipoidea, family Cynipidae) (de Vere Graham & Gijswijt, 1998), not lepidopterans. Thus, if *Torymus* and *Diolcogaster* are competing in some manner, it would likely not be for hosts. Instead, it is likely that the small sample size of *Torymus* is inflating the observed correlation. Finally, I report that the presence of *Diolcogaster* did decrease overall community evenness; however, this is to be expected due to the sheer abundance of the genus.

The European *D. claritibia* was likely recently introduced to North America (Fernández-Triana *et al.*, 2014); however, the specifics on this species' movement and history have yet to be published, and the biology of *D. claritibia* is generally poorly understood (Mason *et al.*, 2022). They are parasitoids of the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae *syn*. Yponomeutidae) (Mason *et al.*, 2022), a common pest whose larval offspring feed on plants in the Brassicaceae (*syn*. Cruciferae) family (Talekar & Shelton, 1993). This species of moth is common worldwide and invasive to Canada, known for its resistance to various pesticides (Talekar & Shelton, 1993). Diamondback moth larvae are particularly known as pests of canola, which may explain why such high numbers of *Diolcogaster* were collected in this survey; the magnitude of *Diolcogaster* spp. that I report is likely not found in other crop types where *P. xylostella* are less plentiful, such as alfalfa or wheat, however future surveys are needed to confirm this.

Other collected genera included species that specifically attack *P. xylostella*, such as *Diadegma* (Ichneumonidae: Campopleginae) and *Diadromus* (Ichneumonidae: Ichneumoninae) (Mason *et al.*, 2022). One species, *Diadromus subtilicornis* Gravenhorst, was positively identified via DNA barcoding as one of the 32 *Diadromus* specimens collected from canola fields; thus, it can be concluded that there are multiple species of parasitoids attacking *P. xylostella* in the sampled southern Albertan canola fields, however future studies are needed to determine if they provide an adequate level of crop protection.

In all, the majority of the collected specimens were known parasitoids of lepidopterans (i.e., *Diolcogaster* (Fernández-Triana, 2010; Mason *et al.*, 2022), *Diadegma* (Mason *et al.*, 2022), *Diadromus* (Mason *et al.*, 2022), *Campoplex* (Leong & Oatman, 1968; Eikenbary & Fox, 1968), *Brachymeria* Westwood (Burks, 1960), *Copidosoma* (Guerrier & Noyes, 2005), etc.). While many lepidopterans are known agricultural pests, the insect order also includes some of the most important pollinators. Thus, it raises the question if parasitoid hymenopterans are impacting pollinator populations. A future study quantifying the exact hosts of the parasitoids, perhaps through rearing lepidopterans, would help answer this question. Such a study may also help determine if *Diolcogaster* is parasitizing lepidopteran species other than *P. xylostella*.

In conclusion, this study demonstrated that edge type has minimal effects on overall parasitoid biodiversity, and that distance from the edge only significantly influences the diversity of parasitoids closest to the edge. The significant differences in Shannon Diversity Indices and genus richness across farms imply that the biodiversity of parasitoids is instead influenced by agricultural practices, landscape-scale factors, or another variable not measured. Here, I also report *Diolcogaster*, likely *Diolcogaster claritibia*, as the dominant genus in sampled canola fields across southern Alberta, disproportionately outweighing all other sampled genera. The emergence of *D. claritibia* as potentially the primary parasitoid in canola could be valuable as a biocontrol agent of the diamondback moth; however, further study on its impact on *P. xylostella* populations and whether it is keeping the pest below threshold levels is needed. Furthermore,

more work is needed to confirm if *Diolcogaster claritibia* is the only *Diolcogaster* species present in southern Alberta canola fields, or if native species such as *D. facetosa* are also present. The findings from this research emphasize the importance of more research into the factors that influence parasitoid diversity, as well as the need for further surveys to understand the composition of parasitoids in our fields.

3.6 Figures

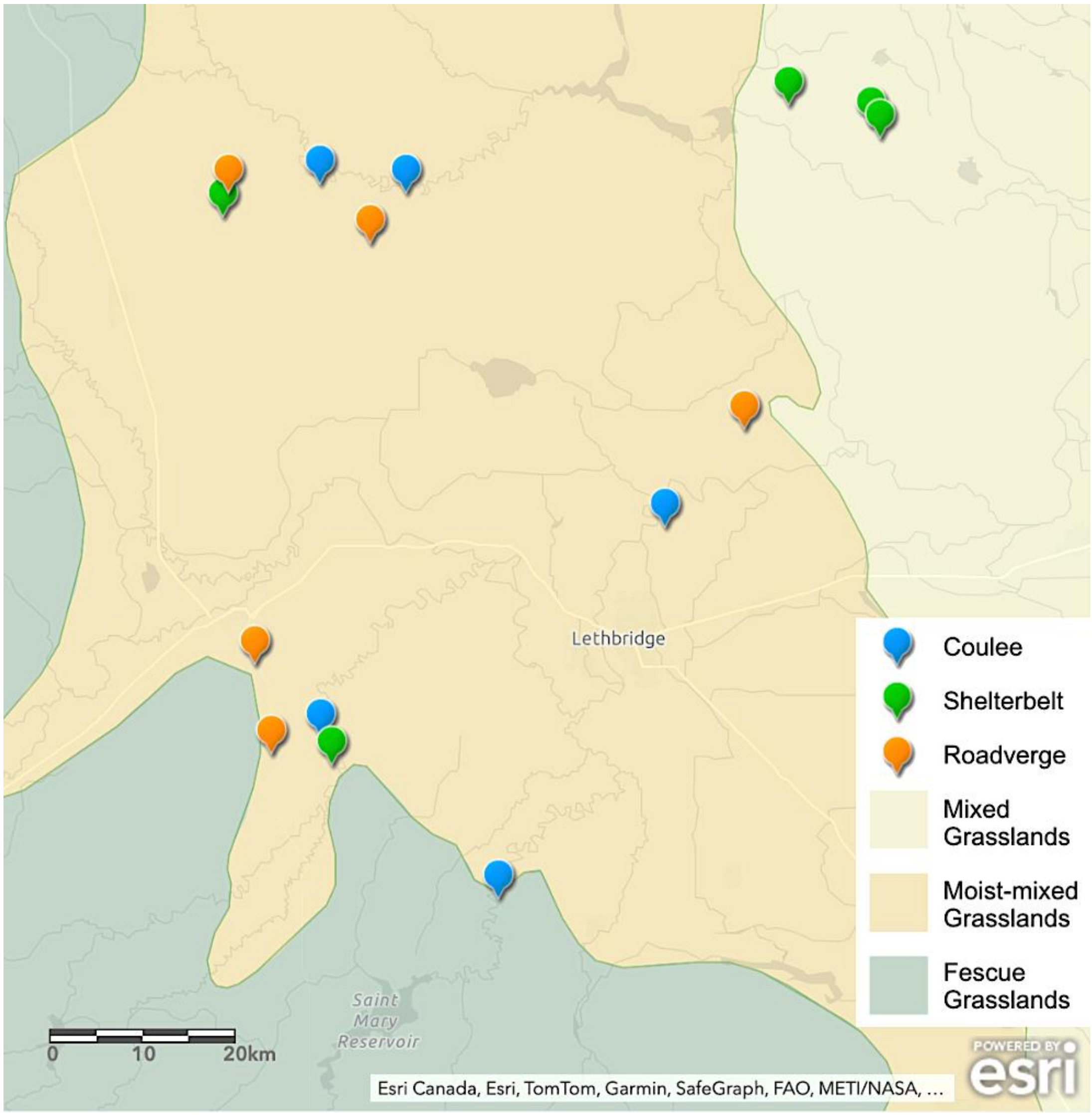

Figure 3. The distribution of study sites across southern Alberta. Blue pins indicate fields that bordered coulees, green pins indicate fields that bordered shelterbelts, and orange pins indicate fields that bordered road verges. The three types of grasslands (Mixed, Moist-mixed, and Fescue) found in the area are also illustrated.

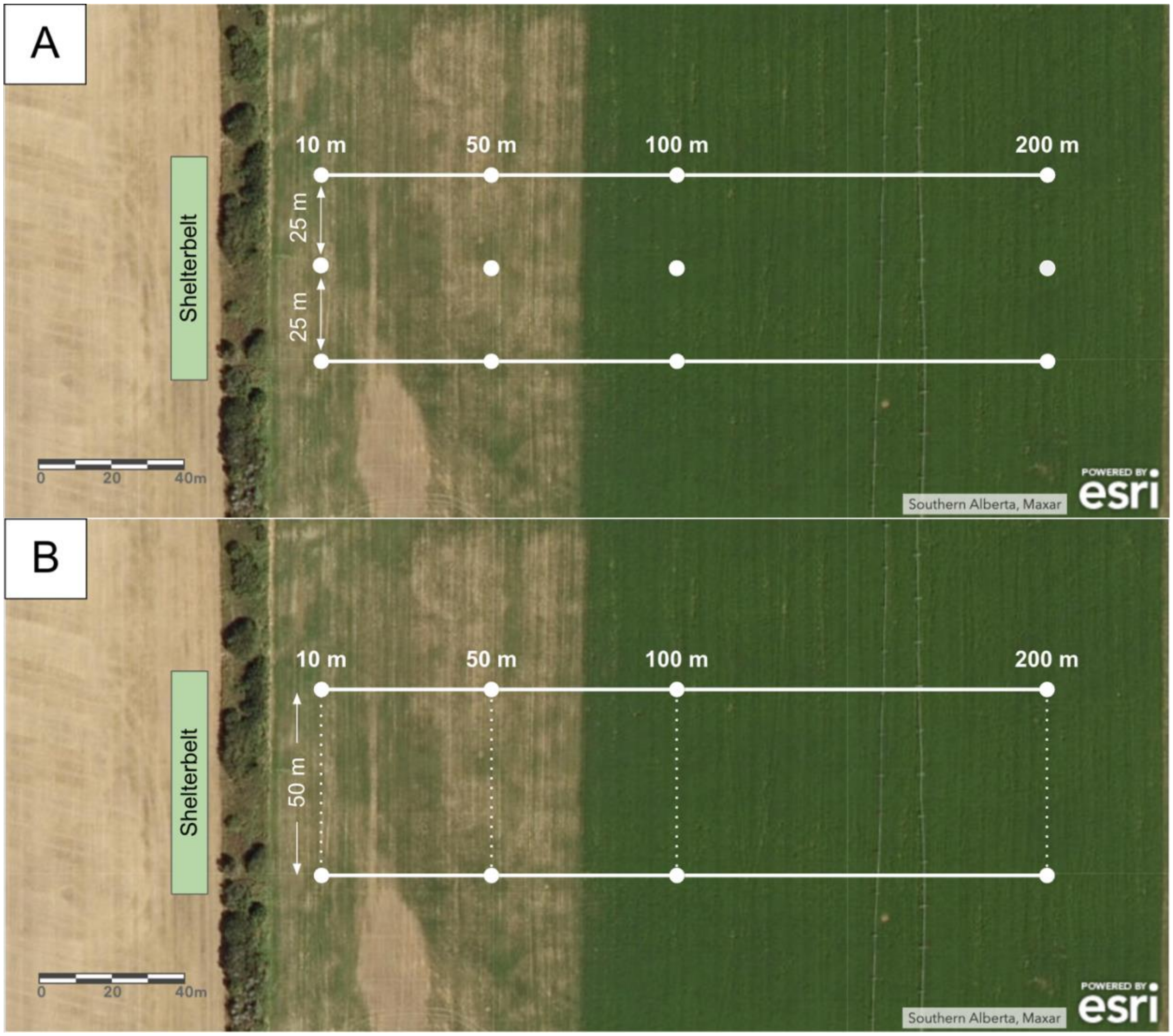

Figure 4. An example of the transect design in a sampled shelterbelt field. **A)** The pan trap sampling layout, occurring during the seedling and bolting stage of canola. Each dot represents a pan trap placement, with three pan traps occurring per sampling point, spaced 25 m apart. **B)** The sweep net sampling layout, occurring during canola's flowering and pod stages. The dotted line represents the path that was followed while sweeping between each sampling point.

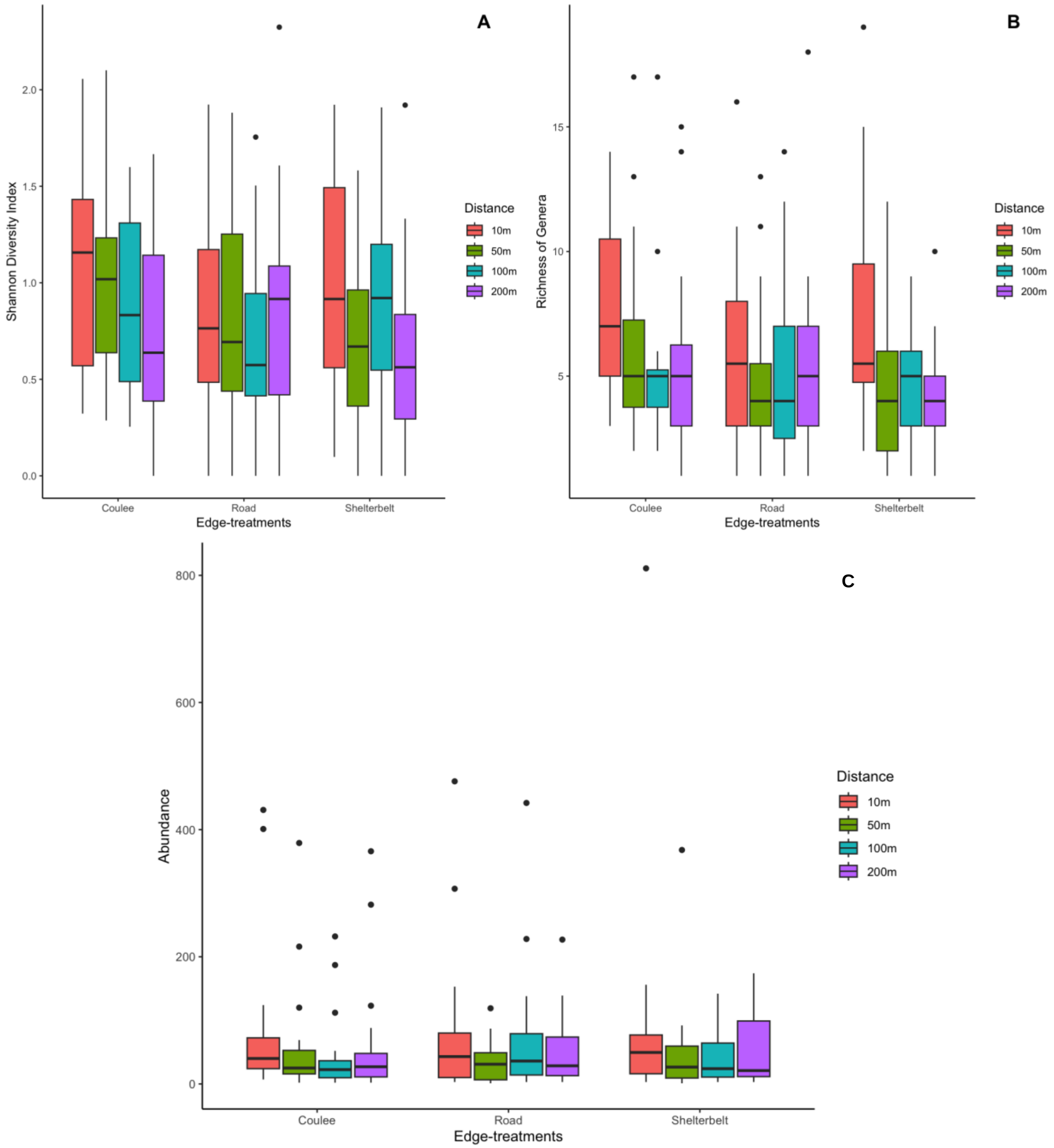

Figure 5. Boxplots illustrating the variation in Shannon Diversity Indices, richness of genera, and abundance of individuals within sampled Parasitoida across the three sampled edge treatments (coulee, road verge, and shelterbelt) at the four distances from the edge (10 m, 50 m, 100 m, and 200 m). **A)** The median calculated Shannon Diversity Indices of treatment and distance samples, showing that diversity is typically highest at the edge and decreases further into the field. **B)** The median richness of genera in sampled edge treatments and distances, showing richness is higher at the edge and has little variation at further distances. **C)** The median parasitoid abundance across all sampled edge types and distances. Abundance was generally <100 individuals, excluding certain outliers.

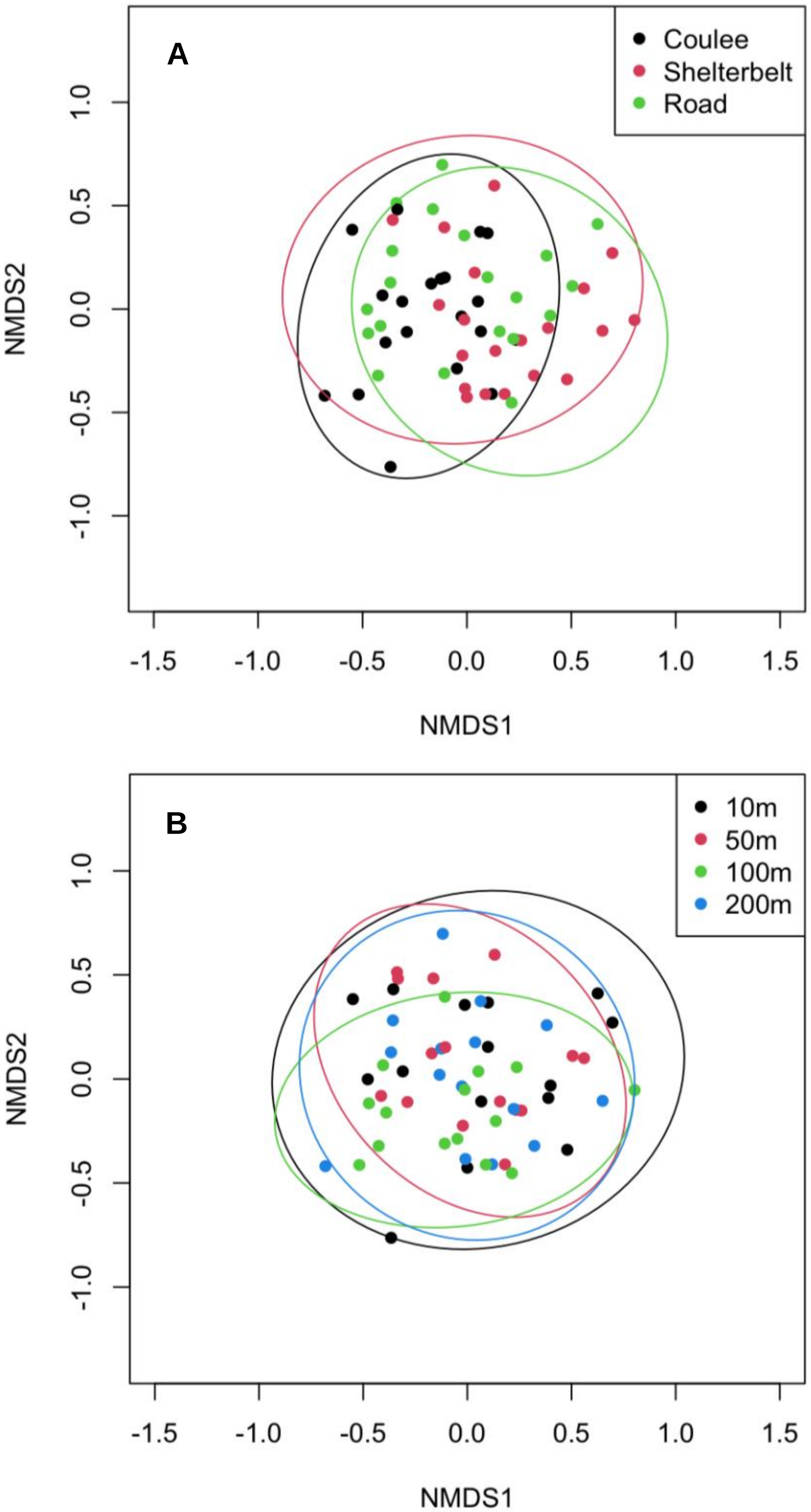

Figure 6. NMDS ordination plots for edge treatment and distance into the field based on the abundance of Parasitoida. The stress level in 3-dimensions is 0.21. **A)** The ordination plot for edge boundaries with samples and corresponding 95% CI ellipses coloured by treatment. **B)** The ordination plot for distances into the field with samples and their corresponding 95% CI ellipses coloured by distance.

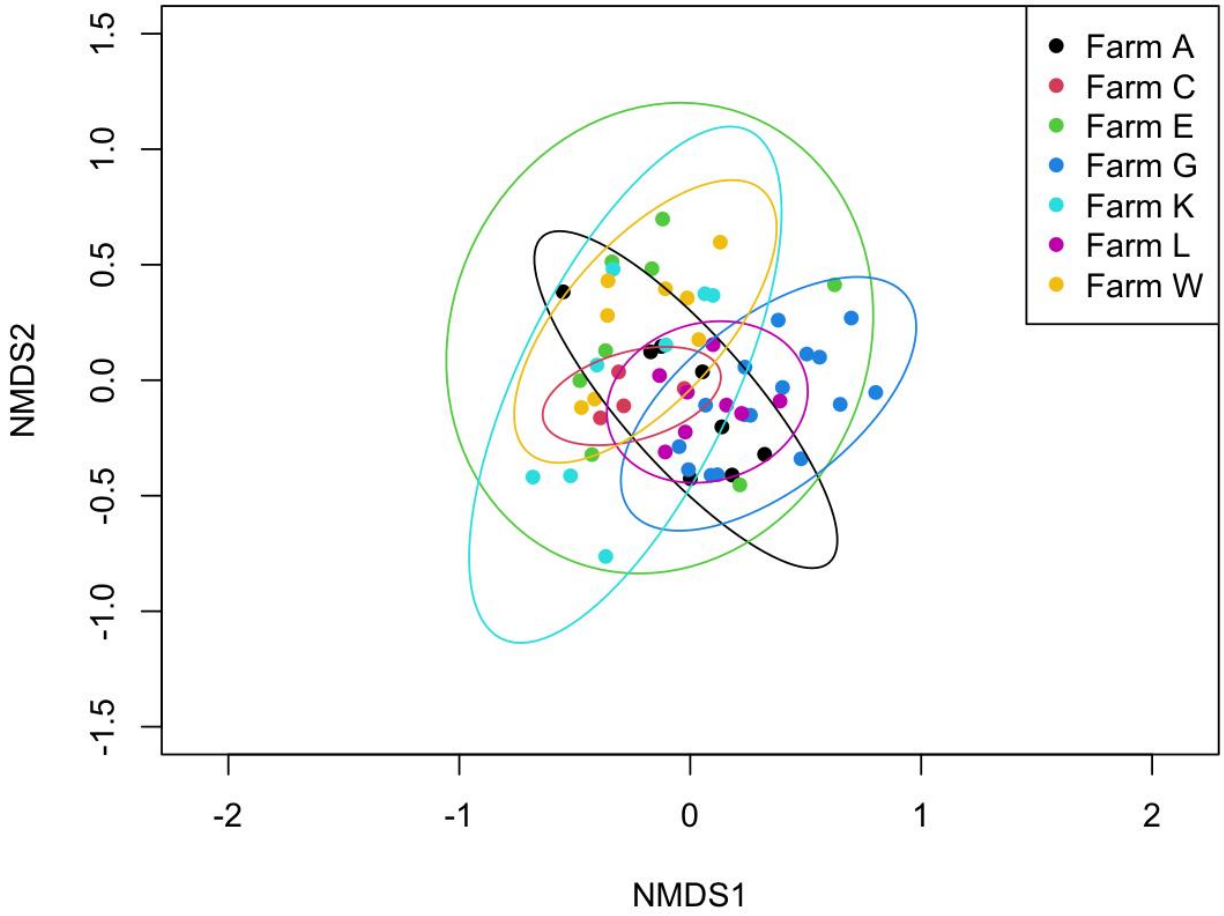

Figure 7. NMDS ordination plots for the seven sampled farms based on the abundance of Parasitoida. The stress level in 3-dimensions is 0.21. Note the clustering of points and dissimilarity between the composition of certain farms.

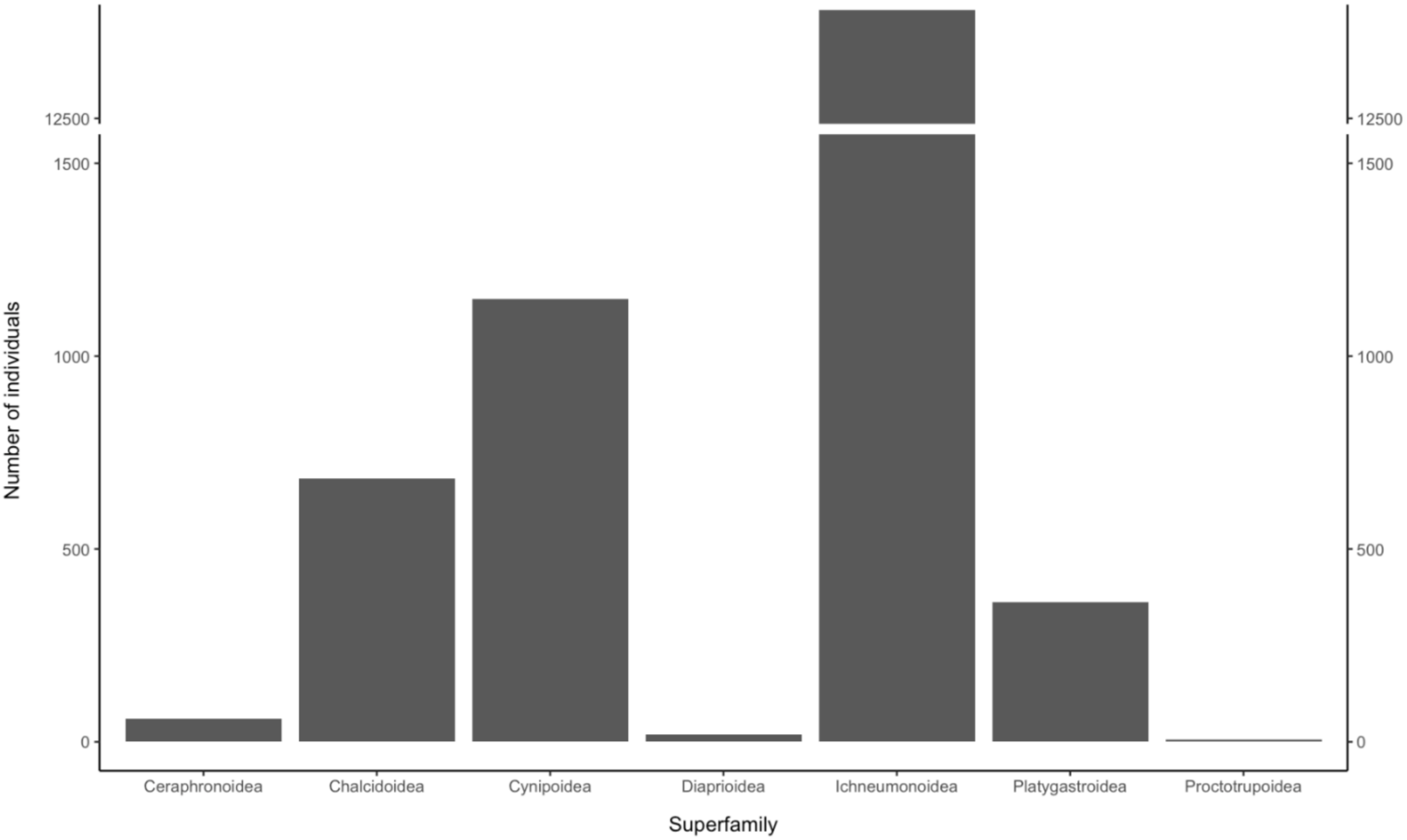

Figure 8. The distribution of the total number of individual specimens collected, categorized by family within each superfamily. Note the break in the y-axis, excluding ~11,000 individuals in the superfamily Ichneumonoidea, family Braconidae.

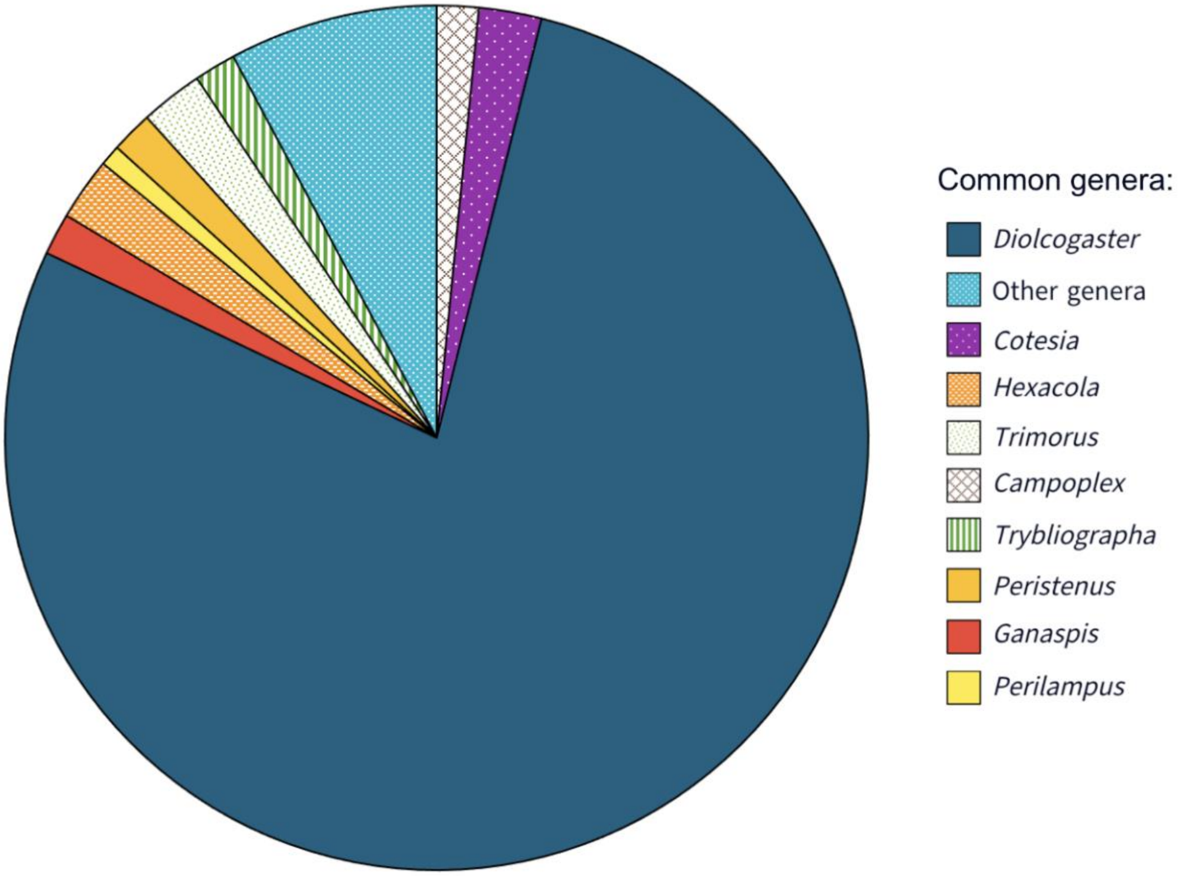

Figure 9. A pie chart illustrating the relative abundance of commonly collected parasitoid hymenopteran genera from sampled canola fields.

3.7 Tables

Table 2. An alphabetical list of common plant species found at the sample edge types (coulees, shelterbelts, and road verges). Both the scientific and common name of the species is listed.

Coulees	**Shelterbelts**	**Road verges**
Agropyron cristatum L. (Crested Wheatgrass)	*Acer negundo* L. (Boxelder)	*Agropyron cristatum* L. (Crested Wheatgrass)
Arctium spp. L. (Burdock)	*Amaranthus retroflexus* L. (Red Root Amaranth)	*Avena* spp. L. (Wild Oats)
Artemisia absinthium L. (Wormwood)	*Bromus inermis* Leyss. (Smooth Brome)	*Bromus inermis* Leyss. (Smooth Brome)
Artemisia tridentata Nutt. (Western Sage)	*Capsella bursa-pastoris* (L.) Medik. (Shepherd's Purse)	*Crepis* spp. L. (Hawksbeard)
Aster spp. L. (Asters)	*Caragana arborescens* Lam. (Caragana)	*Helianthus* spp. (Wild Sunflower)
Avena spp. L. (Wild Oats)	*Crepis* spp. L. (Hawksbeard)	*Kochia scoparia* L. (Kochia)
Bromus inermis Leyss. (Smooth Brome)	*Fraxinus velutina* Torr. (Velvet Ash)	*Melilotus officinalis* L. (Yellow Sweet Clover)
Kochia scoparia L. (Kochia)	*Medicago sativa* L. (Alfalfa)	*Phalaris arundinacea* L. (Reed Canary Grass)
Rosa woodsii Torr. (Wild Rose)	*Populus deltoides* Bartr. (Cottonwood)	*Taraxacum officinale* L. (Dandelion) *Typha* spp. L. (Cattail)
Solidago nemoralis Aiton (Goldenrod)	*Prunus padus* L. (Bird Cherry)	Misc. grasses
Symphoricarpos albus L. (Snowberry)	*Prunus virginiana* L. (Choke Cherry)	
Taraxacum officinale L. (Dandelion)	*Rheum rhabarbarum* L. (Rhubarb)	
Trifolium spp. L. (Clover)	*Solidago nemoralis* Aiton (Goldenrod)	
Misc. wildflowers		

Table 3. The full list of surveyed parasitoid Hymenopteran genera, including morphotypes. Individual specimen counts for each genus are shown in square brackets. Any identified species are included below their respective genera; however, the species listed are not exhaustive and do not include individual counts.

Ichneumonoidea

- BRACONIDAE : Agathidae
  - *Agathus* sp. [2]
- BRACONIDAE : Alysiinae
  - *Asobara* sp. [3]
  - *Aspilota* sp. [13]
  - *Chorebus* sp. [11]
  - *Dinotrema* sp. [60]
  - *Orthostigma* sp. [1]
  - *Phaenocarpa* sp. [8]
- BRACONIDAE : Aphidiinae
  - *Aphidius* sp. [3]
  - *Diaeretiella* sp. [6]
    - *D. rapae*
  - *Trioxys* sp. [1]
- BRACONIDAE : Braconinae
  - *Bracon* sp. [44]
    - *B. cephi*
  - *Habrobracon* sp. [1]
  - Morphotype A [6]
- BRACONIDAE : Cheloninae
  - *Chelonus* sp. [24]
- BRACONIDAE : Euphorinae
  - *Meteorus* sp. [1]
  - *Microctonus* sp. [123]
  - *Peristenus* sp. [193]
    - *P. dayi, P. howardi, P. mellipes*
  - *Perilitus* sp. [1]
- BRACONIDAE : Microgastrinae
  - *Apanteles* sp. [2]
  - *Diolcogaster* sp. [11,149]
    - *D. claritibia*
  - *Microgaster* sp. [1]
  - *Microplitis* sp. [2]
- BRACONIDAE : Opiinae
  - *Opius* sp. [66]
  - Morphotype A [4]
- BRACONIDAE : Rhyssalinae
  - *Acrisis* sp. [4]
  - *Oncophanes* sp. [3]
  - Morphotype A [4]
  - Morphotype B [3]
- BRACONIDAE : UNKNOWN SUBFAMILY
  - Morphotype A [1]
  - Morphotype B [6]
  - Morphotype C [3]
- ICHNEUMONIDAE : Anemabninae
  - *Erigorgus* sp. [37]
- ICHNEUMONIDAE : Banchinae
  - *Banchus* sp. [2]
- ICHNEUMONIDAE : Campopleginae
  - *Bathyplectes* sp. [8]
  - *Campopletis* sp. [11]
  - *Campoplex* sp. [486]
  - *Diadegma* sp. [61]
  - *Eriborus* sp. [2]
  - *Sinophorus* sp. [2]
  - Morphotype A [4]
  - Morphotype B [1]
- ICHNEUMONIDAE : Cryptinae
  - *Aritranis* sp. [3]

ICHNEUMONIDAE : Diplazontinae
  - *Diplazon* sp. [78]
    - *D. laetatorius*
  - *Homotropus* sp. [2]

ICHNEUMONIDAE : Ichneumoninae
  - *Aoplus* sp. [1]
  - *Cratichneumon* sp. [2]
  - *Diadromus* sp. [32]
  - *Diphyus* sp. [3]
  - *Ichneumon* sp. [6]
  - *Spilichneumon* sp. [2]
  - Morphotype A [1]
  - Morphotype B [1]

ICHNEUMONIDAE : Mesochorinae
  - *Astiphromma* sp. [1]
  - *Mesochorus* sp. [14]

ICHNEUMONIDAE : Ophioninae
  - *Ophion* sp. [3]

ICHNEUMONIDAE : Oxytorinae
  - Morphotype A [1]

ICHNEUMONIDAE : Phygadeuontinae
  - *Gelis* sp. [2]
  - *Phygadeuon* sp. [16]
  - Morphotype A [2]
  - Morphotype B [1]

ICHNEUMONIDAE : Pimplinae
  - Morphotype A [3]
  - Morphotype B [1]

ICHNEUMONIDAE : Tryphoninae
  - *Erromenus* sp. [6]

ICHNEUMONIDAE : UNKNOWN SUBFAMILY
  - Morphotype A [2]
  - Morphotype B [2]
  - Morphotype C [1]

Chalcidoidea

CHALCIDIDAE : Chalcidinae
  - *Brachymeria* sp. [106]
  - *Conura* sp. [9]

CHALCIDIDAE : Haltichellinae
  - *Prilochalcis* sp. [1]

ENCRYTIDAE : Encrytinae
  - *Copidosoma* sp. [83]
  - Morphotype A [3]
  - Morphotype B [1]

EUCHARITIDAE : Oraseminae
  - *Orasema* sp. [15]

EULOPHIDAE : Eulophinae
  - *Diglyphus* sp. [28]
  - *Euplectrus* sp. [1]
  - *Necremnus* sp. [8]
  - Morphotype A [1]
  - Morphotype B [3]
  - Morphotype C [2]

EULOPHIDAE : Tetrastichinae
  - *Aprostocetus* sp. [54]
  - *Baryscapus* sp. [1]
  - *Oomyzus* sp. [1]
  - *Tetrastichus* sp. [26]
  - Morphotype A [3]

EULOPHIDAE : UNKNOWN SUBFAMILY
  - Morphotype A [2]
  - Morphotype B [1]

EUPELMIDAE : Eupelminae
  - Morphotype A [2]

EURYTOMIDAE : Eurytominae
  - *Eurytoma* sp. [22]
  - Morphotype A [2]
  - Morphotype B [2]

- Morphotype C [1]

MYRMARIDAE : Mymarinae

- *Gonatocerus* sp. [3]
- *Stephanodes* sp. [1]
- *Lymaenon* sp. [1]

PERILAMPIDAE : Perilampinae

- *Euperilampus* sp. [2]
- *Perilampus* sp. [153]

PIRENIDAE : Pireninae

- *Macroglenes* sp. [54]
  - *M. penetrans*
- Morphotype A [2]

PTEROMALIDAE : Miscogastrinae

- *Halticoptera* sp. [13]
- *Merismus* sp. [11]
- Morphotype A [1]

PTEROMALIDAE : Pteromalinae

- *Catolaccus* sp. [1]
- *Homoporus* sp. [1]
- *Pteromalus* sp. [21]
- *Trichomalus* sp. [16]
- Morphotype A [1]
- Morphotype B [4]
- Morphotype C [2]
- Morphotype D [2]
- Morphotype E [6]
- Morphotype F [3]

SYSTASIDAE : Systasinae

- *Systasis* sp. [1]

TORYMIDAE : TORYMINAE

- *Torymus* sp. [3]
- Morphotype A [1]
- Morphotype B [1]

PROCTOTRUPOMORPHA

Ceraphronoidea

CERAPHRONIDAE : Ceraphroninae

- *Aphanogmus* sp. [6]
- *Ceraphron* sp. [41]

MEGASPILIDAE : Megaspilinae

- *Conostigmus* sp. [1]
- *Dendrocerus* sp. [7]
- *Megaspilus* sp. [4]

Cynipoidea

FIGITIDAE : Eucoilinae

- *Ganaspis* sp. [286]
- *Hexacola* sp. [417]
- *Striatovertex* sp. [51]
- *Trybliographa* sp. [364]
- Morphotype A [3]
- Morphotype B [2]
- Morphotype C [6]
- Morphotype D [2]
- Morphotype E [1]
- Morphotype F [1]

FIGITIDAE : Figitinae

- *Figites* sp. [1]
- *Melanips* sp. [9]
- Morphotype A [4]

Diaprioidea

DIAPRIIDAE : Belytinae

- Morphotype A [10]
- Morphotype B [2]

DIAPRIIDAE : Diapriinae

- Morphotype A [3]

DIAPRIIDAE : UNKNOWN SUBFAMILY

- Morphotype A [5]

Platygastroidea

PLATYGASTRIDAE : Platygastrinae

- *Insotemma* sp. [2]
- *Platygaster* sp. [7]
- *Synopeas* sp. [1]
- Morphotype A [6]

SCELIONIDAE : Scelioninae

- *Calliscelio* sp. [47]
- *Gryon* sp. [59]

SCELIONIDAE : Teleasinae

- *Teleas* sp. [1]
- *Trimorus* sp. [196]
- Morphotype A [3]

SCELIONIDAE : Telenominae

- *Telenomus* sp. [27]
- *Trissolcus* sp. [10]

PLATYGASTROIDEA : UNKNOWN SUBFAMILY

- Morphotype A [1]

Proctotrupoidea

HELORIDAE : Helorinae

- *Helorus* sp. [4]

PROCTOTRUPIDAE : Proctotrupidae

- *Proctotrupes* sp. [1]

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4. CONCLUSION

4.1 Preamble

The overall goal of most research endeavours is to fill gaps in knowledge–a common phrase reiterated throughout many studies, proposals, and publications. The goal of my thesis was to help fill some of the many gaps in knowledge regarding parasitoid hymenopterans, a poorly understood and understudied group of insects. Both data chapters of this thesis examined different angles of parasitoid biology in southern Alberta with the shared goal of better understanding and conserving our native species.

4.2 Chapter 2 discussion, limitations, & future directions

In chapter 2, we evaluated the parasitism rates of native *Peristenus* species on *Lygus* bugs across various cultivated and uncultivated crops in southern Alberta. The data collected from this survey showed no significant difference in parasitism rates across all sampled host plants and showed generally higher mean parasitism rates compared to previous *Peristenus-Lygus* parasitism surveys in the Canadian Prairies (e.g., Braun *et al.*, 2001; Fernández *et al.*, 2018). This study was the first to find that a native *Peristenus* (*P. howardii*) has recently become established in canola fields and is rampant in most annual crops. Furthermore, this study found that parasitism rates differed significantly between instar ages in both hemp and canola, possibly due to factors related to the flowering stages of these plants. Finally, DNA barcoding from this study supported previous research by Fernández *et al.* (2018) regarding the phenology of the three *Peristenus* species in southern Alberta.

In the general introduction of my thesis (chapter 1), I predicted that: 1) parasitism levels would be highest in alfalfa and lowest in canola, and 2) parasitism levels would be generally higher in uncultivated rather than cultivated crops. My first prediction was partially correct, as alfalfa hay produced some of the highest overall parasitism rates (35%), alfalfa seed produced some of the lowest parasitism rates at 7%. However, canola also had a surprisingly high parasitism rate at 32%, contrary to previous literature (e.g. Braun *et al.*, 2001, Fernández *et al.*, 2018) and my initial predictions. Similarly, while the uncultivated species, kochia, had the highest overall mean parasitism rate of 48%, flixweed, another uncultivated species, had no recorded parasitism.

One of the primary limitations of this study was the small sample size of dissected nymphs that were collected from certain host plants, particularly uncultivated plants. Many of the uncultivated plants, and some of the cultivated ones, had a sample size of less than 100. More data will be needed in the future to have a more robust analysis and understanding of *Lygus-Peristenus* parasitism rates in southern Alberta. Thankfully, this limitation is already being corrected as chapter 2's survey of parasitism rates in cultivated and uncultivated plants is still ongoing as of the writing of this thesis. Sampling was conducted in the Summer of 2024, and the data will be incorporated in a larger publication.

This survey is a component of a larger project seeking to relocate the exotic *Peristenus digoneutis* from eastern Canada to the Canadian Prairies for enhanced *Lygus* biocontrol. Related studies on this topic included a competition study between the exotic and native *Peristenus* species, as well as determining the relative abundance of native *Peristenus* spp. to assess the feasibility and potential impact of integrating *P. digoneutis* into southern Alberta ecosystems. Intentionally relocating non-native species into new habitats is a delicate process that requires careful planning and a thorough understanding of its potential implications.

Finally, an interesting observation that arose from this chapter's findings was the interaction between instar age and crop type in hemp and canola. An olfactometer study on either *Peristenus* or *Lygus* plant preference would help explain these findings and expand our knowledge of parasitoid and/or pest biology or perhaps, more realistically, a field study with these crops planted simultaneously and weekly sampling.

4.3 Chapter 3 discussion, limitations, & future directions

Chapter 3 of this thesis examined three topics: 1) How three types of edge habitats (coulees, shelterbelts, and road verges) affect the biodiversity of parasitoid hymenopterans; 2) Whether the community composition of parasitoids differs with distance from the edge; 3) The types and abundance of parasitoids in these fields. There was no statistically significant difference in the richness, abundance, and calculated Shannon Diversity Indices of parasitoid hymenopterans when looking at different edge types, and there was only a significant difference in genera richness closest to the edge relative to distances 50 to 200 m into the field. One possible driver of parasitoid diversity was the farming practice of each producer (e.g., tillage, pesticide use), as diversity varied significantly between farms, although other explanations could exist. Finally, the community composition of parasitoid hymenopterans in southern Alberta was dominated by the genus *Diolcogaster*; those that were identified to species was the exotic *Diolcogaster claritibia,* which was only formally reported in Canada in 2014 (Fernández-Triana *et al.*, 2014).

In my chapter 1 general introduction, I predicted that chapter 3 would have three general outcomes: 1) Biodiversity would be highest in canola fields bordering coulees; 2) Majority of specimens would be of the families Ichneumonoidea and Chalcidoidea; 3) I would collect specimens from all the Parasitoida superfamilies, excluding Mymarommatoidea. While predictions 2 and 3 were correct (although Chalcidoidea was only the third most abundant superfamily), the first prediction was incorrect. This argument was rooted in the idea that coulee sites have high habitat heterogeneity, although this was based on assumptions on resource/niche availability, rather than any concrete studies on coulee ecosystems. Even if coulees do have quantifiably higher habitat heterogeneity compared to shelterbelts and road verges, it is still not the major driver of parasitoid diversity based on the parameters of this study.

One limitation of this study was that collection only occurred for one year during Summer 2023. It is difficult to draw conclusions from a dataset that lacks at least one or two years of replication when conducting ecological experiments. Another limitation of this study worth noting was that, as I did not expect farm management to be such a significant indicator of parasitoid biodiversity, I did not collect any empirical data on the farming practices of each producer I worked with. Had I known, I would have collected information on practices such as tillage, crop rotation, fertilizer use, etc.; however, this limitation on my part opens the doors for future research.

Finally, I cannot conclude my thesis without mentioning a significant barrier I faced when conducting my research: a significant lack of resources on Hymenoptera identification. Keys such as *Hymenoptera of the World* (Goulet & Huber, 1993) were a lifeline when conducting chapter 3's survey, however few other resources on this subject matter exist. Identifying Nearctic specimens to genus, let alone to species, is a challenging endeavour and the majority of superfamilies in Proctotrupomorpha do not have published keys. DNA barcoding was a useful tool but not as reliable as I had hoped, and thus there is a certain margin of unavoidable error in the identifications that must be acknowledged. However, with limitation comes opportunity: there is a need for artists and taxonomists to produce more keys to help future Hymenopteran researchers and enthusiasts alike.

To conclude, this study has led to more questions than answers, as research often does, and has opened the door for many areas of future inquisition. I believe one of the most important future studies should be a more thorough survey of parasitoids in southern Alberta, possibly on the superfamily Cynipoidea. Cynipoids are known to be both pests (Borowiec *et al.*, 2014) and successful biocontrol agents (e.g., Klöppel *et al.*, 2003) and were the second most abundant group collected in my chapter 3 survey. I only collected specimens belonging to Figitidae, a cryptic and taxonomically uncertain family with very little (if any) keys in existence for North American specimens (L. Natasi, personal communication, March 12, 2024). Despite their obvious presence in southern Alberta canola fields, very little is known about these hymenopterans and their possible harm or benefits to our agroecosystems. Of course, I believe that every group of parasitoid hymenopteran (particularly in southern Alberta) warrants further investigation; indeed, little data exists on these insects and many producers and researchers alike do not know what wasps are in their fields. There are likely many undescribed species, some of which may be important natural enemies that have yet to be discovered. Furthermore, there is a need for establishing this baseline information to monitor how parasitoid populations change over time. Discovering how *D. claritibia* is impacting diamondback moth populations, and potentially other Lepidopterans directly and other parasitoids indirectly through competition, is another fascinating angle. Many, including myself, were surprised to see this genus contributing to almost 80% of all sampled individuals and a study rearing Lepidopteran parasitoids may help us understand how these insects are subsisting. Finally, a future study directly related to this chapter should be one investigating why parasitoid diversity differed so drastically between farms; the likely cause is farming management and intensive versus non-intensive agriculture, however the exact mechanisms behind this should be understood.

4.4 Synthesis of chapters 2 & 3

Chapters 2 and 3 of this thesis, while seemingly different topics from one another, share a level of interconnectedness. On a philosophical angle, the survey of *Lygus-Peristenus* parasitism rates was conceptualized based on years of previous research by the Cárcamo lab (and collaborators), allowing for baseline data that is crucial when considering the relocation of non-native species (i.e., *Peristenus digoneutis*) into a new region, or simply understanding the biology of local fauna. Conversely, such baseline data did not exist before the introduction of *Diolcogaster claritibia* into North America, and thus there is a lack of historical comparison that would be a useful for chapter 3 to build on. In a way, Chapter 3 is the "historical data" as it is an empirical record of not just *Diolcogaster*, but Parasitoida richness, abundance, and composition. Thus, while chapter 2 is built upon years of research, Chapter 3 could help develop a new angle of research should anyone take interest.

On a more practical angle, the findings of both chapters can be applied to one another. For one, chapter 3 found that the presence of *Diolcogaster* did not have a negative correlation on the abundance of other sampled parasitoids in canola fields, implying it may not have a negative impact . While it can be difficult to apply these correlational findings to *Peristenus*, it is not outside the realm of speculation that, if the highly abundant and exotic *D. claritibia* can co-exist with native parasitoids, so can the exotic *P. digoneutis*. Furthermore, the finding that edge boundaries have little effect on parasitoid populations has implications on *Lygus* bug management. If parasitoid populations are indeed driven by land management practices, as speculated, then understanding these mechanisms could aid in the biocontrol of these insects; conversely, it could also be driving an increase in *Lygus* populations. Finally, the indicator species analysis for chapter 3 (Appendix A) found that *Peristenus* wasps were a marginal indicator for road verges (p = 0.0566). In other words, *Peristenus* are likely to be found around road verges, information that is useful when trying to collect and conserve the biocontrol agents.

4.5 Concluding remarks

Wasps are a misunderstood group of insects that are generally disliked by the public; however, they play important roles in ecosystems and the agricultural industry. As insect populations continue to decline worldwide, it is important to research ways to promote, conserve, and understand these valuable natural enemies and, occasionally, pests. Advancements in the field of entomology, particularly the study of Hymenoptera, are needed as the world population continues to grow and the agriculture industry continues to expand in accommodation of more and more needs; studies like this one open the door to the fascinating world of wasp diversity and the possibility to incorporate them into farm management to rely more on natural control than insecticides. The key is to balance agricultural productivity with biodiversity conservation.

I sincerely hope that the findings of this thesis can incite not only curiosity for these lesser-loved insects, but also further research and collaboration amongst scientists, producers, and the general public to protect and preserve our natural world.

4.6 References

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Appendix A

Table 4. Significant (p < 0.05) indicator species (genera) for coulee, shelterbelt, and road verge edge boundaries according to an indicator species analysis with 9,999 permutations.

	Genus	Relative frequency	Relative abundance	Total individuals collected	*p*-value
Coulee	*Dinotrema*	0.35	0.09	59	0.0290
	Diplazon	0.60	0.11	74	0.0068
	Opius	0.27	0.06	41	0.0392
	Orasema	0.17	0.02	15	0.0236
Shelterbelt	*Gryon*	0.15	0.12	80	0.0226
	Mesochorus	0.15	0.02	12	0.0439
	Belytinae Morphotype A	0.10	0.01	7	0.0363
Road verge	*Campoplex*	0.42	0.25	172	0.0426
	Peristenus	0.68	0.28	188	0.0566
	Platygaster	0.10	0.01	8	0.0023
	Telenomus	0.18	0.04	27	0.0047