INVESTIGATING POPULATION DYNAMICS OF THE WHEAT STEM SAWFLY (*CEPHUS CINCTUS*) (HYMENOTERA: CEPHIDAE) IN CANADIAN AGROECOSYSTEMS

A Thesis Submitted to the
College of Graduate and Postdoctoral Studies
In Partial Fulfillment of the Requirements
For the Degree of Master of Science
In the Department of Plant Sciences
University of Saskatchewan
Saskatoon

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ABSTRACT

The wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), has been a serious economic threat to wheat production in the northwest Great Plains region of North America for over 100 years. Despite immense research efforts on *C. cinctus* population dynamics, there remain knowledge gaps. The objectives of this research were to determine how winter and early spring abiotic factors influence: 1) *C. cinctus* larval mortality and the spatiotemporal variation in the efficacy of larval mortality factors, and 2) adult life history traits of *C. cinctus* and one of its natural enemies, *Bracon cephi* Gahan (Hymenoptera: Braconidae). Post-harvest samples were collected from fields in southern Alberta to set baselines for the occurrence of live *C. cinctus* and larval mortality due to parasitism, fungal infection, and unknown reasons. Fields were re-visited the following spring (once in 2020 and twice in 2021) to measure changes in *C. cinctus* larval populations. We observed that the number of viable (*i.e.*, living) *C. cinctus* larvae changed significantly between geographic sites but not over time. This conclusion highlights that overwintering and early season abiotic conditions are unlikely to affect *C. cinctus* population dynamics. The field-level variation underscores the importance of scouting individual fields if high *C. cinctus* population densities are expected in a given area. Laboratory experiments were conducted to test the effects of cold temperatures in early spring, that are known to prolong post-diapause development, on adult life history traits (*e.g.*, longevity, body size, lipid content, and egg capacity) of *C. cinctus* and *B. cephi*. For *C. cinctus*, adult longevity and male lipid content significantly decreased when post-diapause development was extended. In contrast, neither *B. cephi* adult longevity nor body size were affected by the prolonged post-diapause development periods. These results provide novel information on how the post-diapause biology of two important native hymenopterans differ in response to temperature. Altogether, this research shows that while post-diapause abiotic conditions may not increase *C. cinctus* mortality, cold temperatures that delay *C. cinctus* adult emergence can greatly impact several adult life history traits that could impact population dynamics. This information will be useful for the development of *C. cinctus* phenology and population forecasting models.

ACKNOWLEDGEMENTS

I would like to thank my supervisors, Dr. Meghan Vankosky and Dr. Chris Willenborg. Over the last two years, despite all the hiccups and issues brought on by the pandemic, both of you were always there to assist in whatever way possible and made my graduate experience much more enjoyable. I would also like to extend my gratitude to the members of my advisory committee – Dr. Sean Prager and Dr. Eric Lamb. On top of also being my graduate class professors, both of you offered several helpful suggestions and criticisms throughout my project that were greatly appreciated. Lastly, I would like to thank my external examiner, Dr. Erika Peirce, for their insightful comments during my defence.

I am grateful for the help that Scott Meers provided with showing me how to properly scout for sawfly-cut stubs. I am especially thankful for the help Shelley Barkley provided over the last two years. If it was not for your assistance in sample collection and contacting producers, I highly doubt this project would have been able to finish on time. I would also like to thank the members of the AAFC Entomology Lab, Ross Weiss and Jonathan Williams. Whether it was helping with experimental design or just being there to chat about anything, I am very glad both of you were always there to lend an ear. Lastly, this work would not have been possible without the wheat producers who allowed me to sample and collect from their fields. Thank you for giving me the opportunity to work alongside you and I hope my work will be useful for your operations someday.

Finally, I would like to acknowledge all he financial support I have received during my time as a graduate student that allowed me to continue working without stressing about unforeseen expenses brought on by the pandemic. First, I would like to thank the Integrated Crop Agronomy Cluster for funding this project and the work I was did over the last two years. I would also like to thank the scholarships I received: the Saskatchewan Wheat Development Commission Graduate Scholarship, the Entomological Society of Canada Dr Lloyd M. Dosdall Memorial Scholarship, and the Indigenous Academic Excellence Award. As someone who has struggled with their cultural identity for some time, it was very special to me having received and being acknowledged for my academic work as an Indigenous individual, and for that I am very grateful.

DEDICATION

I would like to dedicate this thesis to my family who have been so supportive over my time in graduate school: my parents, Barry Sjolie and Hilda Bouvier, my sister and brother-in-law, Danielle and Michael Perry, my step-sister and her partner, Alex Sams and Dan Yereniuk, and lastly in memory of my step-mother, Judy Hurst. Although Judy will not see me graduate, she was always so supportive in every endeavor and adventure I went on. I love you all and thank you for being there.

To all the people who I got to work and collaborate with over the last 9 or so years. There are a few too many people to thank as this group includes everyone I met while at the University of Alberta, CABI-Switzerland, and Texas A&M University, however one person that really helped me find my way as an "early career researcher" was Dr. Ronald Batallas. Thank you to each and every one of you, I definitely would not be the person I am without you all.

To all my friends, old and new, who have sat and listened to my graduate student and researcher woes over the last two years. In particular, I would like to single out the friends I have made while at the U of S: Caleb Bryan, Amanda Fedorchuk, Matthew Wengler, Ariana Forand, Denys Solskyi, Alanna Orsak, Samantha Morrice, and Stefanie De Heij. Thank you all for making this graduate experience much more enjoyable.

Lastly, I dedicate this two very important people in my life: to my loving girlfriend, Randi Spencer, and to the bestest boy there ever was, Nash. Randi, I highly doubt you knew what you were getting into when you decided to meet an "entomology graduate student", but boy am I glad you decided to stick around. You have been my biggest supporter and carried me through the ups and downs on this rollercoaster called grad school. I love you and I am so happy you are part of my life. And to my four-legged best friend Nash, even though you cannot read this, you are the best companion and pandemic lab mate I could have ever asked for. You two are the best things in my life and I cannot wait for our future journeys together.

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1. INTRODUCTION

1.1 Background

The wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), is one of the most long-standing agricultural insect pests in the northern Great Plains region of North America. The host range of wheat stem sawfly includes over 25 non-cultivated grasses and several important plant crops including wheat, *Triticum aestivum* L. (Poaceae), durum wheat, *T. durum* Desf (Poaceae), and barley, *Hordeum vulgare* L. (Poaceae) (Cockrell *et al.* 2017). From the 1920s to 1950s, severe wheat stem sawfly infestations were a common occurrence in northwestern areas of the United States and the Canadian prairie provinces (Ainslie 1929; Criddle 1915). More recently, wheat stem sawfly has become a growing concern for wheat producers in more southern states like Colorado (Cockrell *et al.* 2021), likely due to climate change and increased developmental synchrony of wheat stem sawfly with other cultivated host plants (*e.g.,* winter wheat) (Morrill *et al.* 1992; Morrill & Kushnak 1996).

Larvae are the damaging life stage of wheat stem sawfly. Larvae reside inside wheat stems and feed on parenchymal tissue and vascular bundles throughout the growing season (Holmes 1979). Larval feeding causes reductions in kernel head weight (Holmes 1977), protein content (Holmes 1977, 1979; Beres *et al.* 2007), and photosynthetic capacity (Macedo *et al.* 2005, 2007). As plants begin to senesce and moisture levels decrease, mature larvae travel to the base of the plant to prepare for their obligatory winter diapause (Holmes 1979). The larvae cut the stem to create their overwintering location, leaving plants susceptible to falling over (Ainslie 1920, 1929). Across its geographic range, wheat stem sawfly can cause up to $450 million CAD (using modern commodity pricing) in economic losses when fields experience 50% stem cutting damage (Beres *et al.* 2007, 2017).

Efforts to control and mitigate wheat stem sawfly populations have focused extensively on cultural techniques, reliance on biological control measures, and host-plant resistance, as chemical control is not an effective strategy (Knodel *et al.* 2009). The most effective and recommended method is to plant solid-stem varieties of wheat that negatively affect pest performance and survivorship (Morrill *et al.* 1992, 1994; Cárcamo *et al.* 2005; Beres *et al.* 2013;

Szczepaniec *et al.* 2015), but their adoption is not currently widespread in heavily affected wheat growing areas in Alberta (Agriculture Financial Services Corporation 2021). Biological control methods for wheat stem sawfly management rely primarily on two native parasitoids, *Bracon cephi* Gahan (Hymenoptera: Braconidae) and *B. lissogaster* Muesebeck (Hymenoptera: Braconidae) (Morrill *et al.* 1998; Runyon *et al.* 2002; Weaver *et al.* 2005). Sawfly larvae are also susceptible to fungal infection by several species of *Fusarium* (Sun 2008; Wenda-Piesek *et al.* 2009). Life table analyses (Peterson *et al.*, 2011; Buteler *et al.*, 2015) and population matrices (Rand *et al.* 2017, 2020) highlight that parasitism, host plant resistance, and pathogens have significant impacts on wheat stem sawfly larval mortality and population dynamics.

Despite the great effort and investment in research to understand wheat stem sawfly, there are still research gaps relating to how populations fluctuate from one year to the next. Non-economic levels of sawflies in one year can produce heavy infestation levels the following year (Holmes 1982). It is not clear why or how this happens. Specifically, it is not well understood how weather during the post-diapause phase of sawfly development might influence larval mortality and the efficacy of their larval mortality factors (*e.g.,* parasitism, fungal infection). This project addresses these research gaps and will provide information that will contribute to development of a tool to forecast wheat stem sawfly populations between growing seasons.

1.2 Objectives and hypotheses

This research aimed to explore the population dynamics of wheat stem sawfly in Canadian agroecosystems. More specifically, the overarching objective of this project was to determine the potential effects of winter and early season temperatures on the population dynamics of wheat stem sawfly and its natural enemies. Four hypotheses were developed to answer the address the main research objective:

- Hypothesis 1: Wheat stem sawfly larval mortality will differ based on survey field.
- Hypothesis 2: Within each field, wheat stem sawfly larval mortality will change between sampling periods.
- Hypothesis 3: Wheat stem sawfly adult life history traits will be impacted by prolonged post-diapause periods as controlled by temperature.

- Hypothesis 4: Prolonged post-diapause development will have a smaller effect on *B. cephi* adult longevity as compared to wheat stem sawfly adult longevity.

To test the hypotheses, the following experiments were developed and conducted:

- Experiment 1: Investigation of the causes of wheat stem sawfly larval mortality in the Canadian Prairies (Hypotheses 1 & 2)
- Experiment 2: Determination of the effects of early season temperature on adult life history traits of wheat stem sawfly and *B. cephi* (Hypotheses 3 & 4)

Twelve harvested wheat fields were sampled to explore changes in sawfly larval mortality between growing seasons and in the early spring of the following year. To determine the effects of early season temperature, emergence groups were set up to control the length of the post-diapause development period for both wheat stem sawfly and the native parasitoid *B. cephi*. These experiments were conducted in Saskatoon, SK at the Agriculture and Agri-Food Canada Saskatoon Research and Development Centre or at my personal residence because of the COVID-19 pandemic.

2.0 LITERATURE REVIEW

2.1 History and biology of the wheat stem sawfly

The wheat stem sawfly (*Cephus cinctus* Norton, Hymenoptera: Cephidae) was first described in Colorado from grasses in 1872 (Norton 1872). Specimens were also reared and reported in New York State and California in 1889 and 1890, respectively (Comstock 1889; Ainslie 1920). Although wheat stem sawfly is widely thought to have originated in North America, Ivie (2001) proposed that wheat stem sawfly is an introduced species from Asia, based on biogeographic data and host-plant associations. However, a recent phylogenetic study that compared two mitochondrial regions (*CO1*, *16S*) between wheat stem sawfly and a related species from Asia, *Cephus hyalinatus* Konow (Hymenoptera: Cephidae), highlighted significant genetic divergence between the two species and presented further support for the North American origin of wheat stem sawfly (Lesieur *et al.* 2016).

Fletcher (1886) made the first observations of wheat stem sawfly in wheat stems in Canada near Souris, Manitoba and Indian Head, Saskatchewan in 1885. By 1910, it had moved farther west and a serious infestation was documented in Claresholm, Alberta (Holmes 1979). The first severe infestation and damage to wheat was recorded in 1922 in western Canada, which highlighted the negative implications of wheat stem sawfly for the expanding wheat industry (Criddle 1923). Infestations between 1938 and 1944 caused losses of 560,000 to 700,000 t (tonnes) annually in western Canada (Holmes 1982). High amounts of precipitation, a widespread outbreak of stem rust, and higher levels of parasitism caused a sharp decline in the wheat stem sawfly population in the 1950s (Holmes 1982). Since the 1950s, two major outbreaks were recorded in Canada; one in Alberta in 1998 and 1999 and another in Saskatchewan in 1999 (Holmes 1982; Meers 2005; Beres *et al.* 2007). Populations declined again in the early 2000s, but have been increasing since 2018 in southern Alberta, based on surveys of cut stems in Alberta wheat fields (Alberta Agriculture and Forestry 2021).

Wheat stem sawfly adults have a narrow shiny black body with yellow abdominal bands (Fletcher 1904). Adults are approximately 12 mm long (Fletcher 1904). Wheat stem sawfly have three genomic clusters that are highly correlated with geography (Lesieur *et al.* 2016). Sawflies found in the Canadian Prairies and eastern Montana belong to the Northern cluster (Lesieur *et al.* 2016). Adults live for approximately 7 d in the field, depending on weather conditions (Criddle 1922; Ainslie 1929). In laboratory assays, adults did not live longer when presented with a sucrose or honey solution, but did benefit slightly when given buckwheat (*Fagopyrum esculentum* Moench, Polygonaceae) as a floral resource (Rand *et al.* 2019). Like other Hymenoptera, males are haploid (nine chromosomes) and are the product of unfertilized eggs, while females are diploid (18 chromosomes) and are the product of fertilized eggs (Holmes 1979). Fertilized (female) eggs tend to be laid early in the summer and unfertilized eggs that produce males are typically laid in greater proportions later in the summer (Holmes 1979). This is probably because it is harder for females to find males to mate with as the summer flight season progresses (Holmes 1979). Males typically emerge first, and copulation begins immediately after female emergence (Wallace & McNeal 1966). Female *C. cinctus* are pro-ovigenic, meaning that adult females emerge from the pupal stage with a full complement of mature eggs. Females have an average complement of 33 eggs upon eclosion, but individuals with up to 50 eggs have been recorded (Ainslie 1920; Holmes 1979). The number of eggs depends on conditions experienced during larval development, including host plant quality and crop variety (Holmes 1982; Cárcamo *et al.* 2005).

Oviposition generally takes place during midday, within a 4-day period following adult emergence (Ainslie 1920; Holmes 1979). When selecting oviposition sites, females tend to prefer succulent plants with large diameter stems (Holmes & Peterson 1960). Larger stems typically contain female offspring while smaller stems tend to have a male-biased offspring ratio (Wall 1952; Morrill *et al.* 2000; Cárcamo *et al.* 2005). Females use their saw-like ovipositor to slice into the internode of a wheat stem and lay a single egg (Holmes 1979). Although females only deposit one egg per stem, other sawflies are not deterred from selecting occupied wheat stems, thus, multiple eggs may be laid into a single wheat stem (Criddle 1923; Buteler *et al.* 2009).

Eggs are white or milky-coloured, crescent-shaped, and usually 1.00 to 1.25 mm long, depending on the size of the female that laid them (Ainslie 1920). Eggs hatch after about 8 d and the newly-emerged larvae immediately begin to feed on the parenchymal tissues and vascular bundles of their host plant (Ainslie 1920; Criddle 1923). In cases where multiple females lay eggs in a single wheat stem, the larva that emerges first will cannibalize or destroy all other eggs or larvae (Criddle 1923). It is rare for two viable larvae to be recovered from a single stem (Holmes 1979; Beres *et al.* 2011). Larvae develop through four of five instars while feeding within the stem (Holmes 1979). Feeding continues until the end of the season when diapause is triggered and the larva travels to the base of the plant stem (Holmes 1979). Pre-diapause is triggered by two environmental cues: light penetration of the stem wall and decreased overall plant moisture (Holmes 1979). At the base of the plant stem, the larva cuts a neat V-shaped groove entirely within and around the stem at or slightly above ground level. The larva then creates a plug-like structure using frass (fecal matter) and seals itself into a sheltered cell below the V-shaped groove (Ainslie 1920). After grooving the plant stem, the larva empties its gut, lines the cell with a cocoon, and overwinters (Salt 1947; Holmes & Peterson 1960). Sawfly larvae are cold-hardy and have a supercooling point of -22°C (Salt 1966; Cárcamo *et al.* 2011). As long as the structural integrity of the chamber and cocoon remain intact, the overwintering larva can survive temperatures ranging from -20 to -28°C because temperatures inside the wheat stem remain higher than ambient external winter temperatures (Holmes 1979; Morrill *et al.* 1993). While in the diapause phase, metabolic activity and oxygen uptake is very low and on average 0.03 – 0.05 mm^3/mg live weight per hour (Villacorta *et al.* 1972).

Diapause termination is temperature dependent. In his experiments, Salt (1947) found the ideal daily mean temperature conditions for diapause termination occur somewhere around 5°C to 10°C. At 10°C, the diapause phase ranges from 50 to 110 d, but is variable among individual larvae (Salt 1947). After breaking out of diapause, overwintered individuals end their larval phase and continue with their pupal development (Salt 1947). Post-diapause wheat stem sawfly development is also highly temperature dependent. If temperatures are between 10°C and 15°C, larval metabolic activity increases slightly, but the larvae remain in a post-diapause development quiescence (Villacorta *et al.* 1972; Perez-Mendoza & Weaver 2006). Neither development nor subsequent adult emergence occurs below 15°C (Salt 1947; Church 1955; Perez-Mendoza & Weaver 2006). Larvae can re-enter diapause if temperatures approach 35°C or conditions are extremely dry and larvae will remain in that state until the following spring (Salt 1947; Holmes 1979). Post-diapause, the metabolic activity and oxygen consumption of wheat stem sawfly steadily increases up to adult emergence when temperature conditions are between 15°C and

35°C (Villacorta *et al.* 1972). The ideal conditions for spring development are around 25°C with 60 to 75% relative humidity (RH); however, sawflies can reach the pupal stage in other scenarios (Perez-Mendoza & Weaver 2006). The pupal period lasts between 16 to 22 d beginning in late May (Criddle 1923; Holmes 1979). After pupation, newly eclosed adults chew their way out of the wheat stub either through the frass plug or the side of the stub itself (Holmes 1982). Spring development time can vary based on geographic region and genetic haplotype group. For example, sawflies from eastern Montana (Mountain cluster) have shorter spring development periods as compared to sawflies in western Montana and Canada (Northern cluster) (Perez-Mendoza & Weaver 2006; Rand *et al.* 2016).

2.2 Effect of wheat stem sawfly on host plants

The larval stage of *C. cinctus* is the primary cause of injury to infested host plants. Damage occurs when the larvae eat through the parenchymal tissues and vascular bundles, resulting in decreased photosynthesis, CO_2 exchange, and transpiration (Macedo *et al.* 2005, 2007). Larval feeding damage results in reduced head weight, reduced protein content, and yield loss (depending on host plant variety) (Holmes 1977, 1979; Morrill *et al.* 1992; Beres *et al.* 2007; Szczepaniec *et al.* 2015). Stem girdling or cutting by mature larvae preparing to overwinter further exacerbates yield loss because girdled (or cut) stems are susceptible to lodging (*i.e.*, stems fall over in windy conditions), making the wheat difficult to harvest (Ainslie 1920). The cumulative effect of larval feeding and lodging can contribute to significant yield and economic losses. For example, a survey of producers in Montana projected that losses from wheat stem sawfly in the 1990s exceeded 402,000 t (tonnes) annually (Blodgett *et al.* 1997). In the 2010s, annual losses in Montana ranged between $45 million and $80 million USD (Bekkerman & Weaver 2018). In Colorado, expected economic losses due to sawfly infestations exceed $30 million USD (Peirce *et al.* 2021). On the Canadian Prairies, Beres *et al.* (2017) estimated that 50% stem cutting could result in losses of over $400 million annually, based on a positive correlation between grain loss and stem cutting (estimated in modern commodity prices) (Beres *et al.* 2007).

2.3 Wheat stem sawfly pest management

Significant efforts have been made to mitigate economic losses on wheat production by wheat stem sawfly. Cultural and chemical tactics have been investigated, with varying levels of success. Of all the pest management tools available, host plant resistance and biological control have been most effective. In-field scouting methods, population distribution maps, and a bioclimatic model are presently available for wheat producers. Still, it is not possible to predict *C. cinctus* population densities for upcoming growing seasons. Therefore, making timely decisions to maximize *C. cinctus* control can be difficult for farmers and agronomists.

2.3.1 Cultural control

Several cultural control methods to curtail wheat stem sawfly population growth have been studied. Early attempts involved burning a layer of straw overtop of infested stubble, but this was largely ineffective and was abandoned as a management technique (Ainslie 1920). Tilling infested wheat fields increased larval mortality as stubs were either crushed mechanically or left susceptible to desiccation but did not completely prevent adult emergence (Ainslie 1920; Criddle 1922; Morrill *et al.* 1993). Improper use of tilling can lead to issues associated with soil erosion and water retention (Quisenberry *et al.* 2000), and in many cases, the consequences associated with tilling were not offset by the benefits of reduced sawfly populations. Another consequence of both tilling and stubble burning is the negative impact on the survivorship of beneficial parasitoids housed in other stems (Runyon *et al.* 2002). Several studies showed that tillage had no negative effect on larval survival if the tilling process failed to remove soil from the host plant root crowns (Holmes & Farstad 1956; Weiss *et al.* 1987; Goosey 1999). Overall, tillage alone is not an economically effective wheat stem sawfly control method and should only be done along the field margins in extreme cases.

Seeding rate, row width, and seeding date all influence sawfly infestations. High seeding rates of hollow-stem cultivars combined with narrow row spacing decreased oviposition, probably because increased plant density lowered whole-plant moisture levels, resulting in less attractive plants (Luginbill & McNeal 1958). The same high seeding rate and narrow row spacing treatment with solid-stem wheat caused an increase of stem cutting damage because increased plant density negatively affects expression of the solid-stem trait (Luginbill & McNeal 1958). Wider row spacing and low seeding rate maximize the efficacy of solid-stem varieties as these practices increase exposure to sunlight, ensuring proper pith expression (Beres *et al*. 2012). For solid-stem cultivars of durum wheat, changing the sowing density is not required to reach the optimal pith development threshold to protect against wheat stem sawfly damage (Nilsen *et al.* 2016). Early seeding significantly reduced sawfly infestation levels, likely because stem elongation was not synchronized with the sawfly oviposition period (Jacobson & Farstad 1952; McNeal *et al.* 1955; Morrill & Kushnak 1999).

Nutrition management affects wheat stem sawfly population densities by altering crop canopy structure and host plant health. Plots treated with nitrogen and phosphorus fertilizers saw an increase in stem cutting compared to plants in plots that did not receive fertilizers (Luginbill & McNeal 1954). The authors noted that fertilized plants had a greater growth response relative to non-fertilized plants, and were likely more appealing to ovipositing females as a result (Luginbill & McNeal 1954). Fertilization with nitrogen alone did not affect sawfly infestation or damage levels and applying phosphorous alone resulted in a slight increase in cut stems (Luginbill & McNeal 1954). Conversely, in a greenhouse study, Delaney *et al.* (2010) showed that phosphorus-deficient plants were most susceptible to sawfly damage when compared to plants that received supplemental phosphorus. Fertilization with nitrogen can reduce the development of pith in solid-stemmed varieties when crop canopy cover increases (Beres *et al.* 2012). Other studies have shown inconsistent or no effects of fertilizer treatments on sawfly densities, probably because environmental factors exert greater influence on sawfly host plant selection and larval feeding damage (O'Keeffe *et al.* 1960; DePauw & Read 1960). The relationship between crop nutrition and wheat stem sawfly infestation is complex.

2.3.2 Chemical control

Managing wheat stem sawfly with insecticides is challenging, in part due to the short flight period of adult sawflies and the concealed feeding habitat of the larvae. Thus, relatively few studies have explored the use of chemical insecticides. Several systemic and foliar insecticide applications do not provide sufficient crop protection, including chlordane (Munro *et al.* 1949), DDT (Munro *et al.* 1949), toxaphene (Munro *et al.* 1949), parathion (Munro *et al.* 1949; Holmes & Hurtig 1952), lindane (Munro *et al.* 1949), DDD (Munro *et al.* 1949), heptachlor (Holmes & Hurtig 1952; Wallace 1962; Wallace & McNeal 1966), Furdane 4F (active ingredient = carbofuran) (Blodgett *et al.* 1996), Lorsban 4E-SG (active ingredient = clorpyrifos) (Blodgett *et al.* 1996), thiamethoxam (Knodel *et al.* 2009), and lambda-cyhalothrin (Knodel *et al.* 2009). Two organophosphates, parathion and heptachlor, did provide relatively effective control against sawfly adults and larvae, respectively (Holmes & Hurtig 1952; Wallace 1962; Holmes & Peterson 1963a; Wallace & Butler 1967). However, due to significant toxic effects on non-target organisms and persistence in soil, both products are no longer registered for agricultural use in the US or Canada (US EPA 2000; Beres *et al.* 2011). Applications of synthetic plant defense elicitors (*e.g., cis*-jasmone) as biopesticides cause increased larval mortality and decreased larval body size but do not prevent yield losses (Shrestha *et al.* 2018).

Montana recently approved the use of Thimet 20-G (active ingredient = phorate, organophosphate), for controlling wheat stem sawfly (Wanner & Tharp 2015). There are no insecticides registered for wheat stem sawfly in Canada (Alberta Agriculture and Forestry 2019; Saskatchewan Ministry of Agriculture 2019). Currently, the use of chemical control measures to mitigate wheat stem sawfly damage are not recommended in North America.

2.3.3 Host plant resistance

Solid-stemmed wheat varieties are an effective tool for mitigating wheat stem sawfly damage. Most solid-stemmed varieties inherit this trait from the wheat line S-615 (Platt & Farstad 1946). Cook *et al.* (2004) determined that a single quantitative trait locus (QTL) on chromosome 3BL, *Qss.msub-3BL*, was responsible for most of the variation in stem solidness. ‘Rescue’ was the first solid stem variety adopted in sawfly-affected areas (Platt *et al.* 1948). Single nucleotide polymorphism (SNP) haplotype analyses indicated that sawfly resistant wheat varieties grown in North America all possess the *Qss.msub-3BL* allele derived from ‘Rescue’ (Varella *et al.* 2015; Cook *et al.* 2017). A recent study by Cook *et al.* (2019) demonstrated the potential of a second solid-stem allele, derived from the wheat variety 'Conan', *Qss.msub-3BL.c*, for management of wheat stem sawfly. Another recent study discovered that the gene *TdDof* controls pith development and the overall solid-stemness in wheat (Nilsen *et al.* 2020).

Stem solidness is influenced by photoperiod, where plants exposed to sufficient sunlight will develop more pith in the culm of the stem and be more 'solid' as a result (Eckroth & McNeal 1953; Holmes 1984). Solid-stem cultivars increase egg and larval mortality, reduce female body mass, and reduce the fecundity of females that develop in solid-stemmed plants (Holmes & Peterson 1961, 1962; Morrill *et al.* 2000; Cárcamo *et al.* 2005). Solid-stem varieties experience less larval feeding damage and reduced stem cutting (Talbert *et al.* 2014; Sherman *et al.* 2015). However, solid stems do not affect mortality rates of overwintering *C. cinctus* (Cárcamo *et al.* 2011). The solid-stem trait appears to affect female preference, as female sawflies oviposited more frequently into the hollow-stem spring wheat variety 'CDC Go' compared to the solid-stem spring wheat 'AC Lillian' (Cárcamo *et al.* 2016). A study investigating the effectiveness of the solid-stem cultivar 'Rescue' by Holmes and Peterson (1957) saw significantly reduced sawfly populations when this variety was grown over a 5-year period.

2.3.4 Biological control

There are several species of natural enemies (*i.e.*, predators and parasitoids) that target wheat stem sawfly within its North American range. Nine parasitoids are capable of parasitizing wheat stem sawfly (Cárcamo & Beres 2013), but only two, *Bracon cephi* Gahan (Hymenoptera: Braconidae) and *B. lissogaster* Muesebeck (Hymenoptera: Braconidae), frequently attack sawfly larvae in wheat (Davis *et al.* 1955; Somsen & Luginbill 1956; Runyon *et al.* 2002). Attempts at releasing populations of *B. lissogaster* and *B. cephi* in Montana for biological control have not been successful, as they failed to establish resident populations at release sites (Morrill *et al.* 1998).

The idiobiont ectoparasitoid, *B. cephi*, was first observed parasitizing sawflies in native grasses at very high levels (Criddle 1923; Ainslie 1929). Later, this bivoltine parasitoid synchronized to the sawfly lifecycle within wheat and became a major sawfly population suppressor, both in Canada and the United States (Munro *et al.* 1949; Nelson & Farstad 1953). *Bracon cephi* overwinters as a mature larva within a cocoon in the lower internodes (0 – 30 cm) of the plant (Holmes *et al.* 1963; Meers 2005). The conditions that allow for diapause induction in *B. cephi* are not well understood. The life history of *B. cephi* is similar to *Bracon terebella* Wesm. (Hymenoptera: Braconidae), a parasitoid of the European sawfly, *Cephus pygmaeus* L. (Hymenoptera: Cephidae). However, each species has pre-diapause phase activities that are quite different (Salt 1931; Nelson & Farstad 1953). To survive extreme winter cold temperatures, *B. cephi* larvae synthesize the cryo-protectant glycerol in large quantities (up to 25% of their body mass) to decrease their supercooling point and prevent larvae from freezing (Salt 1959). The conditions for *B. cephi* diapause termination are also not well understood. Adult *B. cephi* emergence is well-synced to that of wheat stem sawfly adult emergence, so perhaps similar temperature conditions also trigger diapause termination in *B. cephi* (Holmes *et al.* 1963). The larvae of a related species, *Bracon mellitor* Say (Hymenoptera: Braconidae), require a two-week exposure period of low temperature followed by a period of 20°C for diapause termination (Adams *et al.* 1969). Once diapause is broken, pre-pupal and pupal development requires about 2 and 6 d, respectively, under laboratory conditions (25°C at 70% RH) (Nelson & Farstad 1953). Adults emerge by chewing a hole through the pupal cocoon and wheat stem wall (Nelson & Farstad 1953). Early laboratory assays determined that adult males live from 10 to 14 d, while females can live up to 28 d (Nelson & Farstad 1953). Female longevity and egg capacity benefit greatly from feeding on a floral resource or aphid honeydew (Reis *et al.* 2019; Rand & Waters 2020). *Bracon cephi* females go through a pre-oviposition period spanning 12 to 21 d (Nelson & Farstad 1953). Females parasitize sawfly larvae by walking along the stem and injecting venom and a single egg via their ovipositor near the host larva (Nelson & Farstad 1953). Once hatched, the parasitoid larva searches for and attaches itself to the immobile sawfly larva where it begins feeding (Nelson & Farstad 1953). Development of the parasitoid larva requires approximately 10 d after which the larva spins a cocoon and begins pupation. Parasitism by the first generation of *B. cephi* can potentially protect grain yields and prevent stem lodging by mature sawfly larvae (Buteler *et al.* 2008; Wu *et al.* 2013). *Bracon cephi* are susceptible to predation by active sawfly larvae when sawfly densities are high (Holmes 1979). The second generation of adult parasitoids emerge in August and, unlike the first-generation adults, can immediately begin ovipositing

(Holmes *et al.* 1963). The success of the second generation of parasitoids depends on the timing of crop maturity and developmental stage of the larval wheat stem sawflies (Holmes *et al.* 1963).

A second idiobiont parasitoid, *B. lissogaster* was discovered in 1949 at Choteau, Montana (Somsen & Luginbill 1956). It is described as a strict parasite of wheat stem sawfly (Somsen & Luginbill 1956). Female *B. lissogaster* will oviposit between one and four eggs into a single wheat stem (Nelson & Farstad 1953; Somsen & Luginbill 1956). Eggs hatch after 66 hours at 23°C and 78% RH (Somsen & Luginbill 1956). Larvae spend 6 to 8 d feeding on their host, or less if multiple parasitoid larvae share a single host, before spinning a cocoon (Somsen & Luginbill 1956). First generation cocoons are brown and loose, while second generation cocoons are more tightly woven and dull white in colour (Somsen & Luginbill 1956). Very little is known about the impacts of weather and other abiotic factors on the population dynamics and parasitism rates of *B. lissogaster*. *Bracon lissogaster* is most common in central and western Montana but it has been recovered in low numbers in Canada (Cárcamo *et al.* 2012), as far north as Vulcan, Alberta from wheat fields and as far south as Colorado from noncultivated grasses (Peirce *et al.* 2021). *Bracon lissogaster* and *B. cephi* are indecipherable as larvae and can only be differentiated using adult morphological traits (Runyon *et al.* 2001).

Other potential biological control agents for wheat stem sawfly include a generalist predatory clerid beetle, *Phyllobaenus dubius* Wolcott (Coleoptera: Cleridae), infection by naturally occurring or foliar applications of entomopathogens, and entomopathogenic nematodes (EPNs) (Morrill *et al.* 2001; Wenda-Piesik *et al.* 2009; Tangtrakulwanich *et al.* 2014; Portman *et al.* 2016). Both adult and larval *P. dubius* can predate upon sawfly larvae within the stem, but their impact on sawfly populations is not known (Morrill *et al.* 2001). Isolates from a complex of *Fusarium* spp. can infect and kill diapausing and developing larvae in greenhouse settings, but no data is available from the field about the efficacy of these potential entomopathogens (Sun 2008; Wenda-Piesek *et al.* 2009). The fungi, *Beauveria bassiana* (Bals.-Criv) Vuill and *Metarhizium anisopliae* (Metschnikoff) Sorokin, can reduce stem cutting damage without significantly affecting yield when applied as a foliar spray (Tangtrakulwanich *et al.* 2014). Studies conducted in Montana wheat fields highlighted the potential use of EPNs as a biocontrol agent for wheat stem sawfly (Tangtrakulwanich *et al.* 2014; Portman *et al.* 2016), but no research has addressed the use of EPNs in Canada.

2.3.4 Population detection and forecasting

Scouting, sampling, and forecasting are important tools in integrated pest management programs that can help farmers make appropriate pest management decisions (Dent 1991). There are no exact economic thresholds for wheat stem sawfly, but solid-stem cultivars and swathing are recommended when wheat fields experience over 15% stems cut due to sawfly larvae (Holmes 1982). Methods for predicting sawfly densities use different types of sampling during the growing season (*e.g.*, splitting wheat stems to count larvae, calculating dark spots below the nodes, sweeping adult sawfly females) (Holmes 1982; Weiss & Morrill 1992; Nansen *et al.* 2005; McCullough 2016). Mid-season assessments can be applied to make harvesting choices, such as straight cutting (direct harvesting) or swathing a given field (Holmes & Peterson 1965; Goosey 1999; McCullough 2016). On the Canadian Prairies, the Prairie Pest Monitoring Network, in partnership with Alberta Agriculture, Forestry and Rural Economic Development, develops wheat stem sawfly distribution maps that can help growers estimate potential risks in their region on an annual basis using an estimate of the percentage of cut stems in randomly selected wheat fields at the end of the growing season (Prairie Pest Monitoring Network 2019). However, these maps estimate distribution and density of the sampled populations, and the information is not robust enough to function as a forecast for damage in the subsequent growing season (Prairie Pest Monitoring Network 2019). A recently developed bioclimatic model for *C. cinctus* used the software program CLIMEX (Kriticos *et al.* 2015) to assess how populations respond to abiotic climate factors across its geographic range (Olfert *et al.* 2019). Although this approach does provide valuable information on how future climate conditions may impact potential *C. cinctus* range expansion, the model cannot forecast population fluctuations between growing seasons (Olfert *et al.* 2019).

3.0 Identifying the causes of wheat stem sawfly larval mortality in the Canadian Prairies

3.1 Introduction

The wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), is a major established North American pest first described in Colorado grasses in 1872 (Norton 1872). Since the 1920s, severe *C. cinctus* infestations have occurred in cultivated cereal crops including common wheat (*Triticum aestivum* L.), durum wheat (*T. durum* Desf.), and barley (*Hordeum vulgare* L.) (Wallace & McNeal 1966; Cockrell *et al.* 2017; Varella *et al.* 2018). *Cephus cinctus* populations impact cereal crop producers in the western Canadian provinces (Alberta, Saskatchewan, Manitoba) and northwest United States (Montana, North Dakota, South Dakota, Nebraska, Colorado, Wyoming) of the North American Great Plains region (Criddle 1923; Holmes 1982; Morrill *et al.* 1993; McCullough *et al.* 2020; Cockrell *et al.* 2021). Adult sawflies emerge from wheat stubble in late spring and females oviposit individual eggs into their host plants from June to July (Holmes 1977). Larvae develop within the plant stem and feed on parenchymal tissue and vascular bundles throughout the summer (Holmes 1977). Early instar larval feeding decreases kernel head weight, protein content, and photosynthetic capacity of the host plant (Macedo *et al.* 2005, 2007). From August to mid-September, as plant moisture begins to decrease, mature larvae travel to the base of the stem and cut the stem to create their overwintering hibernaculum (Holmes 1979). The cereal stems that host *C. cinctus* larvae are susceptible to lodging (*i.e.*, falling over) as the result of winds or other incidental contact, making it difficult for producers to harvest their crops. Using modern commodity prices, economic losses in high *C. cinctus* population years when stem cutting exceeds 50% can be upwards of $400M CAD annually (Beres *et al.* 2007, 2017).

Implementation of resistant solid-stem cultivars and methods that enhance local established populations of natural enemies are the primary tactics used to manage *C. cinctus*. Solid-stem plants develop greater amounts of pith within the stem that increases juvenile sawfly mortality (Holmes & Peterson 1961, 1962) and reduces adult fitness (Morrill *et al* 2000; Cárcamo *et al.* 2005). Several wheat and durum solid-stem varieties like ‘AC Lillian’ (DePauw *et al.* 2005), ‘AAC Stronghold’ (Ruan *et al.* 2019), and ‘CDC Fortitude’ (Pozniak *et al.* 2015) are registered for use in Canada to mitigate sawfly damage but are not widely selected as a preferred variety by producers (Agriculture Financial Services Corporation 2021). Two idiobiont ectoparasitoids, *Bracon cephi* Gahan and *B. lissogaster* Muesebeck (Hymenoptera: Braconidae), are the most common natural enemies of *C. cinctus* in North America (Nelson & Farstad 1953; Somsen & Luginbill 1956). *Bracon cephi* is the predominant parasitoid species in Canada, although *B. lissogaster* has been reported in small numbers southeast of Lethbridge, Alberta (Cárcamo *et al.* 2012) and as far north as Vulcan, Alberta. First generation adult *B. cephi* parasitoids attack sawfly larvae in July before they cut the host plant stem (Nelson & Farstad 1953). The success of the second generation of parasitoids is highly dependent on abiotic factors (*i.e.*, cold, wet conditions) that prolong the growing season and delay host plant senescence (Holmes *et al.*1963). Parasitism rates vary drastically by geographic region, with some areas having over 90% of *C. cinctus* attacked (Morrill *et al.* 1998). Within the stem, *C. cinctus* larvae are susceptible to infection and subsequent death by a complex of *Fusarium* including *F. acuminatum* Ell. And Ev. *sensu* Gordon, *F. avenaceum* (Fr.) Sacc., *F. culmorum* (W.G Smith) Sacc., *F. equiseti* (Corda) Sacc. *sensu* Gordon, and *F. graminearum* (Schwabe) (Sun 2008; Wenda-Piesik *et al.* 2009). Sawfly larvae are also susceptible to predation by the clerid beetle *Phyllobaenus dubius* Wolcott (Coleoptera: Cleridae) (Morrill *et al.* 2001; Meers 2005). Despite extensive research over the last 100 years on *C. cinctus* and its natural enemies, it is not clear how these larval mortality factors are influenced spatially or temporally, nor is clear how abiotic factors (*i.e.*, temperature) impact them.

Insect populations are dynamic and constantly fluctuate over time and space. Availability of suitable hosts (Sétamou *et al.* 2000; Opedal *et al.* 2020), abiotic conditions (Kingsolver 1989; Crozier 2004; Khokhar *et al.* 2019), natural enemy populations (Alyokhin *et al.* 2011, Bouchard *et al.* 2018), and anthropogenic activities (e.g., habitat destruction and chemical pesticide usage) (Ciesla 2015; Wagner *et al.* 2021) can heavily influence population densities. To better understand the population ecology of an insect, researchers and integrated pest management practitioners use species-specific biological parameters and climatic factors to create complex population models (Nietschke *et al.* 2007). For example, mechanistic or process-based population models (*i.e.*, phenology models) and distribution models have been developed for a number of insect pests of Canadian agriculture including *Melanopus sanguinipes* Fabricius (Orthoptera: Acrididae) (Olfert & Weiss 2006; Olfert *et al.* 2021), *Plutella xylostella* L.

(Lepidoptera: Plutellidae) (Li *et al.* 2016), *Aphis glycines* Matsumura (Hemiptera: Aphididae) (Bahlai *et al.* 2013), and *Sitodiplosis mosellana* Géhin (Diptera: Cecidomyiidae) (Olfert *et al.* 2020). These models are excellent integrated pest management tools for researchers as they allow for timely population density predictions on different spatial and temporal scales. In addition, output from these models provides important information to producers and helps them to make appropriate agronomic and pest management decisions to protect their crop yields. To date, a phenology model with predictive capacity within and between growing seasons has not been developed for *C. cinctus*.

The recent increase in *C. cinctus* population densities in areas of southern Alberta (Prairie Pest Monitoring Network 2020) provided an excellent opportunity to examine *C. cinctus* population dynamics in Canadian agroecosystems. Our study aimed to explore how *C. cinctus* larval mortality and the associated causes of mortality change regionally and over growing seasons, as this information is required for the development of a future phenology model and may facilitate the use of fall monitoring data to forecast future pest pressure. Previously developed multiple decrement life tables (Peterson *et al.* 2011; Buteler *et al.* 2015; Achhami *et al.* 2020) and population matrices models (Rand *et al.* 2017; 2020) found that during the summer growing season, larval mortality factors like parasitism and fungal infection can greatly influence *C. cinctus* population dynamics, but their impact drops as they overwinter. Rand *et al.* (2017) and Olfert *et al.* (2019) have pointed out that we do not completely understand the potential effect of these mortality factors on *C. cinctus* during the early spring growing period, when post-diapause larvae are completing their development. Within *C. cinctus*-infested regions, past studies have shown that populations vary spatiotemporally (Sing 2002; Nansen *et al.* 2005a) and *C. cinctus* mortality is not uniform from field to field (Holmes *et al.* 1957; Perez-Mendoza & Weaver 2006). However, these studies did not question if larval mortality factors were also relevant. In the present study, we expected to observe an overall effect of field location on *C. cinctus* larval mortality but a minimal effect of time on the larval mortality factors within each field.

3.2 Methods

3.2.1 Experimental locations and sampling protocol

Commercial wheat fields in southern Alberta with reported sawfly damage were sampled in this study. Fields were initially sampled as part of the annual wheat stem sawfly survey conducted by Alberta Agriculture, Forestry, and Rural Economic Development. Permission to resample the fields for this work was granted by all farmers. All fields were presumed to have been seeded with the hollow-stem hard red spring wheat 'AAC Brandon' as this was the most seeded wheat variety in all sample areas in both years (Agriculture Financial Services Corporation, 2021). In Fall 2019, six harvested wheat fields in Alberta with known sawfly infestations were visited and sampled for sawfly-cut stems (Table 3.1). The sampling procedure followed the survey protocol provided by the Prairie Pest Monitoring Network (https://prairiepest.ca/monitoring-protocols/), but was modified to focus on field edges where wheat stem sawfly larval densities are typically concentrated (Nansen *et al.* 2005b). Using this protocol, the total number of wheat stems, including the number of wheat stubs (cut by sawfly larvae) and long stems (cut mechanically during harvest) were counted along 1 m transects at four locations in each field, with each location separated from the others by 50 m. The protocol ensured that over 200 wheat stems were collected in each of the surveyed fields, providing confidence in our estimations of overall field population densities (Cárcamo *et al.* 2007). All wheat stub samples were collected and transported to the Agriculture and Agri-Food Canada Saskatoon Research and Development Centre in Saskatoon, SK. The number of *C. cinctus*-cut stems and the presence of *C. cinctus* larvae were determined. Stems were dissected to assess the condition of the sawfly larvae inside the stubs. Larval presence was tallied when *C. cinctus* larvae (regardless of condition) or parasitoid pupae were recovered from a dissected stem. The number of live larvae, dead larvae, and probable causes of mortality (*e.g.*, parasitism, fungal pathogen, unknown) were recorded. Unknown mortality may have been due to malnutrition, diseases not detectable using our methods, abiotic conditions including heat stress, cold stress (over the winter), or other factors. The average percentage of wheat stems cut was calculated for each field. In April 2020 the sites (excluding 'Vulcan' where larval populations were very low in Fall 2019) were re-sampled, using the process described above.

In fall 2020, six new fields were selected for the sawfly mortality survey (Table 3.1). Samples were collected between September 8 – 21 from each site to determine the sawfly infestation pressure and larval state prior to winter. In Spring 2021, the fields were re-sampled twice, first in April and then in May. Sampling and stem dissections were conducted using the same protocol as that described for Fall 2019.

Table 3.1: *Cephus cinctus* survey field locations and sampling dates; due to travel restrictions associated with the COVID-19 pandemic, fields could only be sampled once in spring 2020.

Fall 2019 - Spring 2020				
Site	Location	Fall Sample Date	Spring 1 - Sample Date	Spring 2 - Sample Date
Special Area #3	51.29, -110.14	22-Oct	27-Apr	-
Acadia	51.06, -110.20	22-Oct	27-Apr	-
Vulcan	50.20, -113.24	23-Oct	-	-
Willow Creek	49.94, -113.31	23-Oct	27-Apr	-
Warner	49.24, -111.65	23-Oct	28-Apr	-
Forty Mile	49.29, -111.60	23-Oct	28-Apr	-
Fall 2020 - Spring 2021				
Site	Location	Fall Sample Date	Spring 1 - Sample Date	Spring 2 - Sample Date
Willow Creek	49.93, -113.30	21-Sep	19-Apr	17-May
Lethbridge	50.01, -113.19	21-Sep	19-Apr	17-May
Vulcan	50.57, -112.98	21-Sep	19-Apr	17-May
Warner	49.26, -111.69	08-Sep	20-Apr	18-May
Forty Mile	49.30, -111.60	08-Sep	20-Apr	18-May
Special Area #3	51.21, -110.74	28-Sep	19-Apr	17-May

3.2.2 Statistical analysis

Generalized linear models with binomial discrete probability distributions were used to test for effects of field and sampling period on populations of live and dead *C. cinctus* larvae in the two study years. Analyses were performed in RStudio (version 3.6.1; R Core Team 2019) using packages “lme4” (version 1.1-27.1) to run the models, and “multcomp” (version 1.4-17) to determine if fields and sampling periods were significantly different from one another with a probability of 0.05. Within each year, sampling period and field were treated as fixed factors. Differences were compared using a Type II ANOVA. Data were tested and successfully met the assumptions of analysis of variance.

3.3 Results

From September 2019 to April 2020, 4237 stems were collected and dissected from six wheat fields (Table 3.2). 'Vulcan' was the only field that did not have any measurable *C. cinctus* damage or larvae, so it was not resampled in April 2020. The remaining five fields had stem cutting, with the percentage of stems that were cut and that contained *C. cinctus* larvae ranging from 5.36 – 68.57% and 3.30 – 66.16%, respectively (Table 3.2). Field was a significant factor that influenced the percentage of live *C. cinctus* larvae recovered, regardless of when the fields were surveyed ($F_{4,33} = 3.8177$; $p = 0.0117$; Figure 3.1). Within each field, live *C. cinctus* occurrence did not change significantly between sampling periods.

Table 3.2: Total number of stems dissected, percentage (± S.E.) of *Cephus cinctus*-cut stems, and percentage (± S.E.) of stems with *C. cinctus* larvae present between September 2019 and April 2020.

Fall 2019			
Site	# Stems Dissected	Stems Cut % (± S.E.)	Larval Presence % (± S.E.)
Special Area #3	278	31.16 ± 2.78	29.60 ± 2.74
Acadia	401	5.36 ± 1.16	4.67 ± 1.05
Vulcan	657	0 ± 0	0 ± 0
Willow Creek	279	19.49 ± 4.42	23.19 ± 4.97
Warner	374	68.57 ± 4.76	50.56 ± 5.08
Forty Mile	375	66.98 ± 4.34	66.16 ± 4.48
Spring 2020			
Site	# Stems Dissected	Stems Cut % (± S.E.)	Larval Presence % (± S.E.)
Special Area #3	320	22.50 ± 4.72	23.20 ± 4.74
Acadia	447	9.02 ± 1.92	3.30 ± 1.32
Vulcan	n/a	n/a	n/a
Willow Creek	220	14.82 ± 0.26	23.91 ± 0.35
Warner	403	53.60 ± 4.99	48.29 ± 5.07
Forty Mile	483	40.96 ± 3.75	50.86 ± 4.37

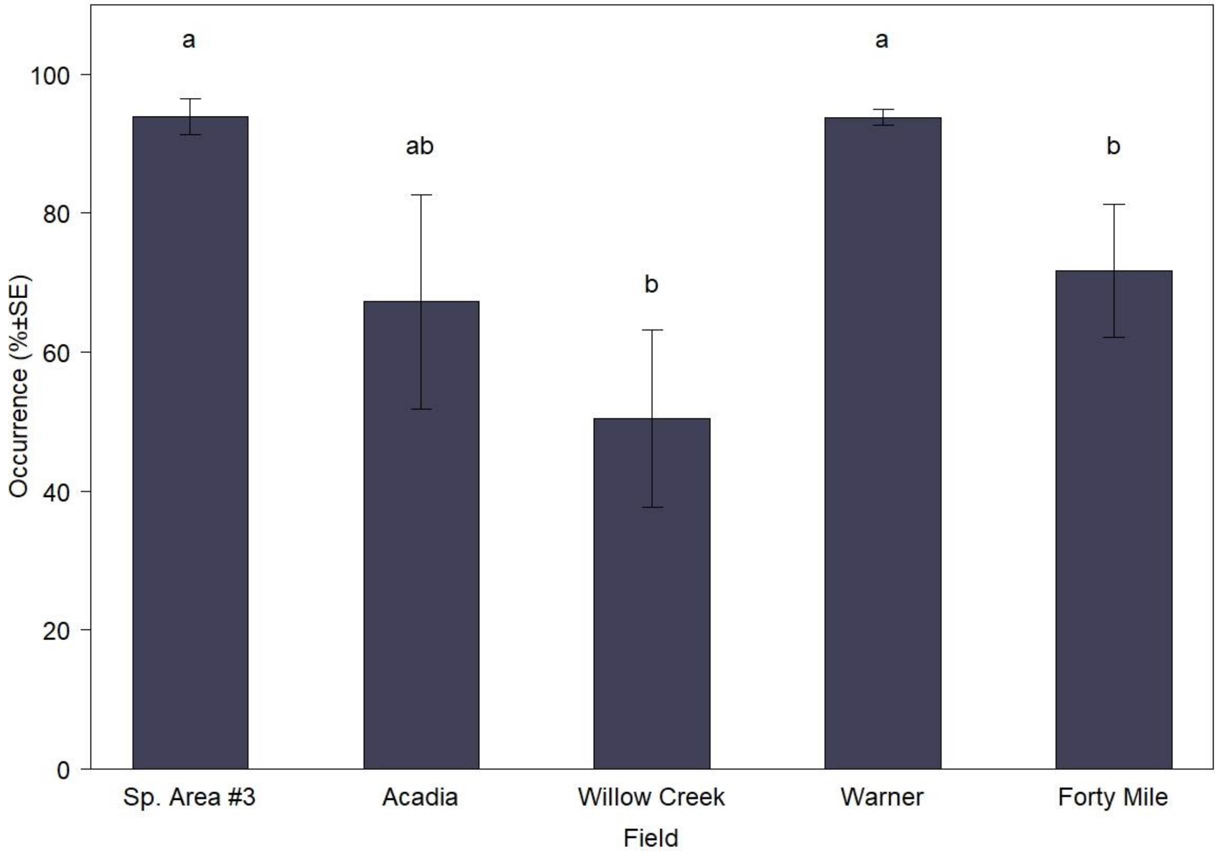

Figure 3.1: Total pooled observations (% ± S.E) of live *Cephus cinctus* larvae from the Fall 2019 and Spring 2020 surveys. Means with different letters are significantly different ($p < 0.05$).

Within each field, mortality did not change significantly from September 2019 to April 2020 (Table 3.3). Larval mortality factors were not uniform across the five fields (Figure 3.2b – d). If parasitoids were present, then parasitoid-associated mortality contributed most to *C. cinctus* larval mortality (Figure 3.2c). Mortality resulting from pathogen infection and unknown causes ranged from 1.14 – 14.24% and 1.56 – 5.21%, respectively in April 2020 (Figure 3.2b,d).

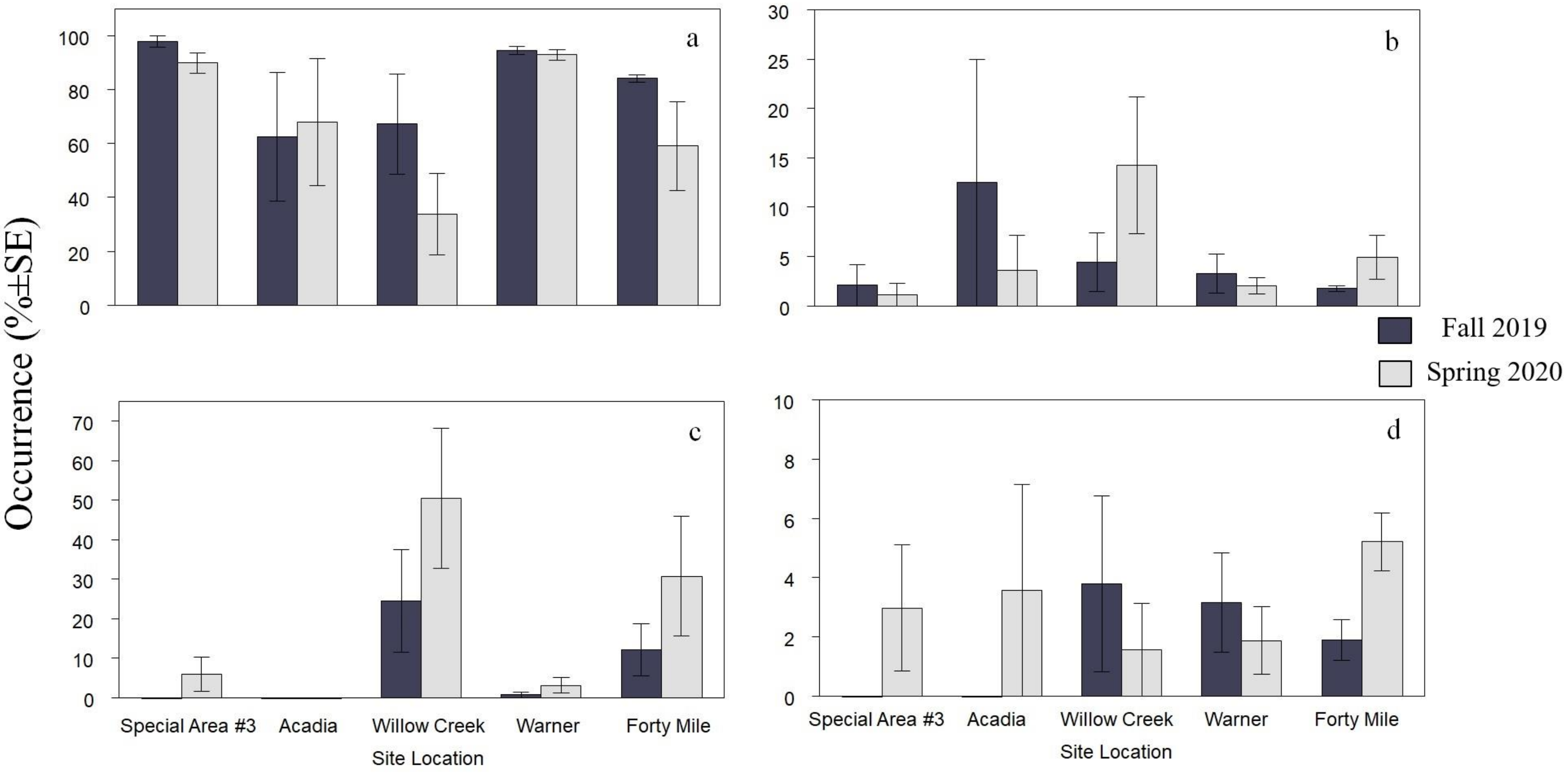

Figure 3.2: Observations (% ± S.E) of (a) live *Cephus cinctus* larvae, (b) fungal-associated larval mortality, (c) parasitism-associated larval mortality, and (d) unknown mortality from the Fall 2019 and Spring 2020 larval mortality surveys.

Table 3.3: Model ANOVA results for the effect of sampling time on larval *Cephus cinctus* mortality observed at five wheat fields surveyed between September 2019 and April 2020 and observed at six wheat fields surveyed in September 2020, April 2021, and May 2021.

Fall 2019 - Spring 2020		
Site	$F_{(1,6)}$	p-value
Special Area #3	3.7332	0.1015
Acadia	0.003	0.959
Willow Creek	1.8356	0.2243
Warner	0.2907	0.6092
Forty Mile	2.1272	0.195
Fall 2020 - Spring 2021		
Site	$F_{(2,6)}$	p-value
Willow Creek	0.6727	0.537
Lethbridge	0.3197	0.7343
Vulcan	0.8103	0.4825
Warner	0.2163	0.8096
Forty Mile	0.5288	0.6066
Special Area #3	4.5533	**0.04303**

A total of 7342 stems were collected from six new wheat fields sampled in September 2020, April 2021, and May 2021 (Table 3.4). All fields at each sampling period had measurable *C. cinctus*-damage or *C. cinctus* larvae, but numbers ranged widely based on where the samples were taken. Stem cutting was lowest at ‘Willow Creek’ and highest at ‘Forty Mile’ during all sampling periods (Table 3.4). At ‘Forty Mile’, the proportion of cut stems with larvae was highest while the ‘Lethbridge’ field had the lowest proportion of stems with larvae (Table 3.4). Larval mortality varied based on field ($F_{5,63} = 34.934$; $p < 0.0001$; Figure 3.3) but was not affected by sampling period at any field except ‘Special Area #3’ (Table 3.3). At this field, mortality was lower in Fall 2020 compared to the two sampling periods in Spring 2020 (Figure 3.4a). Fungal-associated and unknown mortality were irregular in the second year of the study, ranging from 0 – 27.60% and 0 – 8.98%, respectively (Figure 3.4b,d). Notably, the rate of fungal infection at ‘Willow Creek’ was nearly double that of the other fields during the May 2021 sampling period (Figure 3.4b,d).

Table 3.4: Total number of stems dissected, percentage (± S.E.) of *Cephus cinctus*-cut stems, and percentage (± S.E.) of stems with *C. cinctus* larvae present (± S.E.) from surveys conducted in September 2020, April 2021, and May 2021.

Fall 2020

Site	Stems Dissected	Stems Cut % (± S.E.)	Larval Presence % (± S.E.)
Willow Creek	599	1.27 ± 0.67	52.42 ± 4.02
Lethbridge	525	13.66 ± 2.88	22.73 ± 3.55
Vulcan	489	34.33 ± 4.38	49.43 ± 4.53
Warner	398	45.49 ± 5.01	48.27 ± 5.17
Forty Mile	399	72.17 ± 4.31	71.05 ± 4.43
Special Area #3	493	33.68 ± 4.21	57.99 ± 4.68

Spring - April 2021

Site	Stems Dissected	Stems Cut % (± S.E.)	Larval Presence % (± S.E.)
Willow Creek	530	1.77 ± 0.69	9.46 ± 2.08
Lethbridge	488	2.67 ± 1.47	4.20 ± 1.78
Vulcan	634	3.28 ± 0.96	3.72 ± 1.25
Warner	365	79.47 ± 4.34	71.11 ± 4.88
Forty Mile	513	82.31 ± 3.31	80.12 ± 3.42
Special Area #3	320	43.62 ± 5.60	39.10 ± 5.47

Spring - May 2021

Site	Stems Dissected	Stems Cut % (± S.E.)	Larval Presence % (± S.E.)
Willow Creek	361	2.04 ± 1.15	17.09 ± 2.66
Lethbridge	505	2.97 ± 1.32	4.02 ± 1.68
Vulcan	322	4.37 ± 1.45	5.33 ± 1.89
Warner	343	73.26 ± 4.69	53.41 ± 5.26
Forty Mile	356	76.07 ± 4.05	67.95 ± 4.95
Special Area #3	302	45.13 ± 5.47	45.38 ± 5.45

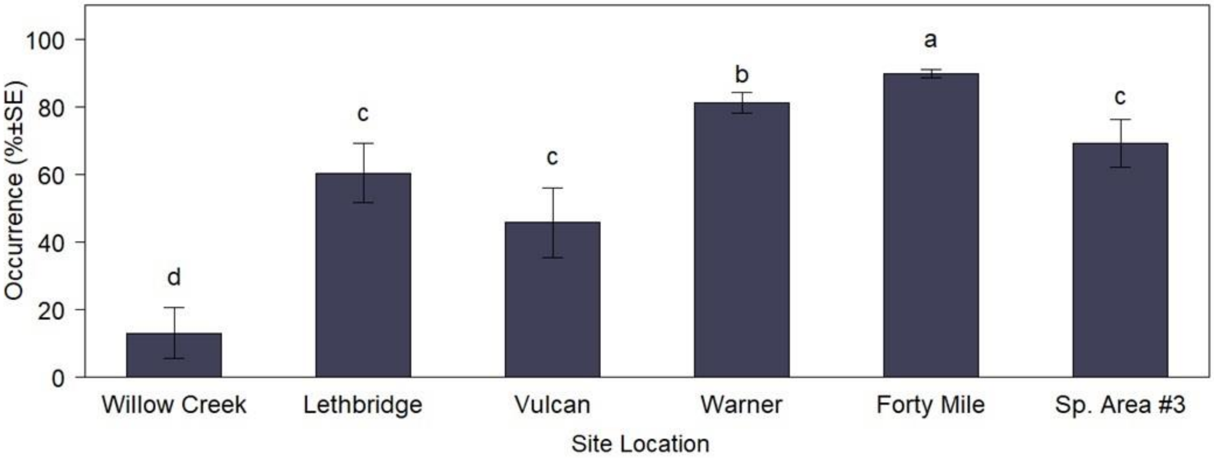

Figure 3.3: Total observations (% ± S.E.) of live *Cephus cinctus* larvae from the Fall 2020 and Spring 2021 surveys. Means with different letters are significantly different ($p < 0.05$).

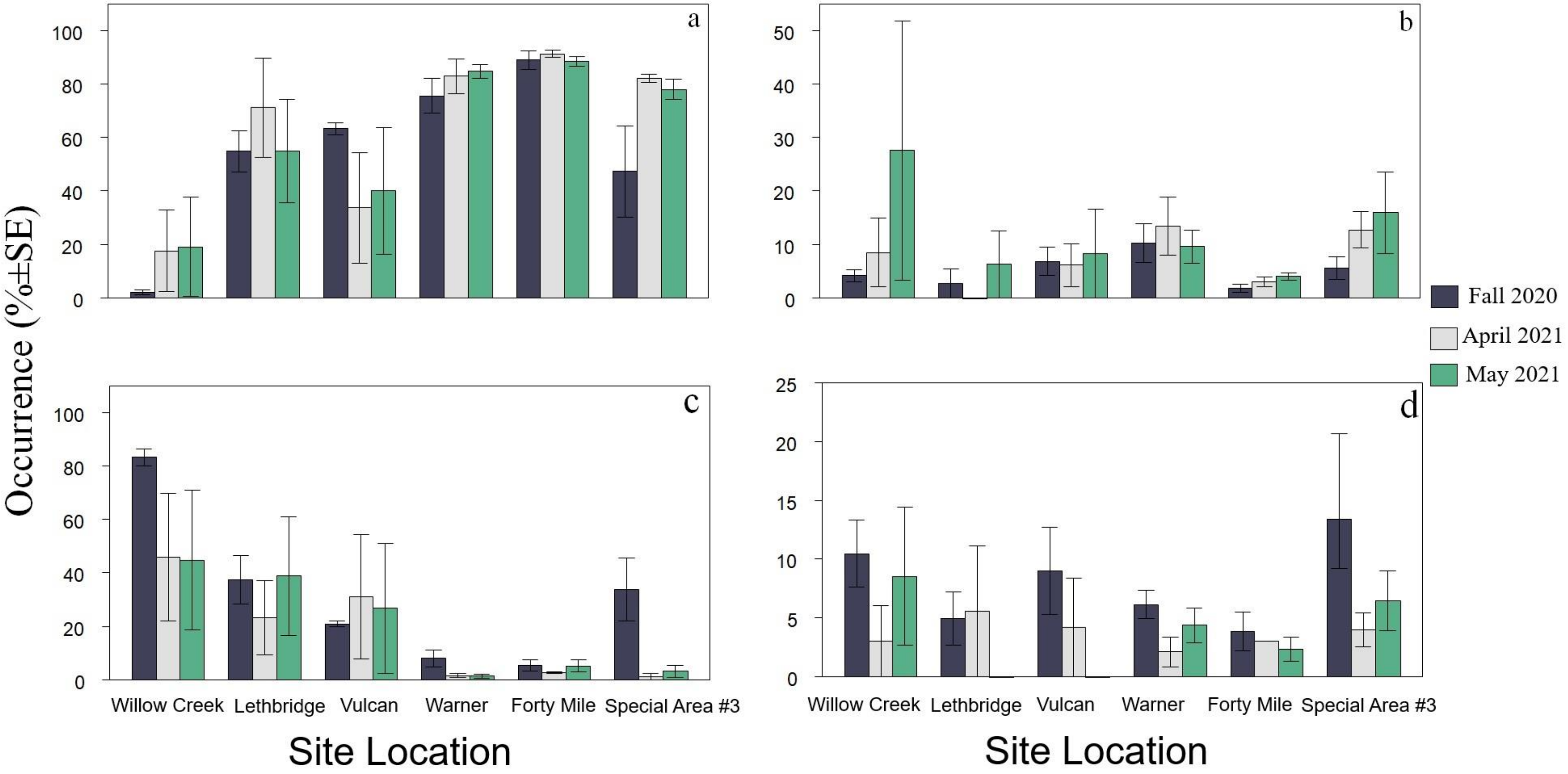

Figure 3.4: Observations (% ± S.E.) of (a) live *Cephus cinctus* larvae, (b) fungal-associated larval mortality, (c) parasitism-associated larval mortality, and (d) unknown mortality from the Fall 2020 and Spring 2021 larval mortality surveys.

3.4 Discussion

Sequential sampling of harvested wheat fields in the fall and following spring yielded two key observations. First, in the vast majority of wheat fields, *C. cinctus* mortality was not affected by sampling time, and the proportion of the larval population alive in the pre-overwintering larval phase was approximately equal to that observed during the spring post-overwintering phase of larval development. In addition, mortality due to unknown factors, which could have included abiotic stress, was minimal in all sampling periods in both years of the study (*i.e.*, <10%). This provides indirect evidence that is it unlikely that winter weather, even extreme conditions, impacts *C. cinctus* populations. These results agree with past studies which also concluded that overwintering abiotic conditions do not affect *C. cinctus* mortality (Morrill *et al.* 1993; Cárcamo *et al.* 2011). Second, the present study highlighted the field-level variability of *C. cinctus* larval densities and mortality levels in southern Alberta wheat fields. Both observations have implications for management of *C. cinctus*. Specifically, if higher *C. cinctus* populations are expected in a given area based on the fall survey, scouting and surveying individual fields will be needed to get the highest level of resolution for potential *C. cinctus* densities in the upcoming growing season, so that appropriate actions (e.g., planting solid stem wheat or planting an alternative crop) can be taken to avoid yield losses. Additionally, both observations provide important information that will contribute to the future development of phenology models and forecasting systems for *C. cinctus* in Canadian agroecosystems. For example, phenology models are initiated in the spring with an estimate of larval density (see Olfert *et al.* 2020). In the case of *C. cinctus*, the density of viable larvae observed in the fall can be used as an estimate of the viable larval density in the spring with confidence. This is because we observed that abiotic conditions have minimal, if any, impact on overwintering survival and that the proportion of viable larvae in the fall is approximately equal to the number of viable larvae found in the spring. Phenology models can also incorporate the impact of natural enemies and other mortality factors (see Olfert *et al.* 2020). Because mortality factors affecting *C. cinctus* varied spatially, it could be difficult to accurately incorporate the effects of mortality due to natural enemies on *C. cinctus* into models for wide geographic areas.

Our study aimed to sample fields in southern Alberta with known *C. cinctus* populations, but due to constraints brought on by the COVID-19 pandemic, we could not obtain information about field histories. Therefore, that information could not be accounted for in our analyses. Despite this, our results allude to the potential effect of field-level diversity in pest management, agronomic practices, and landscape ecology on *C. cinctus* larval mortality. Several past studies have highlighted the effect of field configuration on the survivorship and population densities of *C. cinctus*. For example, cultural methods that influence *C. cinctus* infestation rates and female oviposition behavior such as changing wheat row spacing and seeding rates (Luginbill & McNeal 1958; Beres *et al.* 2012), delaying seeding date (Morrill & Kushnak 1999; Sing 2002), and managing soil nitrogen and phosphorus levels (Luginbill & McNeal 1954; Delaney *et al.* 2010) all decreased sawfly densities within fields. Crop rotation can also affect *C. cinctus* population densities as adults are regarded as poor fliers (Ainslie 1929). Although these studies focused on understanding field-level *C. cinctus* population densities, there is a possibility that cultural control tactics also influence larval mortality and *C. cinctus* natural enemies. Currently, agronomic practices relating to *C. cinctus* larval enemies have primarily focused on parasitoid conservation. For example, overwintering *B. cephi* larvae can be preserved in a field by leaving the bottom third of wheat stems (0 to 30 cm above soil) standing post-harvest (Meers 2005). Parasitoid populations can also be protected from cannibalism by non-parasitized *C. cinctus* larvae when other suitable *C. cinctus* host plants (*i.e.,* wild oats, *Avena fatua* L.) are present to act as population sinks (Weaver *et al.* 2004). Future studies could explore how other agronomic practices influence populations of both *C. cinctus* and their natural enemies to further improve integrated pest management strategies for Canadian wheat producers.

Larval mortality did not change significantly between the sampling periods in either study year except for one field, ‘Special Area #3’, in 2020 – 2021. In this particular field, the proportion of larvae that were alive in the Fall 2020 sampling period was much lower than the two subsequent sampling periods in Spring 2021. Although a significant result, the difference could be a sampling artefact, arising from the destructive nature of the sampling protocol that did not permit the same wheat stems to be examined on all sampling dates. The difference also appears to be driven by a decrease in observed parasitism rates between Fall 2020 (39.13%) and Spring 2021, when parasitism levels dropped to below 5% on both Spring 2021 sampling dates.

Overall, the minimal difference in *C. cinctus* larval mortality over time at the majority of the study fields agrees with past research that found the overwintering larval mortality was low and had little influence on wheat stem sawfly population dynamics (Morrill *et al.* 1993; Cárcamo *et al.* 2011).

The lack of difference in *C. cinctus* larval mortality over time is most likely a result of two factors. First, it is unlikely that cold stress or other abiotic factors associated with winter weather contribute to *C. cinctus* mortality because the level of unknown mortality observed in this study did not increase over time. *Cephus cinctus* larvae overwinter in hibernacula inside wheat stems near the soil surface where they are protected from harsh winter weather and several authors have previously observed that mortality associated with the overwintering period is negligible (Morrill *et al.* 1993; Cárcamo *et al.* 2011). Second, we observed no variation in mortality factors in the samples collected in the fall and subsequent spring. It is thus clear from our results that little to no mortality was attributed to abiotic conditions and the majority of larval mortality was due to other factors. From our samples, three mortality factors were observed: parasitism, fungal infection, and unknown causes, with parasitism the most common mortality factor. There was no evidence of predation on *C. cinctus* larvae by clerid beetles *Phyllobaenus dubius* Wolcott (Coleoptera: Cleridae). To our knowledge, all of the fields sampled grew conventional hollow stem wheat, so we do not expect that any larval mortality arose due to host plant resistance. Parasitism by both parasitoids, *Bracon cephi* and *B. lissogaster*, were grouped together as it is not possible to differentiate the parasitoids in their larval stage (Runyon *et al.* 2001). Nevertheless, the majority of parasitism was likely caused by *B. cephi* as it is the predominant parasitoid species in Alberta (Cárcamo *et al.* 2012). Parasitoids overwinter as larvae within the lower internodes (0 to 30 cm above soil surface) of wheat stubble and resume their development the following spring (Nelson & Farstad 1953). Thus, while parasitoids are an important larval mortality factor for the summer *C. cinctus* larval phase, they are not expected to influence mortality between growing seasons. The next most common mortality factor, fungal infection, was observed in every field, but never at levels above 30%. This result matches the conclusions from a postharvest survey conducted in Montana winter wheat fields that found fungal infection could cause 10 to 30% mean overwintering larval mortality (Sun 2008). Similar to parasitism, it is also unlikely that rates of fungal infection would change between growing seasons because dormant fungal saprophytes overwinter within remaining wheat stubble and other crop residues and only become active again under warm and moist conditions (Sutton 1982). So, although fungal saprophytes may become active again in the early spring months under ideal environmental conditions, the fungus may still not infect the post-diapause *C. cinctus* larvae as the fungal hyphae still need to break through the larva's protective hibernaculum. In scenarios where fungal infection did seem to increase in the spring, like at the 'Willow Creek' field in 2021, fungal infection did not contribute to additional *C. cinctus* larval mortality in that sampling period. Overall, *C. cinctus* larval survivorship from the fall into the early spring months did not change significantly, probably because the major mortality factors were inactive in the winter and early spring and because *C. cinctus* are well-adapted to survive winter weather. Overwintering and early season larval mortality have minimal impacts on *C. cinctus* population dynamics.

3.5 Conclusion

The present study demonstrates the field-level variation of *C. cinctus* populations and sources of larval mortality across southern Alberta, where *C. cinctus* populations have been increasing in recent years. Although not tested explicitly here, the degree of variation we observed between fields could arise from differences in landscape dynamics and agronomic practices (*i.e.*, conservation of wheat stubble at harvest) that influence *C. cinctus* (and *B. cephi*) field population dynamics. Future research is needed to further elucidate the factors driving variation in *C. cinctus* population dynamics across western Canadian landscapes.

The study also highlighted that *C. cinctus* population dynamics are not susceptible to mortality due to abiotic conditions or biotic mortality factors between growing seasons as larvae undergo their overwintering phase and subsequent spring development. This information will contribute to the future development of phenology models and *C. cinctus* population forecasting tools. Recently constructed Leslie matrix population models have emphasized that winter larval mortality has the greatest impact on sawfly population growth rate as individuals are in this life stage the longest (Rand *et al.* 2016, 2020). However, in the absence of consistent and wide-reaching mortality pressure on late-stage *C. cinctus* larvae, as we observed, it is unlikely that overwintering and early-season larval mortality is an important factor in the overall population dynamics of wheat stem sawfly in western Canadian agroecosystems.

4.0 Early season temperature impacts on adult life history traits of wheat stem sawfly *Cephus cinctus* (Hymenoptera: Cephidae) and *Bracon cephi* (Hymenoptera: Braconidae)

4.1 Introduction

In temperate climates, insects cease biological activities during the winter months and commonly undergo a series of physiological processes called diapause (Košťál 2006; Košťál *et al.* 2017). Winter diapause is a mechanism to delay further development and to time specific life stages with more optimal conditions in the future (*i.e.*, when host plants of the correct stage are available and abiotic conditions are ideal) (Tauber & Tauber 1976). Diapause also allows for mitigation of massive energetic losses while individuals are not actively feeding during the winter by substantially reducing their metabolic rate (Hahn & Denlinger 2007, 2011). Diapause consists of three distinct physiological phases: pre-diapause, diapause, and post-diapause (Košťál 2006). The pre-diapause phase begins when environmental cues induce behavioural activities (*i.e.*, migration to an overwintering spot, aggregation of individuals, or accumulation of metabolic resources) that prepare individuals for their upcoming dormancy (Košťál 2006). Following pre-diapause, the diapause phase starts with the initiation of developmental arrest and reduction of the metabolic rate. These changes are maintained over a given period until specific environmental cues change the physiological state of the individual and thus signal the end of diapause (Košťál 2006). The final phase follows diapause termination as individuals may proceed with development under the new environmental conditions, or continue in a state of post-diapause quiescence when conditions are not yet ideal (Košťál 2006).

Although one main goal of undergoing a winter diapause is to preserve valuable metabolic resources while food is not available, diapause is still an energy-draining strategy (Hahn & Denlinger 2007; Margus & Lindström 2020). Prolonged diapause periods can reduce survivorship (Ishihara & Shimada 1995; Irwin & Lee 2000), fat reserves (Ellers & van Alphen 2002; Sinclair & Marshall 2018), fecundity (Kroon & Veenendaal 1998; Sadakiyo & Ishihara 2012), and body size (Matsuo 2006). Individuals that enter diapause in a post-feeding life stage (*e.g.*, pre-pupal larva or pupa) and that do not feed as adults rely solely on the metabolic resources that they stored in the pre-diapause phase for all subsequent adult activities, including dispersal flights and reproduction (Hahn & Denlinger 2007). For these species, long periods of post-diapause development can have consequences on fitness and fecundity (Irwin & Lee Jr 2000). In western Canada, the wheat stem sawfly (*Cephus cinctus* Norton, Hymenoptera: Cephidae), a serious economic pest of wheat, and its primary parasitoid, *Bracon cephi* Gahan (Hymenoptera: Braconidae) both use diapause as a strategy to survive the winter (Ainslie 1929; Nelson & Farstad 1953). In fact, we have demonstrated that *C. cinctus* mortality during the winter is extremely low and largely driven by biotic factors (Chapter 3). The experiments reported in this chapter were designed to investigate the impact of abiotic conditions on *C. cinctus* and *B. cephi* during post-diapause development following the termination of winter diapause.

The life cycle of *C. cinctus* is univoltine with adult emergence occurring in early June to mid July (Criddle 1915). Adults are relatively small-bodied insects (7 to 12 mm) with yellow stripes along the abdomen (Ainslie 1929) that live for 5 to 8 d (Wallace & McNeal 1966. Adult females are noticeably larger than males and possess a “saw-like” appendage at the posterior end of their abdomens (Criddle 1915). Females begin laying eggs shortly after their emergence from the pupal stage and deposit a single translucent, oval-shaped egg per plant (Holmes 1979; Holmes 1982). Each female emerges from the pupal stage with their full egg load complement of about 50 or more eggs (Ainslie 1929; Holmes 1979). Larvae emerge from the eggs by the sixth- or seventh-day post-oviposition and quickly enter the centre of the stems of their host plant (Ainslie 1929). Within the stems, larvae bore up and down through the internodes of the stem until the plant begins to mature and desiccate (Holmes 1977). At this time, mature larvae are triggered into an obligatory diapause by infrared light penetration through the stem wall and overall reduction in plant moisture content (Holmes 1975). The larvae travel down to the base of the plant, cut a V-shaped groove around the entirety of the stem, and create a frass plug to encase themselves (Holmes & Peterson 1960). Within their hibernaculum, the prepupal larvae are well insulated and can survive severe temperatures as low as -28°C (Ainslie 1929). Larvae resume with their post-diapause development after a 90-day exposure period at 10°C (Salt 1947; Holmes 1982). Once diapause is broken, larval metabolic activity also increases significantly (Villacorta *et al.* 1972). The transition from a larvae to pupa takes no longer than 7 d (Villacorta *et al.* 1972), and the overall pupal period is about 18 to 20 d (Criddle 1923; Holmes 1979). The newly eclosed adults then chew a small hole through their frass plug and emerge out of the wheat stub (Holmes 1982).

Two native parasitoids, *Bracon cephi* Gahan (Hymenoptera: Braconidae) (Nelson & Farstad 1953) and *B. lissogaster* Muesebeck (Hymenoptera: Braconidae) contribute to control of *C. cinctus* populations (Somsen & Luginbill 1956). In Canadian agroecosystems, both parasitoids are present but *B. cephi* is more common (Cárcamo *et al.* 2012). *Bracon cephi* is an idiobiont ectoparasitoid that can have two generations per year depending on weather patterns (*i.e.*, cold, wet conditions in June and July) that can extend the growing season (Holmes *et al.* 1963). Adult female *B. cephi* emerge in early June and require a 12 to 21 d period before they can oviposit (Nelson & Farstad 1953). Females locate sawfly larvae within wheat stems using host plant volatiles and movement vibrations produced by the sawfly larvae (Mankin *et al.* 2004; Pérez 2009). Females utilize a venom toxin to paralyze the sawfly larva and then deposit an egg close to the paralyzed larva inside the stem (Holmes *et al.* 1963). The newly emerged parasitoid larva will attach itself to the immobile sawfly larva and consume the entire sawfly larva throughout its 10-d larval development period (Nelson & Farstad 1953). If weather conditions do not permit for a second generation, the mature larva will spin a cocoon and overwinter in the lower internodes (0 to 30 cm) of the wheat stem (Holmes *et al.* 1963; Meers 2005). *Bracon cephi* larvae synthesize glycerol to decrease their supercooling points which allows them to survive extreme cold temperatures as low as -47°C (Salt 1959). The mechanisms of diapause termination for *B. cephi* are not well-studied, but their post-diapause development is well synchronized to that of *C. cinctus*, allowing for the new generation parasitoid adults to emerge just as *C. cinctus* adults are also emerging and beginning to lay eggs (Nelson & Farstad 1953; Holmes *et al.* 1963).

Abiotic factors impact several phases of *C. cinctus* development, including the post-diapause period in the spring following the end of the winter diapause. Post-diapause larvae will not develop when temperatures are below 15°C or above 35°C, and will sometimes re-enter diapause outside of this optimal temperature range (Salt 1947; Church 1955; Villacorta *et al.* 1971). Within the optimal range, lower temperatures prolong post-diapause development, reduce adult emergence, and increase overall mortality (Perez-Mendoza & Weaver 2006). The non-lethal consequences of prolonged post-diapause development on *C. cinctus* adult life history traits have not been extensively explored. Similarly, this aspect of *B. cephi* life history is also poorly understood. The goal of the experiments reported here was to identify how the length of the post-diapause period, as controlled by temperature, effects several adult life history traits (*i.e.*, longevity, body size, lipid content, and egg load) of *C. cinctus* and *B. cephi*. Previous studies in other systems showed that extended development can result in negative effects on energy reserves when diapause and post-diapause individuals do not acquire new metabolic resources (Danks 1987; Ellers & Van Alphen 2002). Adult *C. cephus* do not commonly exhibit active foraging behaviour (Wallace & McNeal 1966), gain relatively little from sugar resources (Rand *et al.* 2019), and ultimately rely heavily on the reserves collected as larvae to fuel their adult activities. In contrast, *B. cephi* adults actively forage for floral resources and their longevity greatly increases when a sugar resource is readily available (Nelson & Farstad 1953; Reis *et al.* 2019). Based on the ability of adult parasitoids to make up for lost resources resulting from extended post-diapause development, we expect that adult life history traits of *C. cinctus* will be more heavily impacted by extended periods of post-diapause development compared with adult *B. cephi*. Investigating this part of the development of *C. cinctus* and *B. cephi* will provide important insight on how abiotic conditions influence their development, their subsequent host-parasitoid interactions, and their impact on wheat yields in western Canada.

4.2 Materials and methods

4.2.1 Adult longevity experiments

Wheat samples were collected on 27 and 28 April 2020 from five wheat fields (Chapter 3, Table 3.1) in southern Alberta where *C. cinctus* were known to occur and brought back to Saskatoon, SK to be processed. Intact individuals from dissected wheat stubs were separated by site and set up in 1 L plastic containers. Containers were placed under two temperature regimes to control *C. cinctus* post-diapause development and delay adult emergence: i) an early emergence regime set at 22°C ± 5°C that would allow for post-diapause development to continue at its normal rate, and ii) a delayed emergence regime set at 12°C ± 2°C for 4 weeks and then 17°C ± 5°C to let individuals continue with their post-diapause development. The number of parasitoid and *C. cinctus* larvae collected from some sites was quite low. Thus, only sawfly larvae from 'Warner' and 'Forty Mile' and parasitoids from 'Forty Mile' were available for the delayed emergence temperature regime. Eclosed adults were transferred individually into 50 mL plastic vials and held at 22°C ± 5°C. Adult parasitoids were provided with honey droplets, as adults greatly benefit from having access to a sucrose resource (Reis *et al.* 2019). Wheat stem sawfly adults were not provided any source of sucrose or water due to issues with accidental mortality. Past work has shown that sugar water does not significantly extend adult sawfly longevity (Rand *et al.* 2019). Adults were monitored from late May to early August and adult lifespans were recorded. All emerged parasitoids were identified with a key (Runyon *et al.* 2001) to differentiate between *B. cephi* and *B. lissogaster*.

In 2021, the experiment was repeated at the Agriculture and Agri-Food Canada Saskatoon Research Development Centre (AAFC-SRDC) in Saskatoon, SK. Overwintered parasitoids and sawflies were collected on 19 and 20 April 2021 from ‘Willow Creek’ and ‘Forty Mile’ respectively, as these sites had the highest relative abundance based on sampling the previous fall (Chapter 3, Table 3.1). Wheat samples were transported to AAFC-SRDC and carefully dissected to determine the presence and state of parasitoid or sawfly larvae. Prior to the start of the experiment, intact individuals were evenly divided up by species into 12 500 mL glass emergence containers (nine for wheat stem sawfly and three for *B. cephi*) and set up at 15°C to slow down post-diapause development. To set up three distinct emergence groups (first, second, and third for the purposes of treatment names), three *C. cinctus* emergence containers (one for longevity, one for lipid content, and one for egg load) and one *B. cephi* emergence container were placed into separate cells of a thermal gradient plate (TGP) set at 25°C on three dates 14 d apart (3, 17, and 31 of May 2021). Cells of the TGP are controlled individually and precisely; the technology is used for studies of insect, weed, and plant pathogen development. For additional information about the design and function of the TGP, please see Stumborg *et al.* (2020). Emergence containers in TGP cells were monitored daily and newly eclosed adults were placed individually into 2 mL Eppendorf screw top tubes (Thermo Fisher Scientific, Waltham, MA, USA). After adult eclosion, *B. cephi* adults were given dental wick soaked in a 30% (w/v) sucrose solution and *C. cinctus* adults were given dental wick soaked in distilled water. Honey and water wicks were not replaced during the experiment to prevent individuals from escaping or other incidents while handling adults. Tubes were placed in the TGP set at 25°C and monitored daily throughout their lifespan to measure adult longevity.

4.2.2 Adult body size measurements

To approximate overall adult *C. cinctus* body size, forewing length (Cárcamo *et al.* 2008) was measured using either a dissecting microscope (magnification = 0.63; Leica MZ75) or ImageJ (Version 1.53m) (Schneider *et al.* 2012). For *B. cephi* body size approximations, the length of the hind tibia (Reis *et al.* 2019) was measured using a dissecting microscope.

4.2.3 Adult *C. cinctus* lipid content and egg load measurements

Newly eclosed adult *C. cinctus* were placed in 2 mL Eppendorf tubes without a water source and allowed to mature for 24 h before being frozen at -20°C (Cárcamo *et al.* 2005) in preparation for lipid content and egg load assays. Females from egg load containers were dissected to determine egg numbers. Individual female egg counts were done under a dissecting microscope (magnification = 0.63; Leica MZ75) by making an incision along the side of the abdomen to expose eggs freely floating within the abdominal cavity. Adult *C. cinctus* lipid extractions followed a gravimetric assay approach described by Cuff *et al.* (2021). Individuals were dried in an oven at 58°C for 24 h and subsequently weighed to obtain pre-extraction dry weights. Dried individuals were then placed in a 2 mL Eppendorf vial with 0.5 mL of 1:12 chloroform:methanol solution (Sigma-Aldrich, St. Louis, MO, USA). After 24 h the first extraction solution was discarded and individuals were submerged in another 0.5 mL of 1:12 chloroform:methanol solution for an additional 24 h to ensure all lipids were removed. Once the two extractions were complete, adults were dried again for 24 h in an oven at 58°C and weighed to determine post-extraction dry weights. The difference between pre- and post-extraction dry weights was calculated to estimate the total adult lipid content.

4.2.4 Statistical Analysis

The effect of prolonged post-diapause development on adult life history traits (successful emergence, longevity, lipid content, and egg load) for wheat stem sawfly and *B. cephi* were analyzed with separate mixed-effects models. Emergence rate, longevity, and egg load data were tested using generalized linear models with binomial (emergence rate) and poisson (longevity, egg capacity) discrete probability distributions. Analyses were performed in RStudio (version 3.6.1; R Core Team 2019) using packages "lme4" (version 1.1-27.1) and "nlme" (version 3.1-152) to run the models, and "multcomp" (version 1.4-17) to determine which treatments were significantly different from one another with a probability of 0.05. Emergence groups, sex, and site locations (2020 analysis only) were treated as fixed effects. All datasets met the assumptions for analysis of variance and their respective model.

4.3 Results

4.3.1 Adult longevity

In total, 215 of 303 adult *C. cinctus* and 39 of 50 *B. cephi* successfully emerged in 2020. *Cephus cinctus* adult longevity was affected by emergence group ($F_{1,211} = 48.8139$; $p < 0.0001$), sex ($F_{1,211} = 18.3667$; $p < 0.0001$), and the interaction of emergence group and sex ($F_{1,211} = 7.4534$; $p = 0.0069$). The interaction effect was driven by the difference in longevity between the sexes, where males in the second emergence group had shorter lifespans compared to female sawflies (Figure 4.1). For *B. cephi*, male parasitoid longevity was shorter than that of female parasitoids, regardless of emergence group ($F_{1,31} = 21.6208$; $p < 0.0001$; Figure 4.1). Emergence group did not have a significant effect on the longevity of either sex ($F_{1,31} = 0.0010$; $p = 0.9754$) and there was no interaction between sex and emergence group ($F_{1,31} = 1.0314$; $p = 0.3177$).

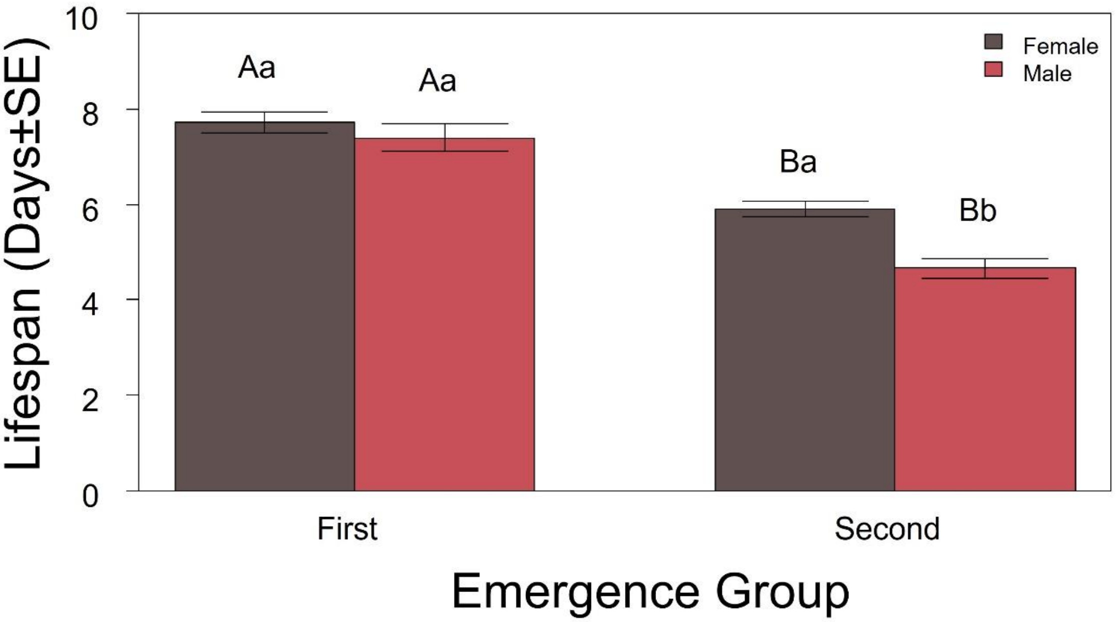

Figure 4.1: Mean lifespan of 2020 adult *Cephus cinctus* from two post-diapause emergence groups. Means with different letters are significantly different ($p < 0.05$).

In 2021, 154 of 174 adult *C. cinctus* and 33 of 54 adult *B. cephi* successfully emerged and were used in the adult longevity experiments from the three emergence groups. For *C. cinctus*, the longevity results were contradictory to the 2020 findings as there was no effect of emergence group ($F_{2,148} = 2.8657$; $p = 0.0601$) and no significant interaction between sex and emergence group ($F_{2,148} = 2.6608$; $p = 0.0732$). Sex did affect adult *C. cinctus* longevity ($F_{1,148} = 17.5916$; $p < 0.0001$), but contrary to the results in 2020, adult males (8.85 ± 0.28 days) lived longer on average than females (7.44 ± 0.20 days). For *B. cephi*, there was no difference in adult longevity between sexes ($F_{1,27} = 0.0682$; $p = 0.7960$) or emergence groups ($F_{2,27} = 0.6300$; $p = 0.5402$), and no interaction effect ($F_{2,27} = 1.0921$; $p = 0.3499$).

4.3.2 Adult body size

In the 2020 experiment, female *C. cinctus* had longer wings than males ($F_{1,211}$ = 185.8845; $p < 0.0001$; Figure 4.2). Emergence group did not have a significant effect on wing length ($F_{1,211}$ = 0.8010; p = 0.3718) and there was no interaction between sex and emergence group ($F_{1,211}$ = 1.9833; p = 0.1605). For *B. cephi*, neither sex ($F_{1,31}$ = 0.8861; p = 0.3538; Figure 4.2), emergence group ($F_{1,31}$ = 1.798; p = 0.1897), nor the interaction between the two factors ($F_{1,31}$ = 2.306; p = 0.1390) significantly affected hind tibia length.

In 2021, adult female *C. cinctus* were larger than their male counterparts ($F_{1,420}$ = 390.7484; $p < 0.0001$; Figure 4.3). Neither emergence group ($F_{2,420}$ = 2.0467; p = 0.1304) nor the interaction between sex and emergence group ($F_{2,420}$ = 0.9600; p = 0.3837) affected overall wing length. There was no effect of emergence group ($F_{1,27}$ = 2.3090; p = 0.1187) or the interaction of emergence group and sex ($F_{2,27}$ = 2.8949; p = 0.0726) on *B. cephi* hind tibia length. Females had longer hind tibias compared to males ($F_{1,27}$ = 32.6331, $p < 0.0001$; Figure 4.3).

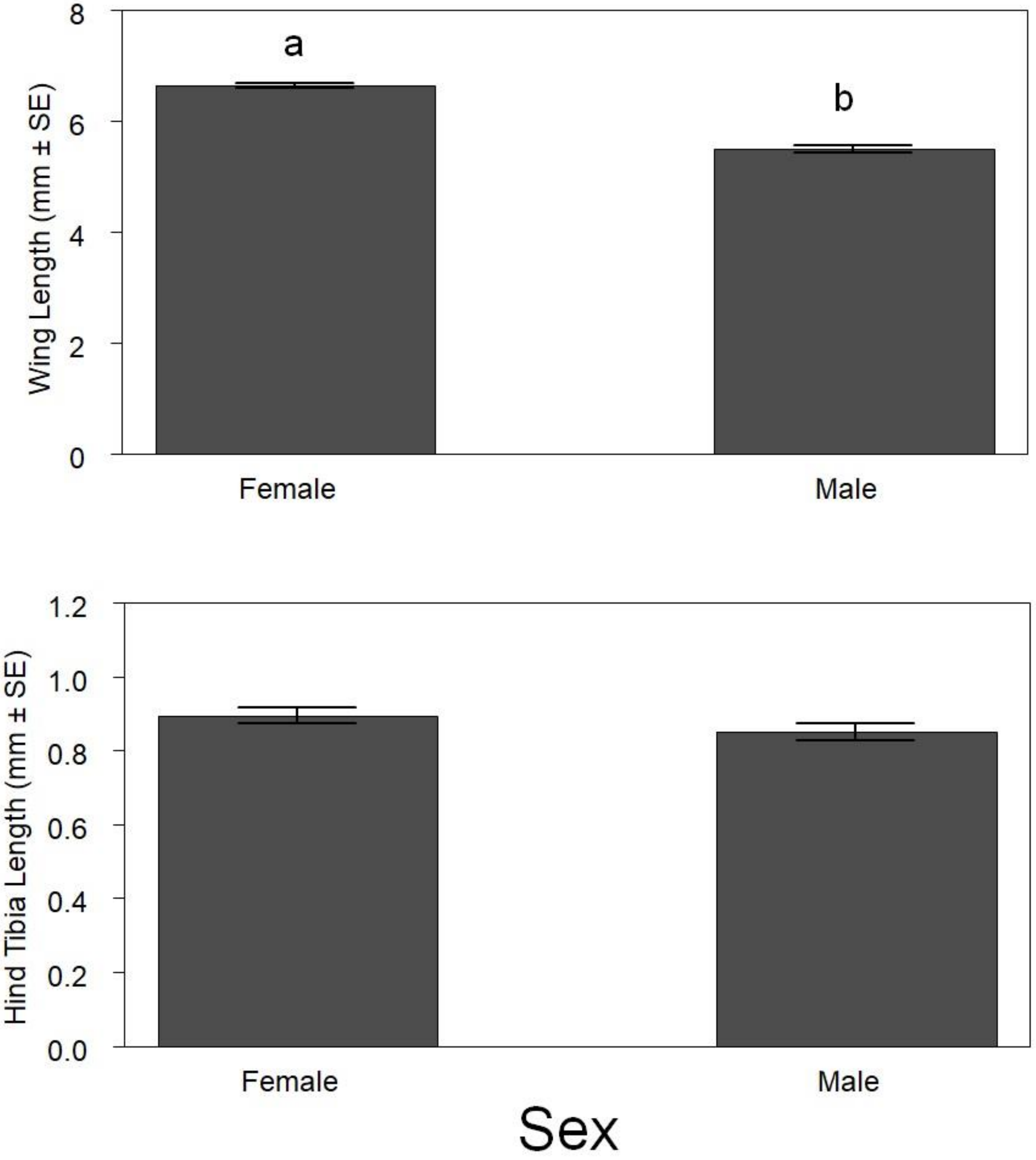

Figure 4.2: Mean (± S.E.) adult female and male *Cephus cinctus* wing length (top) and *B. cephi* hind tibia length (bottom) measurements in 2020. Means with different letters are significantly different ($p < 0.05$).

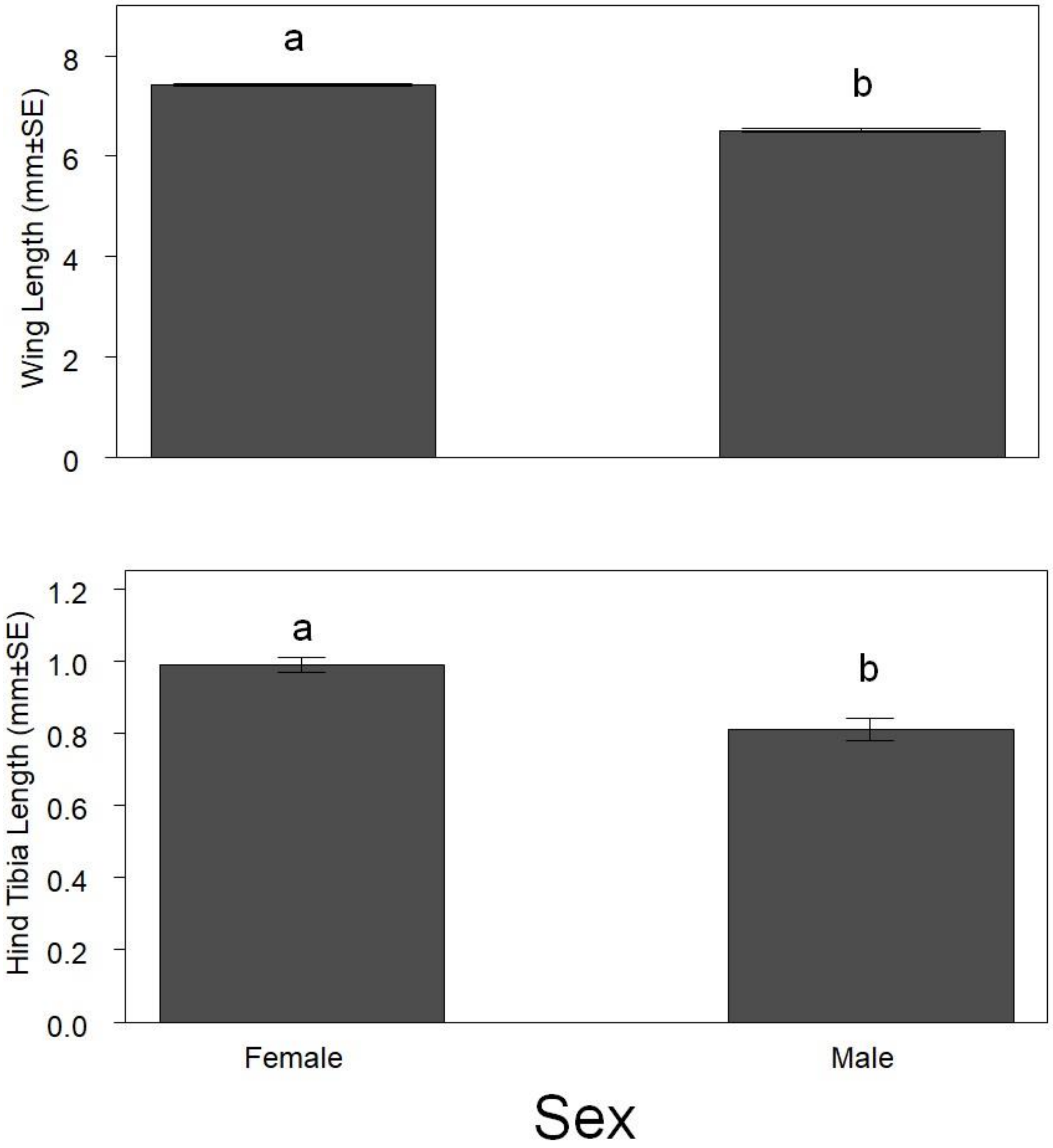

Figure 4.3: Mean (± S.E.) adult female and male *Cephus cinctus* wing length (top) and *B. cephi* hind tibia length (bottom) measurements in 2021. Means with different letters are significantly different ($p < 0.05$).

4.3.3 Adult *C. cinctus* lipid content and egg load

Lipid content varied significantly among emergence groups, but only for male sawflies. Individuals that emerged earliest had more lipids than males that emerged later ($F_{2,62} = 3.9103$; $p = 0.02516$; Figure 4.4). There was no significant difference in the amount of lipids between emergence groups with female sawflies ($F_{2,94} = 2.0808$; $p = 0.1305$), but females from first and second emergence groups contained, on average, greater amounts of lipids as compared to females from the last emergence group (Figure 4.4). There was no difference in egg load observed between emergence groups ($F_{2,107} = 1.6461$; $p = 0.1976$). Similar to the lipid content results, females from the first two emergence groups contained more eggs (47 ± 1.53 eggs and 47.95 ± 1.18 eggs respectively), on average, than females from the last emergence group (44.35 ± 1.56 eggs). Lipid content and egg load of adult *C. cinctus* were only measured in 2021.

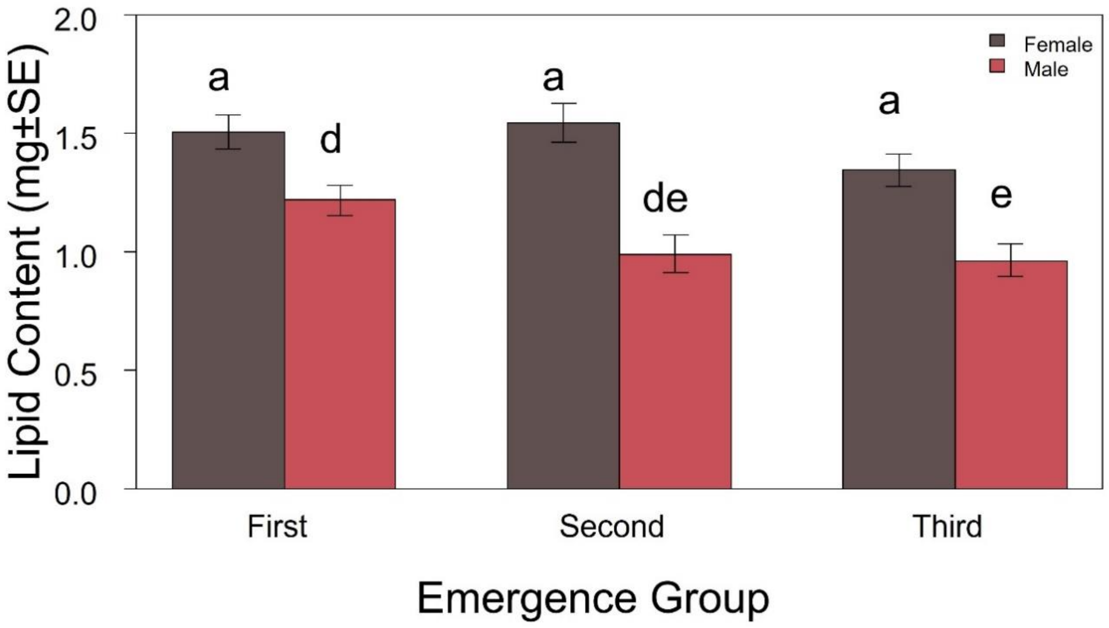

Figure 4.4: Mean lipid content of adult *Cephus cinctus* from three different post-diapause emergence groups. Means with the same letters are not significantly different ($p > 0.05$). Significance between male and female longevity is not displayed.

4.4 Discussion

The laboratory studies we conducted demonstrated that prolonging the post-diapause developmental period by manipulating temperature affected adult longevity and life history traits of *C. cinctus*. Several life history traits (longevity, lipid content) of adult *C. cinctus* were negatively impacted when the post-diapause development period increased, although the effects of prolonged post-diapause were not consistent in both years of the study. Conversely, *B. cephi* adult life history traits were not impacted by different post-diapause development periods, suggesting that *B. cephi* can tolerate prolonged post-diapause development using resources collected before the winter or following adult emergence. Altogether, our findings provide new information on how early season abiotic weather factors like temperature can influence adult *C. cinctus*. This information may have important implications for modeling and forecasting *C. cinctus* populations in Canadian agroecosystems.

Adult *C. cinctus* emergence was delayed by colder early season temperatures. Moreover, adult longevity decreased when post-diapause development was extended. Regardless of sex, individuals from the first emergence group in the 2020 longevity experiment lived about 2 to 3 d longer than adults that emerged later. Contrary to our expectations based on the 2020 experiment, manipulating the post-diapause development period did not impact adult *C. cinctus* longevity, and males lived longer than females in the 2021 experiment. In 2021, emerged *C. cinctus* adults were held in smaller vials that might not have been big enough for the larger-bodied females and may have resulted in stress that smaller-bodied males did not experience. In particular, we observed that the females seemed to be more active than males; females exhibited constant movement within the tubes. Elevated activity rates and stress may have reduced female energy resources and resulted in premature mortality for the adult females, as has been previously noted (*e.g.*, Heslop & Ray 1959). Repeating the experiment with the same sized vials as used in the 2020 experiment is recommended to determine if vial size did or did not impact *C. cinctus* female longevity.

Other life history traits (body size, lipid content, female egg load) were measured to further explore the effect of prolonged post-diapause development on *C. cinctus*. Emergence group did not affect adult *C. cinctus* forewing length in either study year. Our results agree with previous work that found temperature was not a significant factor affecting adult *C. cinctus* body weight (Perez-Mendoza & Weaver 2006). In that study, Perez-Mendoza & Weaver (2006) did not measure wing length. However since adult body weight and wing length are positively correlated (Cárcamo *et al.* 2005), we can assume that wing length likely did not change significantly under different early season post-diapause temperature conditions in the study reported by Perez-Mendoza & Weaver (2006).

Interestingly, the average wing length of sawfly adults in 2021 (7.41 ± 0.02 mm for females and 6.52 ± 0.04 mm for males) was greater than the average wing length of sawfly adults in 2020 (6.64 ± 0.05 mm for females and 5.49 ± 0.06 mm for males). This year-to-year variation could be a result of weather conditions from the prior growing season, as unfavorable weather in the summer months during larval development is known to have potential non-lethal effects on the adults that emerge in the next spring (Seamans 1945; Holmes 1982). We did not record field-level weather measurements in our study, but the average temperatures between June to August were hotter in 2019 compared to 2020 (Agriculture Financial Services Corporation 2020, 2021) and possibly could have been detrimental to larval development.

Total lipid content decreased in males between the first and third emergence group when post-diapause development was prolonged in the 2021 experiment. Although not significant, a slight decrease was also observed in adult female lipid content between the first and third emergence groups in the same year. Egg load was not different between emergence groups, with each female containing on average 44 to 48 eggs.

Overall, several of the adult life history traits were not significantly affected in our study, but decreasing trends were observed with time. The lack of significant effects (statistical or biological) may have arisen because the post-diapause treatments were not long enough. For example, Holmes & Farstad (1956) observed that sawflies that broke out of diapause in early July were markedly smaller in body size and wing lengths and the sawflies in our experiments likely broke out of diapause in early May. They hypothesized that adults that emerged later could have used nutrients for the extended diapause and post-diapause periods instead of for adult tissue production (Holmes & Farstad 1956). Our results support this hypothesis and suggest a potential trade-off between post-diapause development length and adult life history traits in *C. cinctus*. Future studies could expand on our results and explore how even longer post-diapause development periods can impact adult life history traits.

In contrast to *C. cinctus*, neither male nor female *B. cephi* adult longevity changed when adult emergence was delayed. Curiously, parasitoid sex only affected longevity in one study year. This is peculiar as male *B. cephi* are known to live only half as long (on average 14 d) as their female counterparts who can live up to 4 weeks (Nelson & Farstad 1953). The absence of a consistent sex effect on longevity may have been a result of the experimental design and our decision to not replenish the sucrose resource during the experiment. The absence of the expected sex effect could also be an artefact of the relatively low number of individual parasitoids available for the experiment (39 in 2020 and 33 in 2021) and subsequent small sample sizes in each treatment group. Similar to *C. cinctus*, body size did not change as post-diapause development was extended. Overall, our findings suggest that in a changing climate, *B. cephi* adults are not likely to be heavily impacted by early season cold temperatures that extend their post-diapause development period.

Reduced adult *C. cinctus* longevity may impact female oviposition rates and overall population densities within a region, resulting in altered population dynamics and varying degrees of economic impact on wheat production. Females typically emerge after males and soon begin mating (Jacobson & Farstad 1952; Holmes & Peterson 1963b). Sex determination in *C. cinctus* follows an arrhenotokous parthenogenesis system, meaning fertilized eggs will be female and unfertilized eggs result in male offspring (McGinnis 1950; Mackay 1955). Over their lifespan, a single female can lay between 30 to 50 eggs (Ainslie 1929). Taking all of this into consideration, a hypothetical situation where early season temperatures extend post-diapause development could decrease the window of opportunity for female *C. cinctus* to locate a mate and then find suitable host plants for oviposition. In this scenario, the amount of time for oviposition would most likely decrease, resulting in an overall decrease in the number of eggs laid. As well, since male longevity also decreases in this scenario, the number of mating partners available may be reduced. This would mean the overall the rate of unfertilized eggs oviposited would likely increase and result in a male-biased population in the following year. This potential scenario highlights how one aspect of adult life history could end up affecting an entire population of *C. cinctus* in a region. Overall, it is important that this information be used to create models that forecast *C. cinctus* populations, as models can provide important information for Canadian wheat producers and assist them in making informed agronomic decisions.

Lipids are vital for several activities including mobility (Beenakkers *et al.* 1985), embryogenesis (Van Handel 1993), pheromone production (Blomquist *et al.* 2018), and immune response (Cheon *et al.* 2006). Our findings show that early season cold temperatures during post-diapause development are linked to total adult lipid content of *C. cinctus*. In the field, decreased adult lipid content could have negative impacts on *C. cinctus* fitness, although our experiments were not designed to assess those fitness characters. Future studies could address if adult *C. cinctus* total lipid content affects adult processes and overall populations densities. For example, flight is crucial for adult females to find mating partners and host plants. Adults are poor fliers but are still capable of flying distances up to a mile or more to find a suitable oviposition host (Ainslie 1929; Wallace & McNeal 1966). If females do not have the energy stores to facilitate flight, they may choose suboptimal hosts (*e.g.*, wild oats, *Avena sativa* L. or flax, *Linum usitatissimum* L.) which decrease their progeny's growth and survivorship (Farstad 1944; Sing 2002). Future studies in the laboratory using flight mills, for example (see Evenden *et al.* 2015; Wijerathna & Evenden 2020), could investigate the effect of adult lipid content on *C. cinctus* flight.

Although the quantity of eggs did not change, we did not examine their quality in the experiments reported here. Despite several studies that report how external stressors impact *C. cinctus* female egg capacity (Morrill *et al.* 2000; Cárcamo *et al.* 2005; Beres *et al.* 2013), there has yet to be a study on how similar stressors influence the quality of individual eggs. Morphological traits like egg volume, length and shape can influence the speed of larval development and chance of survival (Fox & Czesak 2000). For example, larger eggs and progeny have increased survivorship and better resistance to environmental stresses (see Harvey 1985; Fox 1994; Azevedo *et al.* 1997). An investigation of how environmental factors and agronomic practices influence egg quality could provide new insights on how to improve *C. cinctus* pest management strategies.

The synchronization of a specialist parasitoid with its host is a crucial factor that determines parasitoid population dynamics (Godfray *et al.* 1994). Abiotic conditions can negatively affect parasitoids and de-synchronize their development from that of their host (Van Nouhuys & Lei 2004; Wetherington *et al.* 2017). Fortunately, our study indicates relatively minor, if any impacts of cold early season temperatures and prolonged post-diapause development on adult *B. cephi* life history traits, suggesting that *B. cephi* life history would remain in-sync with that of *C. cinctus* in these conditions. Adult *B. cephi* emergence occurs in relatively the same time frame as *C. cinctus* adult emergence, beginning in early June (Holmes *et al.* 1963), and is followed by a 12 to 21 d pre-oviposition period. The exact reason for this period is not known, but perhaps it gives female parasitoids time to acquire nutrients for egg production and allows *C. cinctus* larvae the time grow large enough to be detected and support parasitoid larval development (Nelson & Farstad 1953; Holmes *et al.* 1963). Alterations in the synchrony between *B. cephi* and *C. cinctus* could reduce the effectiveness of the parasitoids in managing *C. cinctus* populations and catastrophically decrease the density of parasitoids in a given area. Our results show that temperature is unlikely to cause any disruptions between *B. cephi* and *C. cinctus*, although other factors (*i.e.*, relative humidity) should be investigated. This information will help to better our understanding of how *C. cinctus* populations change over time due to *B. cephi* parasitoid pressure and could be included in *C. cinctus* phenology models.

4.5 Conclusion

Our study provides novel insight about the effect of temperature during the post-diapause developmental phase on the adult life history traits of *C. cinctus* and its overall population dynamics in Canadian agroecosystems. Adult *C. cinctus* longevity decreased in both sexes when their post-diapause period was extended, whereas *B. cephi* parasitoids did not have any measurable difference in adult longevity under the same conditions. Total lipid content in male *C. cinctus* adults was also reduced under prolonged post-diapause development periods, which could have other impacts on *C. cinctus* life history traits later in their lifecycle. Other abiotic factors (*i.e.*, relative humidity, precipitation) which affect post-diapause development could have similar effects on adult *C. cinctus* life history traits and should be explored in follow-up studies. It is our hope that the information presented here will be useful in the development of *C. cinctus* population forecasting tools and phenology models that can be used to help protect Canadian wheat producers from heavy economic losses arising from *C. cinctus* infestation.

5.0 General discussion and future work

Increasing levels of abiotic stresses, due in large part to climate change, pose real problems for wheat producers and jeopardize the stability of the Canadian agricultural industry. The rise of agricultural pests (pathogens, insects) in historically non-impacted growing regions is a key issue requiring attention. Abiotic conditions are becoming more advantageous for some pest populations to thrive and expand their influence onto field crops in considerable portions of the agricultural area. A bioclimatic model developed for *C. cinctus* suggests that a 1°C increase in temperature above the current long-term average temperature results in the overwhelming majority of growing regions in the Canadian Prairies being favorable for *C. cinctus* populations to establish and reach densities that could be damaging to wheat crops (Olfert *et al.* 2019). On top of increasing the use of solid-stem wheat varieties, which deter *C. cinctus* growth (Cárcamo *et al.* 2005), wheat producers need other tools like phenology and population forecasting models to help mitigate severe yield losses. Unfortunately, knowledge gaps still exist that prevent the development of such models. Thus, the primary goal of this project was to address how early season temperatures impact several facets of *C. cinctus* development and their overall population dynamics.

Over two years, sawfly-cut samples were collected from harvested wheat fields in Alberta to investigate the spatial and temporal variation in larval *C. cinctus* mortality and the associated mortality factors. Our results conclude that larval *C. cinctus* mortality greatly differed between fields which agreed with previous studies (Sing 2002; Nansen *et al.* 2005a). Although not accounted for in our study, these differences are likely due to individual field histories and agronomic practices (*e.g.,* harvesting with a stripper header (Meers 2005)) that influence the abundance of *C. cinctus* and their natural enemies. Our findings also highlight the importance of scouting individual fields if high *C. cinctus* populations are expected to achieve the highest resolution and most accurate information. One future hurdle for wheat producers that we experienced in this study is the large time commitment required to gather and process over 200 wheat stubs from each field. A pre-harvest sampling protocol by Nansen *et al.* (2005b) estimates an individual field would require 9.5 work hours. Despite the differences between their protocol and the one used in our study (ten 30 cm transects versus four 1 m transects), this estimate is comparable to the time needed in this study. One option for producers would be to hire crop consultants or agronomists to conduct this work, however other protocols should be tested in the future that are more practical for all wheat producers. Another potential problem with solely relying on post-harvest sampling is the underestimation of parasitism activity. While this approach allows agronomists and field research technicians to sample fields on their own schedule without disrupting farming operations, this approach likely underrepresents the number of parasitoids within a field. *Bracon cephi* adult activity in Canada ranges from June to September (Nelson & Farstad 1953) and is largely reliant on abiotic conditions (*i.e.,* high precipitation) that delay crop ripening and allow for a second generation (Holmes *et al.* 1963). As such, parasitoids rarely attack *C. cinctus* larvae after the stem is cut (Holmes *et al.* 1963; but see Rand *et al.* (2011)) and signs of parasitism (*e.g.,* small holes in the wheat stem, *C. cinctus* larval cadavers, parasitoid pupae) are likely destroyed during harvest if stubble is not conserved. Without this knowledge, producers may not fully understand the true levels of *C. cinctus* and *B. cephi* population densities within their field. A sampling protocol for *C. cinctus* larvae that also considers parasitoid population densities would give agronomists and wheat producers further information on how strong *C. cinctus* population activity is within a given area.

The larval mortality factors found in our samples (parasitism, fungal infection, and unknown) did not change between growing seasons, pointing to the minimal influence of winter and early spring abiotic conditions on *C. cinctus* population dynamics. This outcome means samples collected in the fall (pre- or post-harvest) can provide wheat producers with an accurate estimate of *C. cinctus* in their fields in the following year. Other mortality factors such as host-plant resistance and predation were not expected to contribute to mortality in either survey year. Host-plant related mortality occurs when solid-stem wheat plants express higher levels of pith within the stems, which leads to increased late instar larval desiccation (Holmes & Peterson 1962). In our study, we were unlikely to observe mortality from plant responses for two reasons. First, solid-stem varieties are not commonly seeded in southern Alberta (Agriculture Financial Services Corporation 2021). Second, larval desiccation normally occurs prior to harvest and any remnant cadavers would likely be destroyed during harvest and not detected with a post-harvest sampling protocol. This agrees with the life table analyses by Buteler *et al.* (2015) who showed that mortality from host plant responses was not measurable for the winter larval life stage.

Predation by a generalist clerid beetle, *Phyllobaenus dubius* Wolcott (Coleoptera: Cleridae), occurs throughout the summer as adults and larvae feed on *C. cinctus* larvae within growing wheat stems (Morrill *et al.* 2001). Their occurrence in Canada has been recorded multiple times, either from collecting live specimens or observing larger entry holes through the frass plug of sawfly-cut stems (Beres *et al.* 2009; Cárcamo *et al.* 2011). In the present study, we found no instances of predation in the over 11,000 stems dissected, pointing to the probable lack of impact that predation has on *C. cinctus* population dynamics. This result again agrees with Buteler *et al.* (2015) who reported almost no mortality associated with predation in the presence of other mortality factors. Together with the minimal change in the previously mentioned larval mortality factors, the lack of influence of other factors between growing seasons highlights that *C. cinctus* populations are unlikely to decrease significantly once larvae enter their overwintering phase.

Early season temperatures that prolong the post-diapause development period length negatively influence several *C. cinctus* life history traits (adult longevity, male lipid content). *Cephus cinctus* adults do not benefit from sucrose feeding (Rand *et al.* 2019) and are likely incapable of acquiring enough resources to compensate for expended energy reserves from their post-diapause development period during their relatively short lifespans. As such, *C. cinctus* adults heavily depend on the metabolic resources from their larval stage, which are likely to decrease when post-diapause development is extended. Although our study only found significant differences in adult longevity and male lipid content between emergence groups, other traits (female total lipid content and egg load) were on average greater in the first emergence group than the third emergence group. There was a possibility that emergence group could have been significant factor if our study prolonged development further (see Nelson & Farstad 1956). Adults emerge over a four-to-six-week period from early June to July in Canada (Criddle 1922, Philip *et al.* 2018). In 2021, there were no adult sawflies that emerged later than 25 June. Future studies could look to extend post-diapause development for a longer period to verify if life history traits are continually impacted. As well, our results highlighted the potential the effect of temperature on several adult processes (*i.e.*, oviposition, flight movement) that should be investigated further. Altogether, we demonstrated that there are consequences for *C. cinctus* adult life history traits when post-diapause development is delayed due to temperature. This information will be critical for *C. cinctus* phenology model development and help to outline how abiotic factors influence their development after overwintering.

Extended post-diapause development time did not influence *B. cephi* adult longevity. A major explanatory factor is that *B. cephi* adults actively forage and benefit greatly when provided sucrose and floral resources (Reis *et al.* 2019). Thus, although larval metabolic stores may be expended if their post-diapause development is prolonged, the depleted reserves can be recuperated if adults seek out floral resources over their lifespan. Future studies could take a metabolomic approach to test this hypothesis. As well, an exploration into which nutrients benefit *B. cephi* adults the greatest could give wheat producers insights in how to alter the composition of the agricultural landscape to benefit and conserve parasitoid populations. Lastly, there remain several questions to answer about *B. cephi*, as their post-diapause larval biology is not well understood. Nelson & Farstad (1953) report that the time from post-diapause larva to adult eclosion is about 8 d under laboratory settings (25°C at 70% RH), but they do not mention how, nor do they mention the time needed for larvae to terminate diapause. *Cephus cinctus* and *B. cephi* adult emergence are well-synced (Holmes *et al.* 1963), so perhaps the abiotic factors that trigger diapause termination and post-diapause development in *C. cinctus* would be similar in *B. cephi.* This hypothesis would also explain the lack of response of adult body size from both species to the tested early season temperature conditions. Further research should be done to better understand the exact parameters that trigger *B. cephi* larval diapause termination and if they match the parameters of *C. cinctus* larval diapause termination.

This thesis explored how abiotic factors between growing seasons affected *C. cinctus* and their natural enemies, and the overall importance of abiotic conditions on *C. cinctus* population dynamics by testing four hypotheses with the following key conclusions:

1. Wheat stem sawfly larval mortality differed significantly between field locations.
2. Wheat stem sawfly larval mortality did not change over time between growing seasons or spring sampling periods.
3. Wheat stem sawfly adult longevity and male lipid content were significantly impacted by prolonged post-diapause development as controlled by cold early season temperatures.
4. In contrast to wheat stem sawfly, adult *B. cephi* longevity was not affected when post-diapause development was extended.

Together, the findings outlined in this thesis provide information related to knowledge gaps in *C. cinctus* overwintering and post-diapause development. This research will be helpful for modelling *C. cinctus* populations and their potential for range expansions in Canadian agroecosystems in future climates.

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