

**EFFECTS OF LANDSCAPE STRUCTURE AND NATURAL ENEMIES ON THE
ABUNDANCE OF CEREAL LEAF BEETLE, *OULEMA MELANOPUS* L.
(COLEOPTERA: CHRYSOMELIDAE), IN WHEAT FIELDS IN SOUTHERN
ALBERTA**

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By

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ABSTRACT

EFFECTS OF LANDSCAPE STRUCTURE AND NATURAL ENEMIES ON THE ABUNDANCE OF CEREAL LEAF BEETLE, *OULEMA MELANOPUS* L. (COLEOPTERA: CHRYSOMELIDAE), IN WHEAT FIELDS IN SOUTHERN ALBERTA

By

Arash Kheirodin

In recent years, the cereal leaf beetle (CLB) *Oulema melanopus* (Coleoptera: Chrysomelidae), an important pest of wheat, barley, and oat throughout the world, has become a serious pest of cereal crops in Western Canada. Following CLB invasion, *Tetrastichus julis* (Hymenoptera: Eulophidae), the most efficient larval parasitoid of CLB, was introduced into the Canadian Prairies. I investigated the effect of landscape complexity, ranging from high (semi-natural habitats > 50%) to low (semi-natural habitats < 30%) on the abundance of CLB and its parasitism in southern Alberta. Cereal leaf beetle abundance and the parasitism rate of *T. julis* responded positively to the proportion of CLB major hosts (wheat and barley) in the current and previous years at various spatial scales (0.5 to 2 km). Landscape diversity was negatively associated with CLB abundance. Cereal leaf beetle parasitism increased when there was a higher proportion of canola and alfalfa in the landscape. Cereal leaf beetle parasitism also positively responded to CLB abundance in cereal fields, indicating a density-dependent response. Overall, I concluded that diversification of crops and semi-natural habitats in the surrounding landscape are an important factor to reduce CLB numbers in the Canadian Prairies.

Laboratory and field predation trials revealed for the first time the contribution of various predators to CLB control. Several species of *Hippodamia* (Coleoptera:

Coccinellidae), carabids (Coleoptera: Carabidae) and nabid bugs (Hemiptera: Nabidae) were among the best predators of CLB immature stages under laboratory conditions, that also included *Coccinella septempunctata* (Coleoptera: Coccinellidae) and Staphylinidae. I found an average 24.5% of predation on sentinel eggs in wheat fields in 24 h trials. I developed a set of species-specific primers to detect CLB DNA in the gut content of generalist predators, which confirmed that *Nabis americoferus* and several *Hippodamia* species are the most promising predators of CLB in wheat. *Nabis americoferus* was the most abundant predator in the Lethbridge area and had 0.35 proportion positives for CLB DNA. Altogether, the predation studies highlight the importance of predators in CLB control, which has been neglected to date but can have important roles in sustainable pest management programs for CLB in cereal crops.

Key words: Landscape complexity, concentration hypothesis, natural enemy hypothesis, integrated pest management, biological control, molecular gut content analysis, half-lives, cereal leaf beetle, *Nabis americoferus*, *Coccinella septempunctata*, cereal crops, wheat.

DEDICATION

To my dear mother Mitra and my dear Father Hamid

To my dear sister Bahar

To my dear brother Amir

To my dear wife Thais

For their unconditional love, standing beside me in hardest days, their support, encouragement, and understanding, which has always pushed me to move towards my career path.

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CHAPTER 4 – Manuscript I: Laboratory and field tests of predation on the cereal leaf beetle, *Oulema melanopus* (Coleoptera: Chrysomelidae). A revised version of this manuscript is published in the journal Biocontrol Science and Technology.

CHAPTER 5 – Manuscript II: Consumption of cereal leaf beetle, *Oulema melanopus* L. (Coleoptera: Chrysomelidae) by generalist predators in wheat fields detected by molecular analysis. A revised version of this manuscript is accepted for publication in the journal Entomologia Experimentalis et Applicata.

CHAPTER 1: General Introduction

Introduction

The cereal leaf beetle (CLB), *Oulema melanopus* L. (Coleoptera: Chrysomelidae) is native to Europe and recently invaded western Canada. It feeds on a variety of cereal crops including wheat, oats, and barley (Dosdall et al., 2011; Dysart et al., 1973; Kher et al., 2016; Leibee & Horn, 1979). In Canada, CLB expanded its range in the last few years and had successfully spread to Alberta, Saskatchewan and more recently in Manitoba (Dosdall et al., 2011; Gavloski, 2014). The majority of cereal production in Canada is located in the western part of the country including British Columbia, Alberta, Saskatchewan and Manitoba (McCallum et al., 2007), which has adequate climatic and environmental conditions for CLB establishment (Dosdall et al., 2011). Cereal leaf beetle can cause crop losses of 75, 55, 38 and 23% in barley, oat, winter wheat, and spring wheat respectively, in the northern U.S.A (Royce & Simko, 2000; Webster & Smith, 1979).

Several attempts to suppress CLB with classical biological control by introducing parasitoids from Europe have been made since its arrival in North America (Evans et al., 2006; Wellso, 1982). In Canada, the most efficient introduced parasitoid is *Tetrastichus julis* (Walker) (Hymenoptera: Eulophidae), first detected in British Columbia (1988), and now present in Alberta and Saskatchewan (Dosdall et al., 2011; Dysart et al., 1973; Stehr, 1970). In Utah, Evans et al. (2006) reported rates of parasitism by *T. julis* ranging from 20 to 65%, at different temperature regimes, showing the high efficiency of this parasitoid to control CLB. Dosdall et al. (2011) reported a steady increase of CLB parasitism by *T. julis* from 9.4 to 32.8% between 2007 and 2009 in wheat fields near Lethbridge, Alberta, where it is considered established.

Several studies indicate that plant and animal abundance and diversity are influenced by both agricultural landscape complexity and local management practices (Altieri, 1999; Tscharntke et al., 2005). Landscape simplification due to agricultural intensification by turning natural habitats to agricultural fields has resulted in the loss of natural habitats and biodiversity in agricultural landscapes (Benton et al., 2003). Landscape habitat diversity is linked to various ecosystem services such as nutrient cycling, insect biological control, and pollination (Bommarco et al., 2013; Crowder & Jabbour, 2014; Tscharntke et al., 2005). In a review paper, Bianchi et al. (2006) report a lower abundance of natural enemies in simple landscapes (landscapes with low proportions of semi-natural habitats, which includes areas of the landscape that are not under cultivation, such as pastures, woodlands and grasslands) relative to complex landscapes (landscapes with high proportions of semi-natural habitats), resulting in higher pest pressure in simple agricultural landscapes. Habitat diversity within agricultural landscapes can result in higher biological control rates for various pests by providing natural enemies with alternative prey and overwintering sites (Chaplin-Kramer et al., 2011; Gardiner et al., 2009; Kremen, 2005; Rusch et al., 2016; Schlapfer et al., 1999; Tilman et al., 2002). However, recent studies have found that landscape complexity does not always increase pest control (Chaplin-Kramer et al., 2011; Karp et al., 2018), suggesting that the association between landscape complexity and pest suppression is specific for each agro-ecosystem. The impacts of landscape complexity on CLB abundance and parasitism by *T. julis* have not been evaluated.

Generalist predators are likely to contribute to CLB suppression, but their importance has not been studied to date. Although *T. julis* is a very efficient parasitoid and able to decrease CLB abundance over time, the parasitoid does not kill the host until pupation, and

thus CLB larvae can still feed and damage the cereal plants (Dysart et al., 1973). On the contrary, generalist predators cause immediate mortality and contribute to pest suppression within the field season. Generalist predators have been reported to be important mortality factors for chrysomelids such as *Leptinotarsa decemlineata* (Say) (Greenstone et al., 2010; Hazzard et al., 1991) and *Microtheca ochroloma* Stål (Montemayor & Cave, 2012), and have been reported to feed on CLB immature stages in cereal crops (Shade et al., 1970).

Determining the diet of generalist predators or tracking them in the environment with direct observations is a difficult task, especially in the case of small insects. Therefore, laboratory choice and no-choice feeding trials (Sebolt & Landis, 2004; Vankosky et al., 2011) and gut analysis using species-specific primers for insect pest DNA (Greenstone et al., 2010; Harwood et al., 2004; King et al., 2008) are commonly used methodologies to determine potential predators of different insect pests (Greenstone et al., 2005; King et al., 2008).

Thesis organization

There are six chapters included in this thesis including General Introduction (Chapter 1), Literature Review (Chapter 2), Research chapters (3, 4 and 5), and the General Discussion (Chapter 6). The background information along with the rationale for each chapter is addressed in the general introduction. In the literature review section, the information regarding the importance of *Oulema melanopus* as a major pest of cereals and its potential natural enemies are included. Further, I included information regarding the landscape structure associations with various pests and natural enemies. There are three manuscripts included in the research sections (Chapters 3-5). Each manuscript consists of an abstract, introduction, materials and methods, results and discussion sections. The findings of all research chapters are discussed in relation to each other in the general discussion section (Chapter 6), with overall conclusions discussed.

CHAPTER 2: Literature Review

CHAPTER 2

Literature Review

2.1 Distribution and range expansion of cereal leaf beetle, *Oulema melanopus* L. in North America

The cereal leaf beetle (CLB), *Oulema melanopus* L. (Coleoptera: Chrysomelidae) is native to Europe and Asia, and has become an important pest of cereal crops on a global scale (Philips et al., 2011). The species is a common cereal crops pest in Belgium (Stilmant, 1995; Van de Vijver et al., 2019), Poland (Ulrich et al., 2004), France (Stilmant, 1995), Serbia (Dimitrijevic et al., 1999), Italy (Morlacchi et al., 2007), Germany (Schmitt & Ronn, 2011) and The Netherlands (Daamen & Stol, 1993). In 1962, CLB was first detected in Michigan and caused economic damage to cereal crops (Castro et al., 1965; Haynes & Gage, 1981). In the U.S.A, CLB later expanded its range from Michigan to Indiana (Shade et al., 1970; Webster, 1977), Tennessee (Grant & Patrick, 1993), Virginia (McPherson, 1983b), Alabama and Georgia (Buntin et al., 2004), Missouri and Iowa (Bailey et al., 1991), Utah (Hodgson & Evans, 2007), Washington and Oregon (Royce & Simko, 2000), and finally to California, in 2013 (Dowell & Pickett, 2016).

The first report of CLB in Canada was in Ontario in 1965 (Battenfield et al., 1982). About three decades later, CLB was reported in the Maritime Provinces (New Brunswick, Nova Scotia, and Prince Edward Island) in 1994, and British Columbia in 1998 (LeSage et al., 2007). In recent years, following a gradual pattern of expansion, CLB was reported from cereal fields in Alberta in 2005, and in Saskatchewan and Manitoba in 2008 and 2009, respectively (Dosdall et al., 2011; Kher et al., 2011). In Alberta, the abundance of CLB increased slowly between 2006 and 2010 (Dosdall et al., 2011). In Manitoba, CLB was first detected in the Swan River area in

2009 (Kher et al., 2011), and near Roblin, Brandon, Treherne, Roseisle, Pilot Mound and La Riviere by 2014 (Gavloski, 2014).

2.2 Biology and field population dynamics of cereal leaf beetle

Several researchers have investigated the biology and field dynamics of CLB across different regions and agro-ecosystems, including southern Alberta (Gutierrez et al., 1974; Hoffman & Rao, 2011; Kher et al., 2016). Cereal leaf beetle has one generation per year in Alberta (Kher et al., 2011), but a second generation has been reported in Virginia (McPherson, 1983b). Cereal leaf beetle preferred oat and barley as oviposition hosts relative to wheat (Gallun et al., 1966), suggesting that host plant species can affect CLB oviposition potential. Cereal leaf beetle overwinter as adults in grassland and woodland habitats adjacent to fields previously planted with cereal crops (Casagrande et al., 1977; Gutierrez et al., 1974; Philips et al., 2011; Ulrich et al., 2004). In northern Michigan, Casagrande et al. (1977) found that CLB adults had the highest overwintering survival rate at the edge of woodlots (i.e., stands of mature trees with litter ground cover), followed by sparse or dense woods, suggesting that these are the most important overwintering habitats for CLB adults. Overwintering adults emerge in mid-April when temperatures reach 14 °C, disperse and feed on field border and pasture (Casagrande et al., 1977; Sawyer & Haynes, 1985). After breaking the winter diapause, overwintering adults continue to feed on grasses and mate before moving to cereal crops for oviposition (Castro et al., 1965). Typically the beetle is active from March to July with oviposition peaking in late March to mid-April in Michigan (Wellso, 1973). In southern Alberta, the overwintered generation of CLB is active from mid-April to late July, and oviposition peaks in mid-May when suitable cereal crops become available (Kher et al., 2011; Kher et al., 2016) (Fig. 1). The new generation

adults emerge in early August and feed on available crops and grasses before dispersing to the overwintering sites in September (Kher et al., 2011).

Several environmental factors affect CLB oviposition potential in agro-ecosystems. Plant quality and characteristics are shown to affect CLB fecundity. For instance, Hoffman and Rao (2011) found that CLB laid more eggs on younger (later planted) oat plant leaves, and were deterred from laying eggs on older oat plants. The same authors also reported that females laid more eggs on younger and lower tiller leaves compared with flag leaves. Similarly, CLB laid more eggs on younger wheat plants (late planted) over old ones (Casagrande et al., 1977). Moreover, the lower width of wheat leave blades reduces CLB oviposition by 8-fold, showing the importance of cereal leaf width on CLB oviposition rate (Wellso et al., 1973). Cereal leaf beetle feeding before and during oviposition, and plant quality can affect oviposition capacity. Well-fed overwintered females laid on average up to 12 eggs per day and oviposited for a longer time than individuals fed a poor diet, which laid on average up to 8 eggs per day (Wellso et al., 1973). Furthermore, Wellso et al. (1975) reported that females mated once weekly laid on average 2.2 times more eggs and consumed a significantly higher amount of food compared to unmated and once-mated females. This result suggests that the frequency of mating and the amount of available food are important factors affecting oviposition. Gutierrez et al. (1974) found that females infected with *Beauveria bassiana* (Balsalmo) (Hypocreales: Clavicipitaceae) had significantly lower fecundity compare to un-infected individuals. Under optimum conditions, a female CLB can lay 50 to 275 eggs during its lifetime (Kher et al., 2016; Schmitt, 1988). Females typically lay single, or a small groups of two to three eggs (touching end to end) on the adaxial surface of cereal leaves along the mid-vein (Helgesen & Haynes, 1972), and at 21 °C eggs typically hatch within 5 to 9 days depending on the host crop variety (Kher et al., 2016).

The eggs hatched within 5 to 6 days on wheat, barley and oats and up to 9 days on corn and rye (Kher et al., 2016). The eggs start to hatch at $\geq 10^{\circ}\text{C}$ and optimal hatching occurs between 12 to 32 $^{\circ}\text{C}$ (Gutierrez et al., 1974). The eggs require 86.7 degree days to complete its development (Guppy & Harcourt, 1978). Overall, these studies suggest that CLB fitness is affected by various abiotic and biotic factors such as temperature, food availability, host plant species, quality, phenology, and diseases.

Cereal leaf beetle larvae undergo four instars and typically complete its larval development in 10-14 days at favorable temperatures, ranging from 22 to 32 $^{\circ}\text{C}$ (Guppy & Harcourt, 1978; Philips et al., 2011). Each of the first three larval instars require 33 degree days to complete development, but the fourth instar needs 46.7 degree days (Gutierrez et al., 1974). Larvae are not very mobile and typically complete all their instars on the same plant (Kher et al., 2011). In Alberta, larvae are generally found in cereal crops between May and July (Kher et al., 2011). Larvae drop to the soil after completing their 4th instar development, dig into the soil approximately 5 cm and undergo a pre-pupal stage, in which it loses its fecal coat, forms a cocoon using soil materials (Wellso et al., 1973), and subsequently pupates (Philips et al., 2011). Pupation occurs from July until August in southern Alberta (Kher et al., 2011). Adults emerge in approximately 17 to 25 days after pupation depending on soil temperature (Philips et al., 2011). In southern Alberta, adults emerge from early August until mid-September, feed on late cereal crop plants and soon after they move to overwintering habitats (e.g., adjacent woodlands and grasslands) (Kher et al., 2011; Philips et al., 2011).

2.3 Identification characteristics of the CLB

Adults are 4.5 to 6.2 mm in length and characterized by their red to orange pronotum color with occasionally black posterior and interior margins (Bezdek & Baselga, 2015). Cereal

leaf beetle eggs are 1 mm in length, elongated with yellowish-orange color that gradually becomes darker before hatching (Philips et al., 2011). The larval head size is wider than the body and gradually widens with each molt. Female larvae having wider head capsule than males (Hoxie & Wellso, 1974). Cereal leaf beetle larvae are characterized by the accumulation of fecal material over the body (Ruppel & Stehr, 1975). Pupae are on average 5 mm in length, elongated in shape, and yellow. Pupation occurs under the soil surface, and the pupa turns black as it ages (Philips et al., 2011).

2.4 The damage potential of cereal leaf beetle

2.4.1 Feeding damage

Cereal leaf beetle adult and immature stages are capable of damaging cereal leaves through skeletonization (Haynes & Gage, 1981). The yield loss due to CLB skeletonization has been reported for several cereal crops including up to 44.8% on oats (Merritt & Apple, 1969), 75% on barley (cultivar Partizan, Dimitrijević et al., 2001), 23% on winter wheat (cultivar Monon, Gallun, Everly, & Yamazaki, 1967) and 23 to 49% on spring wheat (Webster et al., 1972) when no management tactics were applied. Adult feeding results in elongated holes on the leaf surface (Herbert & Van Duyn, 2009). However, adult damage is rather cosmetic and does not typically lead to yield loss (Herbert & Van Duyn, 2009; Philips et al., 2011). Late larval instars cause majority of damage (e.g., approximately 70%) by removing the leaf chlorophyll down to the cuticle and between the veins (Gallun et al., 1966; Wilson et al., 1969). Larval damage impacts yield differently based on the location of damaged leaves, with damage to the flag leaf resulting in the highest yield loss by lowering the seed number per spike (Dimitrijević et al., 2001). The flag leaf plays an essential role in cereal plants during the seed filling process, provides tolerance against environmental stress and has a high impact on cereal yield by

increasing seeds per spike through photosynthesis (Dimitrijević et al., 2001). Although less detrimental, damage to other leaves can also impact yield by lowering kernel weight (Buntin et al., 2004).

The relative impact of CLB damage on yield depends on environmental conditions. For instance, Buntin et al. (2004) reported that the effect of skeletonization on yield is not constant across fields and regions. In Alabama, they found 1% higher yield loss per every 1% increase in skeletonization, but in Georgia, they only found 7% yield loss in plants with 43% skeletonization. They concluded that environmental stress and plant quality affect this relationship, with plants under stress being more susceptible to skeletonization than healthy plants (Buntin et al., 2004). Similarly, Webster and Smith (1983) suggested that optimal environmental conditions such as sunlight, soil moisture and fertility are linked to higher tolerance of wheat to CLB. Altogether, these studies suggest that the skeletonization by CLB larvae, in particular, fourth instar larvae can result in a high cereal yield loss, and it depends on various environmental conditions and plant stress levels.

Various economic thresholds have been suggested for CLB, depending on crop and region. In Canada, the economic threshold for wheat is three or more eggs and larvae per stem prior to the boot stage or one or more larvae per flag leaf at the heading stage (Webster & Smith, 1983). Ihrig et al. (2001), reported up to 21.6% yield loss due to the presence of one 4th instar larva on the wheat stem, suggesting that the initial economic threshold reported by Webster and Smith (1983) might not be suitable for all regions in North America. A more recent study suggested a threshold of 20 eggs or larvae per 100 tillers (Herbert & Van Duyn, 2009), which is lower than the one reported by Webster and Smith (1983). In addition, Buntin et al. (2004), suggested an economic threshold of 0.5 larva per stem at the anthesis stage, based on up to

12.6% yield loss due to one larva per stem that indicated the need of a lower threshold. Overall, the results of these studies suggest that the economic threshold depends on the crop phenology, and should be estimated regionally.

2.4.2 Virus transmission by cereal leaf beetle

Cereal leaf beetle is capable of indirect damage to its host by transmitting viruses from infected plants to healthy cereal plants. Gaborjanyi and Szabolcs (1987) reported CLB could transmit Brome Mosaic Virus to its winter wheat hosts for up to 24 h, due to the presence of the virus on their mouthparts. Abbrook and Benigno (1972) further confirmed transmission of Phleum Mottle Virus and Cocksfoot Mottle Virus by adult CLB to barley. Also, CLB and other chrysomelid adults are potential vectors of Maize Chlorotic Mottle Virus to corn (Nault et al., 1978). Despite this potential for virus transmission, to date I am not aware of any study reporting significant yield losses or damage due to viruses transmitted by CLB, suggesting that direct damage is the main concern with CLB.

2.5 Management practices for CLB

Soon after the accidental introduction of CLB in Michigan, attempts to detect and eradicate the species started (Haynes & Gage, 1981). The quarantine and eradication attempts using extensive pesticide applications and mass releases of an egg parasitoid, *Anaphes flavipes* (Foerster) (Hymenoptera: Mymaridae) failed (Castro & Guyer, 1963; Haynes & Gage, 1981). Soon after, a biological control program was launched by the University of Michigan and Purdue University to find the egg and larval parasitoids of CLB in Europe and release them in the United States as a part of a classical biological control program (Haynes & Gage, 1981). The control approaches used to date are discussed next.

2.5.1 Chemical control

Chemical control was the primary method of CLB management after it was found in North America (Wellso, 1982). The eradication attempts were not successful even when carbaryl was applied to 654,298 hectares in Illinois, Indiana, and Michigan (Jantz, 1967). Chemical control was recommended when CLB larvae density exceeded the economic threshold of one larva per flag leaf, but many growers sprayed their fields regardless of CLB density (Philips et al., 2011). Carbofuran and endosulfan with 100 and 98.8% control in 7 days were reported as an effective insecticide against CLB immature stages (Webster et al., 1972). Due to their potential non-target effects on human and detriments to environmental health, the application of carbofuran and endosulfan was banned in Canada in 2011, although dimethoate is still in use in Canada. The application of broad-spectrum insecticides such as carbofuran and dimethoate kills natural enemies of CLB (Alexander et al., 2013; Coats et al., 1979) and can disrupt CLB control by its biological control agents, and therefore it should be limited to use when the CLB density exceeds the economic threshold (Kher et al., 2011; Philips et al., 2011).

2.5.2 Cultural control

Relatively few researchers have evaluated the impact of cultural control methods on CLB management. Some studies suggested early planting (Philips et al., 2011), improving plant vigor in the fields (Kher et al., 2014; Walenta & Roberts, 2012), low seeding rate (Webster et al., 1978), tillage (Leibee & Horn, 1979), and nitrogen application prior to the plant stem elongation growth stage (Reisig et al., 2012) as potential cultural controls for CLB. Kher et al. (2014) reported a positive association between soil phosphorous and CLB establishment, and negative associations with soil potassium levels and CLB establishment in winter wheat. The same authors reported that maintaining constant plant vigor using fertilizer could reduce the impact of

CLB on cereal yield. Although plants with higher vigor attracted more CLB, they also attracted a higher number of *Tetrastichus julis* (Walker) (Hymenoptera: Eulophidae), which resulted in higher parasitism. Leibe and Horn (1979) reported up to 93.8% mortality of CLB pupa due to tillage (plow and disc). However, they also found that tillage can also cause up to 89% mortality on CLB parasitoids, and disrupt the biological control of CLB. Overall, these studies suggest that numerous cultural control techniques can be implemented to lower the CLB damage to cereals, although their practical integration in modern cropping systems can be a challenge, and in some cases their use is not widespread (see Webster et al. (1978) & Leibe and Horn (1979)).

2.5.3 Biological control

Biological control efforts started in Michigan in 1966 by screening CLB eggs and larvae for evidence of parasitism by native species such as *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) (Maltby et al., 1969). However, due to the low-density of *Trichogramma* spp. and the low rates of egg parasitism found in cereal fields, this generalist parasitoid was not considered an effective control for CLB. Soon after the initial efforts, several larval parasitoids such as *T. julis* (Stehr, 1970), *Lemophagus curtus* Townes (Hymenoptera: Ichneumonidae) (Stehr et al., 1974) and *Diaparsis carinifer* (Thomson) (Hymenoptera: Ichneumonidae) (Stehr & Haynes, 1972) were imported from Europe as part of a classical biological control program and introduced in the United States.

2.5.3.a The primary larval parasitoid, *Tetrastichus julis*

Tetrastichus julis, a multivoltine gregarious larval endo-parasitoid from Europe was introduced in Michigan in 1967. *Tetrastichus julis* has high host specificity and only attacks CLB (Hervet et al., 2016). By 1972, the establishment of *T. julis* was confirmed in 18

counties of Michigan, showing the expansion of its range to new CLB infested areas (Logan et al., 1976). In Canada, *T. julis* established first in southern Ontario, moving adventively from Michigan, in 1975 (Harcourt et al., 1977). In 2002, the species was introduced and became established in British Columbia (Philip, 2002), and it is now also present in Alberta (Dosdall et al., 2011; Kher et al., 2011), Saskatchewan (Dosdall et al., 2011), and was relocated to Manitoba in 2009 (Gavloski, 2014). This species proved to be the most efficient introduced parasitoid for the control of CLB in North America (Dysart et al., 1973; Evans et al., 2006; Logan et al., 1976; Stehr, 1970). The establishment of *T. julis* with an average of 75% parasitism was reported in Ontario (Harcourt et al., 1977). Further, in Utah, Evans *et al.* (2006) reported up to 60% parasitism rates by *T. julis*. The efficiency of *T. julis* has been linked to its high specificity, synchronization with CLB and efficiency locating and tracking CLB population across its host's geographical range.

In Alberta, *T. julis* is active from mid-May to August, and peak parasitism occurs in mid-June (Kher et al., 2011). The second peak of parasitism by the recently emerged new generation adults occurs in mid-July (Kher et al., 2011). The species overwinters in the field as larvae inside CLB pupa buried in the soil (Dysart et al., 1973). Females prefer earlier larval stages and lay up to 6 eggs per CLB larvae, of which some enter diapause, and the rest emerge in July to parasitize late CLB larval instars, completing a second generation (Dysart et al., 1973; Gage & Haynes, 1975). Dosdall et al. (2011) reported 9.4, 17.8, and 32.8% CLB parasitism in Lethbridge, during 2007, 2008 and 2009, respectively. In summary, *T. julis* is the most successful parasitoid of CLB and is established in British Columbia, Alberta, Saskatchewan, Manitoba and Ontario, contributing substantially to CLB suppression.

2.5.3.b The larval parasitoid, *Lemophagus curtus*

Lemophagus curtus (Hymenoptera: Ichneumonidae) is a multivoltine, solitary larval parasitoid of CLB from Europe. This parasitoid was released in Michigan in 1971 (1,400 individuals). It was established in Michigan by 1972 (Stehr et al., 1974), but further recovery rates were low. In 1991, another study in Iowa and Missouri suggested that this species did not establish in these states (Bailey et al., 1991). Similarly, in a field survey, Philips et al. (2011) reported that the species was not present in Virginia and North Carolina in 2010. In a recent review, Roberts (2016) further reported that *L. curtus* failed to establish in Washington in 2004. These results suggest that *L. curtus* did not establish in most of the USA, and likely is not an important parasitoid of CLB in North America.

2.5.3.c The larval parasitoid, *Diaparsis carinifer*

Diaparsis carinifer (Hymenoptera: Ichneumonidae), a univoltine solitary larval parasitoid of CLB, was initially released in Michigan in 1967 and recovered in low numbers in 1970 (Stehr & Haynes, 1972). In 1971, the parasitism by *D. carinifer* was negligible in Michigan and suggested a relatively low impact on CLB control in North America (Dysart et al., 1973).

2.5.3.d The egg parasitoid, *Anaphes flavipes*

Anaphes flavipes (Hymenoptera: Mymaridae) is an important gregarious egg endoparasitoid of CLB in Europe (Dysart, 1971). The species was initially released in large numbers in 1967-1968 and it was established in the spring of 1968 in Michigan and Indiana (Maltby et al., 1971). The species did not establish in Washington, Oregon, Virginia and North Carolina despite many releases (Philips et al., 2011; Roberts, 2016; Walenta & Roberts, 2012). In Washington, the rate of parasitism increased to 30% in 2005 and dropped to 0% in 2006 and

2007. Therefore, the biological control program to establish it in the western United States was terminated (Roberts, 2016).

2.5.3.e Generalist predators

While numerous studies reported the impact of egg and larval parasitoids on CLB management, few studies have investigated the impact of generalist predators on CLB suppression. A field observation by Shade et al. (1970), investigating generalist predators of CLB, listed several lady beetle species (Coleoptera: Coccinellidae) including *Coleomegilla maculata* (De Geer, 1775), *Hippodamia convergens* Guerin-Meneville, *H. parenthesis* (Say, 1824) and *H. tredecimpunctata* (Linnaeus, 1758) as CLB egg predators. The same authors reported predation on CLB larva by *C. maculata* and *H. convergens*, suggesting a potential impact of lady beetles in CLB control. Similarly, Walenta and Roberts (2012) and Yun and Ruppel (1964) also reported *C. maculata* as a potential predator of CLB. However, CLB predation by lady beetles in Michigan was assumed to be negligible due to their low density, and considered to pose little or no competition for CLB parasitoids (Dysart et al., 1973). To date, there are no other published studies on the predation potential of generalist predators on CLB. Predators will have a more immediate impact on CLB populations since parasitoids do not cause immediate death and therefore CLB continue to cause damage to cereals following parasitization. As a result, predators should be more effective to reduce CLB damage to cereals in the same growing season. Generalist predators act as a key mortality factor of insect pests in various crops (Sunderland, 2002; Sunderland et al., 1997; Symondson et al., 2002); thus their impact on CLB suppression should be studied.

2.6 Use of molecular gut content analysis for identification of natural enemies of insect pests

The identification of natural enemies of most insect pest taxa is a demanding task and is important in ensuring the success of insect biological control, particularly conservation biological control (Kogan, 1998; Symondson et al., 2002). Several techniques, such as field observation (Cloutier & Bauduin, 1995; Culliney, 1986; Stuart & Greenstone, 1990), laboratory feeding trials (Grodén et al., 1990; Vankosky et al., 2011), enzyme-linked immunosorbent assay (Boreham & Ohiagu, 1978; Stuart & Greenstone, 1990) and, more recently, molecular gut content analysis (Greenstone et al., 2007; King et al., 2008), have been used to investigate how different trophic levels (natural enemies, insect pests, and plants) interact. In the mid-1980s, the development of a polymerase chain reaction (PCR) enabled accurate and fast biological diagnosis for human diseases such as Sickle Cell Anemia by targeting B-globin gene (Saiki et al., 1985). Soon after, entomologists started using various PCR based techniques to accurately screen predator species on specific pest taxa using molecular gut content analysis (King et al., 2008). Several species-specific primers for important pest taxa, such as Colorado potato beetle, *Leptinotarsa decemlineata* Say 1824 (Coleoptera: Chrysomelidae) (Greenstone et al., 2007), rape pollen beetles, *Meligethes aeneus* (Fabricius, 1775) (Coleoptera: Nitidulidae) (Oberg et al., 2011), codling moth, *Cydia pomonella* (Linnaeus, 1758) (Lepidoptera: Tortricidae) (Unruh et al., 2016), flea beetles, *Phyllotreta* spp. (Coleoptera: Chrysomelidae) (Ekbom et al., 2014), cereal aphid, *Rhopalosiphum* spp. (Hemiptera: Aphididae) (Chen et al., 2000), soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae) (Harwood et al., 2007), black bean aphid, *Aphis fabae* Scopoli, 1763 (Hemiptera: Aphididae) (Traugott & Symondson, 2008) and diamondback moth, *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae) (Ma et al., 2005), became

available and were applied to screen field-collected predators for evidence of predation on these species (Gariepy et al., 2007; Symondson et al., 2002). Several species-specific primers have been designed for important generalist predators such as lady beetles (Coleoptera: Coccinellidae) *Harmonia axyridis* (Pallas, 1773) (Harwood et al., 2007), *Coccinella septempunctata*, *Coleomegilla maculata*, *Propylea quatuordecimpunctata* (Linnaeus, 1758) (Gagnon et al., 2011), *Adalia bipunctata* (Linnaeus, 1758) (Rondoni et al., 2015), *Oenopia conglobata* (Linnaeus, 1758) (Rondoni et al., 2015), and several bugs (Hemiptera) such as *O. insidiosus* (Say, 1832) (Anthocoridae) (Harwood et al., 2007), *Podisus maculiventris* (Say) (Pentatomidae) (Greenstone et al., 2007), *Perillus bioculatus* (Fabricius) (Pentatomidae) (Greenstone et al., 2010). The goal of these studies has been to test for evidence of intraguild predation among these predators, which can compromise their potential in biological control. Similarly, species-specific primers have also been designed for parasitoids such as *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Aphidiidae) (Traugott & Symondson, 2008), *Ephedrus plagiator* (Nees, 1811), and *Aphidius ervi* Haliday (Hymenoptera: Braconidae) and *Dendrocerus carpenter* (Curtis) (Hymenoptera: Megaspilidae) (Traugott et al., 2012) to screen predators for the evidence of predation on parasitized larvae. To date, no study has provided the molecular tools required to screen generalist predators for the evidence of predation on CLB.

2.7 Landscape structure effects on herbivore control in agroecosystems

Agricultural intensification has led to loss of habitat diversity due to simplification of landscapes through increasing crop field sizes, use of monocultures, and loss of natural habitats in an aim to meet food demands required by human population growth (Landis, 2017; Vankosky et al., 2017). Ecological intensification is an alternative approach to increase crop yield and production while minimizing the negative effects on the environment (Bommarco et al., 2013).

This approach relies on maximizing ecosystem services delivered by biodiversity within agricultural fields (Cassman, 1999). Several studies suggest that insect diversity is influenced by both agricultural landscape complexity and local management practices (Altieri, 1999; Tscharntke et al., 2005). Biodiversity loss can negatively affect insect biological control and crop pollination services (Bianchi et al., 2006; Crowder & Jabbour, 2014; Landis et al., 2000; Potts et al., 2010). Biological control is defined as the use of living organisms (e.g., predators and parasitoids) to suppress the population of a specific pest organism with the ultimate goal to make them less damaging (Hajek & Eilenberg, 2018). It is an important ecosystem service, which has a strong impact on the populations of insect herbivores in agricultural landscapes (Symondson et al., 2002). However, excessive application of pesticides and fertilizers due to agricultural intensification can negatively affect insect biological control, crop pollination and several other ecosystem services such as soil productivity (Bommarco et al., 2013; Desneux et al., 2007; Devine & Furlong, 2007), and reduce the resilience of the agricultural systems. Thus, it is important to understand how agricultural intensification at the landscape level can affect the abundance of herbivores and their natural enemies, and further influence their interaction in agricultural landscapes, to determine its consequences for insect biological control services.

Complexity at the local (e.g., polycultures, addition of flower strips) and landscape level (e.g., high proportion of semi-natural habitats, high habitat diversity) may increase the abundance of natural enemies by the provision of alternative prey/hosts, overwintering habitats, refuge from disturbance, and alternative food resources (e.g. nectar, pollen) (Bianchi et al., 2006; Gurr et al., 2017; Landis, 2017; Landis et al., 2000; Rusch et al., 2016). Complex landscapes with a high proportion of semi-natural habitats can provide numerous advantages for natural enemies, promoting their efficiency, and can also be adverse to insect herbivores (Tscharntke et

al., 2012). These advantages include the build-up of a resilient meta-population for natural enemies that can spillover from semi-natural habitats to adjacent crop fields, resulting in biological control (Tscharnkte et al., 2012). In a meta-analysis, Letourneau et al. (2011) confirmed the positive impacts of local habitat diversity on herbivore suppression and natural enemy abundance, with 54% higher pest mortality and 44% higher natural enemy abundance in the highly diversified cropping systems relative to the simple cropping systems. Similarly, Tschumi et al. (2015) found a higher natural enemy number and lower CLB adult and larval density in winter wheat fields adjacent to flower strips, suggesting that local scale complexity is an important factor promoting CLB biological control. However, a recent study by Pollier et al. (2019) indicated that addition of wildflower strips in the vicinity of wheat fields did not affect the abundance of CLB, although it increased the abundance of natural enemies and aphid biological control in these fields. At the landscape level; Bianchi et al. (2006) reported that in 74% of reviewed studies natural enemy abundance was higher in complex landscapes (high proportion of semi-natural habitats), which resulted in lower pest pressure in 45% of the studies. These findings are potentially explained by high proportion of croplands in simple landscapes (reduction in the area of semi-natural habitats in these landscapes), which can be adverse to natural enemies due to disturbances such as pesticide applications, tillage, the lack of alternative foods and overwintering sites (Bianchi et al., 2006; Landis et al., 2000). These reviews are in line with a recent meta-analysis by Rusch et al. (2016) who reported a consistent negative association between landscape simplification and aphid biological control, with simple landscapes having 46% lower aphid control relative to complex landscapes. However, another meta-analysis, Chaplin-Kramer et al. (2011) showed that despite a general increase of natural enemies in complex landscapes there was not a significant effect on herbivores. Moreover, Karp et al.

(2018) analyzed a dataset of 132 studies across 31 countries and reported inconsistent trends of associations between the proportion of semi-natural habitats in the landscape and insect biological control. They concluded that the effect of natural habitats is not always positive on insect pest control and should be considered system- and species-specific. Moreover, semi-natural habitats may act as reservoirs for herbivores to colonize surrounding crop fields, which might result in higher pest pressure in crop fields (Van Emden, 1964). Also, Phillips & Gardiner (2016) reported higher spotted cucumber beetle, *Diabrotica undecimpunctata* (Linnaeus, 1758) (Coleoptera: Chrysomelidae) predation in simple landscapes and suggested that higher habitat complexity was negatively associated with predation. Tscharrntke et al. (2016) suggested several potential explanations behind the failure of natural and semi-natural habitats to promote biological control. These included the potential for insufficient amounts of natural habitats or being too far from agricultural fields to support natural enemy populations, or low quality habitats due to drought that cannot provide food resources for natural enemies. Further, they linked this lack of associations to the absence of effective natural enemies in the landscape or in cases that crops can provide more resources for natural enemies than natural habitats. Further, they suggested that natural habitats can occasionally provide a better food source for herbivores than natural enemies. Altogether, these studies suggest that although diversification at the landscape and local scales is a key to increase natural enemy abundance and diversity, it may not necessarily result in increased herbivore control. Therefore, it is important to determine how landscape complexity affects CLB abundance and parasitism to increase CLB biological control in agricultural landscapes.

2.8 Landscape composition effects on herbivore abundance in agroecosystems

Landscape simplification has resulted in an increase in the area of suitable crops for various insect herbivores, which can result in increased herbivore abundance in agricultural landscapes, as suggested by the resource concentration hypothesis (Root, 1973; Veres et al., 2013). Based on this hypothesis, herbivores are likely to find and stay in patches with a high abundance of their suitable hosts and achieve high overall abundance in these patches (Root, 1973). For instance, Riggi et al. (2017) reported higher abundance of rape pollen beetle, *M. aeneus* in a landscape dominated by oilseed rape crops, supporting the resource concentration hypothesis. Similarly, Rand et al. (2014) found that landscapes dominated by wheat (suitable hosts) increased the infestation of wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae) in wheat fields, potentially due to higher connectivity between suitable host patches within these landscapes. By contrast, a higher proportion of suitable hosts in the landscape may dilute herbivore abundance due to their dispersion within available suitable host patches (Tscharntke et al., 2012). Earlier studies in the same rape pollen beetle system provide support for the dilution hypothesis, where a high proportion of oilseed rape crops in the landscape reduced the abundance of rape pollen beetles (Scheid et al., 2011; Schneider et al., 2015; Zaller et al., 2008). A review by Veres et al. (2013) reports the species-specific response of different herbivores to the proportion of their suitable hosts in the landscape. They found that out of 18 studies, six found no significant responses, seven found positive responses (resource concentration effect) and five found negative responses (dilution effect) to the proportion of suitable hosts in the landscape, suggesting that herbivore response is species-specific. Kunin (1999) linked the contrasting response of herbivore to the proportion of suitable hosts to their range of accepted foods, biology, fecundity, and dispersal capabilities. For example, specialist

herbivores are more likely to respond to the area with host plants in the landscape than generalist herbivores (resource concentration hypothesis). As CLB is a specialist pest of cereals, it is important to determine if this species will respond to the proportion of cereals in the landscape to develop effective habitat management strategies in western Canada.

2.9 Landscape configuration effects on herbivores and their natural enemies

The spatial distribution of habitats within landscapes (landscape configuration) may affect herbivores and natural enemy abundance due to changes in habitat connectivity (Berger et al., 2018; Boiteau et al., 2008; Fahrig et al., 2011; Woltz & Landis, 2014; Zaller et al., 2008). Increased habitat fragmentation resulting from the conversion of natural habitats into agricultural fields or urban habitats affects the flow of resources and energy between and within habitat patches (Rutledge, 2003). Habitat fragmentation can result in lower mean patch size, increased number of patches, and an increased amount of border between patches (Rutledge, 2003). The responses of herbivores to habitat fragmentation varied from positive to negative. Some species respond positively to fragmentation due to shorter distances and ease of movement between habitats, which can increase resource complementation (Fahrig, 2003; Fahrig et al., 2011). For example, Berger et al. (2018) found a higher occurrence of cabbage stem flea beetle, *Psylliodes chrysocephala* (Linnaeus, 1758) (Coleoptera: Chrysomelidae) and minute seed weevil, *Ceutorhynchus erysimi* (Fabricius) (Coleoptera: Curculionidae) in oilseed rape fields (canola in Canada) close to the forest and concluded that increasing distance from the forest could reduce herbivore abundance in oilseed rape fields. By contrast, Zaller et al. (2008) found that the abundance of rape pollen beetles was positively associated with the isolation of its suitable host (oilseed rape) in the landscape. Other species are negatively affected by the increased amount of field borders that may act as barriers for movement, and by small patch sizes that are insufficient

to hold local populations (Fahrig, 2003). Finally, Woltz and Landis (2014) found a high abundance of lady beetles in landscapes with small field sizes (configuration) and a high proportion of semi-natural habitats (composition), suggesting that both landscape composition and configuration are affecting lady beetle abundance in agricultural landscapes. Altogether, these studies suggest that configurational heterogeneity is an important component of landscape structure that can contribute to explaining variation in the abundance of herbivores and their natural enemies in agricultural landscapes, although patterns of response to these factors are species-specific.

2.10 Landscape and local scale complexity effects on parasitism

Parasitoid impacts on insect pests are affected by agricultural landscape complexity (Thies et al., 2005; Thies et al., 2003; Thies et al., 2008; Thies & Tscharnkte, 1999), local scale complexity (Tscharnkte & Kruess, 1999; Zaller et al., 2009), and host density (Gunton et al., 2016). Plecas et al. (2014) found a positive association between landscape complexity and aphid parasitism in two out of four years of their study, suggesting temporal variation in the response of parasitoid to the landscape complexity. Similarly, Grab et al. (2018) found lower tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae) parasitism by *Peristenus* wasps (Hymenoptera: Braconidae) in strawberry fields within simple landscapes dominated by agricultural fields (e.g., landscapes with low proportion of semi-natural area), suggesting that semi-natural habitats may provide parasitoids with important resources (nectar) that elevate their levels of parasitism. Zaller et al. (2009) reported that higher crop abundance, pollen beetle abundance, and length of the roadside ditch were positively associated with rape pollen beetle parasitism at the 250 m spatial scale, suggesting that local scale complexity is important in explaining herbivore abundance in oilseed rape fields. In the same system, Thies et al. (2003)

and Rusch et al. (2011) found that landscapes with a higher proportion of semi-natural area at the 1.5 - 2 km spatial scales increased parasitism of oilseed rape pollen beetle, most likely due to the provision of overwintering sites, undisturbed habitats, alternative hosts, and sugar resources for parasitoids. Schneider et al. (2015) did not find such a positive association between pollen beetle parasitism and the proportion of semi-natural habitats, suggesting that landscape complexity did not promote parasitism of rape pollen beetle. However, the authors acknowledged that their study included relatively few landscapes with semi-natural habitats and this may have prevented them from detecting any strong positive associations. In a different system, Rand et al. (2014) also did not find any associations between the proportion of semi-natural habitats in the landscape and wheat stem sawfly parasitism in wheat fields. In an extensive review, Karp et al. (2018) found that natural enemy response to the semi-natural area in the landscape is inconsistent, with an almost equal number of negative and positive responses. These authors used data collected from 31 countries across the world, which makes this study a strong contribution to understanding the role of semi-natural habitats in herbivore suppression. These results suggest that semi-natural habitats may not always promote parasitism, and its effect depends on species, regions, and the quality of semi-natural habitats (Tscharrntke et al., 2016).

Parasitoids show variable density-dependent responses to their hosts (Gunton et al., 2016). For instance, the two braconid parasitoids in a field study by Costamagna et al. (2004) responded differently to larval densities of *P. unipuncta* on corn plants. While, *Glyptapanteles militaris* (Walsh) parasitized more larvae when host density was low, *Meteorus* spp. parasitized more *P. unipuncta* larvae under high host densities, suggesting negative density-dependent and positive density-dependent responses, respectively. Kher et al. (2014) reported that *T. julis* positively responded to the density of CLB in wheat patches, where patches with higher CLB

density had a higher percentage of parasitism. This result suggests that CLB density in cereal fields can be an important factor explaining its parasitism in agricultural landscapes. Therefore, landscape complexity might indirectly alter insect parasitism in agricultural landscapes due to its direct effect on herbivore density that triggers lower or higher levels of parasitism.

2.11 Objectives of the research

The objectives of my studies were to identify and assess the relative importance of natural enemies in CLB management in cereal fields and the influence of landscape on CLB and *T. julis*. I investigated (1) how landscape complexity affects the abundance of CLB and its percentage parasitism, (2) how the proportion of suitable hosts in the current and previous years affects CLB abundance and parasitism, (3) how CLB density in cereal fields affect its parasitism, (4) what predator species predate CLB immature stages under laboratory conditions, and (5) what predator species predate CLB under field conditions. For this last objective, I developed novel species-specific primers to detect CLB DNA in the gut content of field-collected predators.

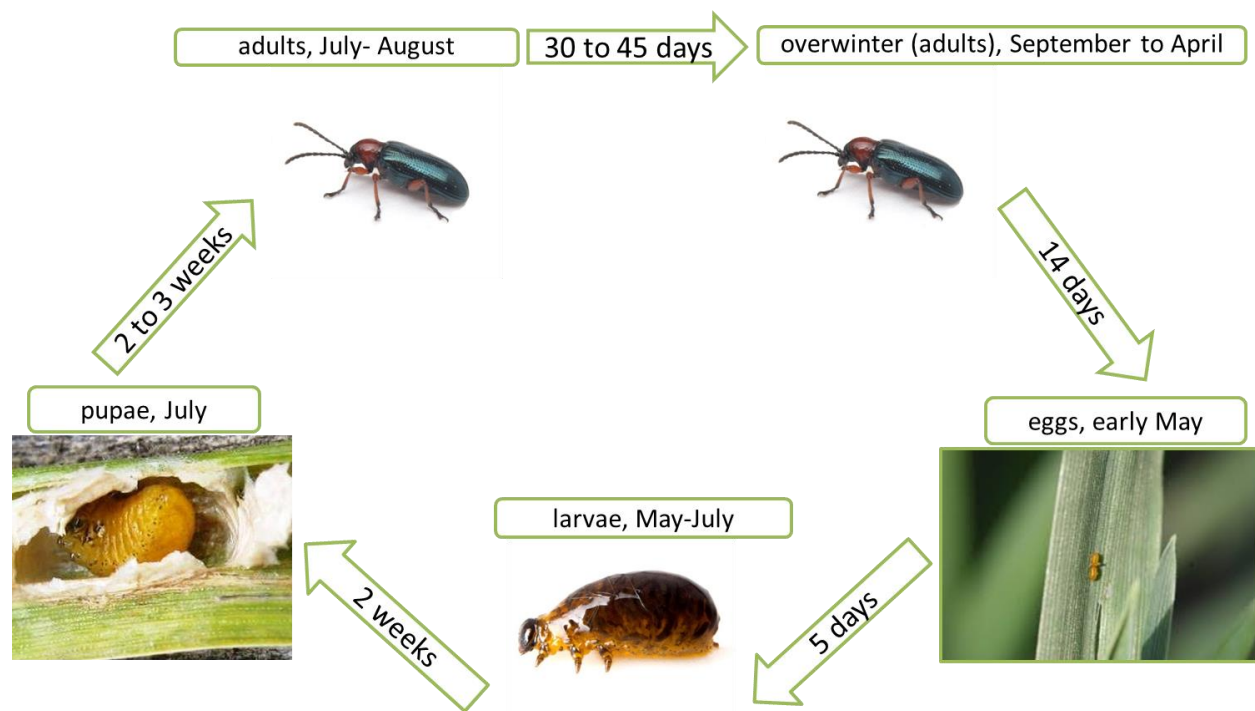


Figure 1. Cereal leaf beetle (CLB) life cycle in southern Alberta, Canada. Photos: Aldo Rios Martinez (CLB larvae and adult), and John Gavloski (CLB pupa).

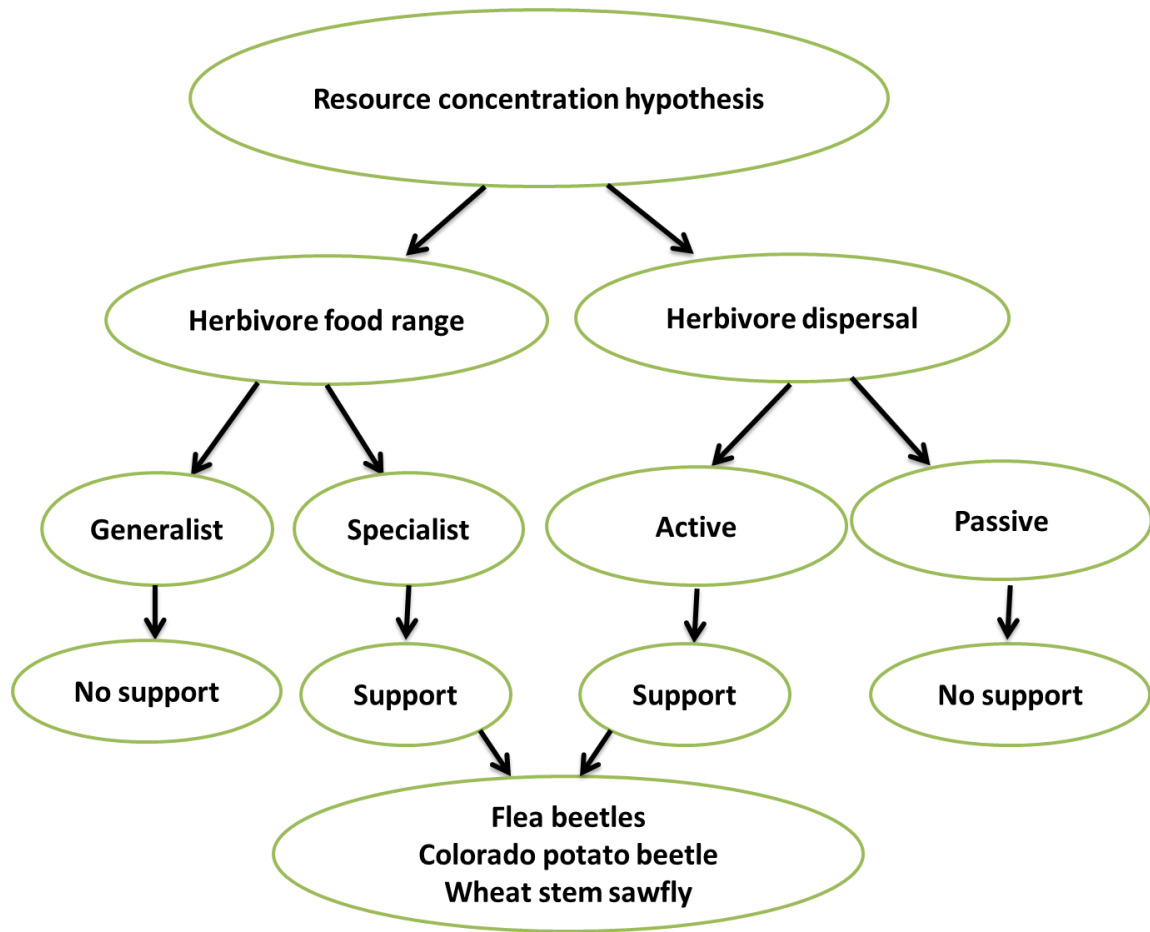


Figure 2. Predictions of the resource concentration hypothesis related to herbivore food range (host specificity) and dispersal.

CHAPTER 3

Effects of major host crops and landscape diversity on the cereal leaf beetle, *Oulema melanopus* L. (Coleoptera: Chrysomelidae) and its parasitism by *Tetrastichus julis* (Walker) (Hymenoptera: Eulophidae)

In this chapter, the association between landscape structure and abundance of CLB and parasitism by the parasitoid *T. julis* is addressed. Further, the associations between the proportion of cereals in the previous year's landscape and abundance of CLB in the study year are examined. The associations between landscape variables and CLB abundance and its parasitism are reported at various scales, and the potential mechanisms behind the significant variables are discussed.

3.1 ABSTRACT

Landscape complexity has direct and indirect effects on herbivores in agroecosystems, but effects on pest control services are variable. Carryover effects of landscape composition in previous years on the abundance and parasitism of herbivores has been seldom assessed. In this study, I investigated the effect of agricultural landscape complexity on cereal leaf beetle (CLB) *Oulema melanopus* L. (Coleoptera: Chrysomelidae) and its parasitism by the introduced parasitoid *Tetrastichus julis* (Walker) (Hymenoptera: Eulophidae). During 2014 and 2015 I assessed CLB abundance and parasitism by *T. julis* in 54 wheat fields located in landscapes ranging from high (more than 50% of semi-natural habitats) to low (less than 30% of semi-natural habitats) complexity. My hypothesis was that landscape complexity affects CLB abundance and its parasitism in agricultural landscapes. My predictions were 1) landscapes with high proportions of host crops increase CLB abundance in wheat fields, 2) landscapes with high proportions of semi-natural habitats increase CLB parasitism, and 3) landscapes with high proportions of overwintering habitats increase CLB abundance in wheat fields. The primary semi-natural habitats were pasture, grasslands, and woodlands. The crops included were CLB major plant hosts (wheat and barley), minor plant hosts (corn, rye, and oats), and non-cereal crops, such as canola, and potato. The proportion of CLB major hosts had a positive association with CLB abundance and parasitism at multiple spatial scales (buffers of 0.5 - 2 km radius), supporting the resource concentration hypothesis. The proportion of cereals in the previous year was also positively associated with current CLB abundance, but not with parasitism. Cereal leaf beetle and its parasitism were negatively associated with the proportion of pasture (a major semi-natural habitat). CLB parasitism increased in landscapes with higher proportion of canola and alfalfa in low CLB abundance years. Cereal leaf beetle parasitism was positively associated with

CLB abundance in cereal fields, suggesting a density-dependent response by *T. julis*. My results suggest that host crop area in the current and previous years increases the abundance of a specialist herbivore, while parasitism increases also in response to other crops in the landscape that provide additional resources.

Keywords: landscape composition, semi-natural habitat, resource concentration hypothesis, natural enemy hypothesis

3.2 Introduction

Landscape complexity has a major effect on the populations of herbivores, natural enemies, and their interactions (Bianchi et al., 2006; Chaplin-Kramer et al., 2011; Tschamntke et al., 2007). Initial studies suggested that complex landscapes (typically defined by a high proportion of semi-natural habitat) enhance the abundance of natural enemies by providing alternative foods, overwintering sites, and refuge from insecticides, which in turn increase herbivore suppression in agricultural landscapes (Bianchi et al., 2006; Landis et al., 2000). Landscape simplification and increased agricultural intensity reduce semi-natural habitats and increase disturbance, and has been associated with declines in pest control services (Grab et al., 2018; Rusch et al., 2016). However, recent work suggests a less clear association between landscape complexity and herbivore suppression. The Chaplin-Kramer et al. (2011) meta-analysis suggested positive associations between landscape complexity and natural enemy abundance, predation, and parasitism rate, but no strong direct association with insect herbivore abundance. Using data from 31 countries, Karp et al. (2018) reported inconsistent associations between pest control and semi-natural habitats in the surrounding landscapes, suggesting that the semi-natural habitats in the agricultural landscape do not always enhance biological control of herbivores. Furthermore, some crops, including alfalfa (Costamagna et al., 2015) and wheat

(Samaranayake & Costamagna, 2018), and crop diversity (Redlich et al., 2018) have been associated with increased pest control services, suggesting that crop habitats may also function as temporary sources of natural enemies (Schellhorn et al., 2015).

Landscape complexity affects herbivore abundance directly by providing them with resources (Denno et al., 2005; Maisonhaute et al., 2017) and is often explained in terms of the resource concentration hypothesis (Root, 1973). According to this hypothesis, a low habitat diversity with the dominance of a suitable host in the landscape increases herbivore abundance due to a higher probability of finding and remaining in the host crop (Root, 1973; Tscharncke et al., 2012). For example, Rand et al. (2014) reported a higher infestation of the wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae) in landscapes dominated by wheat. They attributed it to the availability of suitable host, which increased the connectivity of suitable habitat in these landscapes. Alternately, a higher proportion of suitable hosts might decrease the abundance of herbivores due to a dilution effect (Tscharncke et al., 2012). In this case, herbivores spread out throughout suitable crop patches, diluting their abundance (Tscharncke et al., 2012; Veres et al., 2013). For instance, Schneider et al. (2015) found a lower abundance of rape pollen beetle, *Meligethes* spp. (Coleoptera: Nitidulidae) in agricultural landscapes dominated by oilseed rape crops. Finally, a literature review by Veres et al. (2013) reported no clear trends in the response of herbivores to high proportions of suitable crops in the landscape. The species-specific response of herbivores to their primary hosts has been related to their host range, their biology, and their dispersal potential (Kunin, 1999; Poveda et al., 2012; Rand et al., 2014). These studies suggest that effects of landscape complexity on host-parasitoid systems are species and region-specific and require local study.

Several studies have reported indirect effects of landscape complexity on herbivores through its effects on natural enemies (Bianchi et al., 2006; Chaplin-Kramer et al., 2011). Thies et al. (2003) reported positive associations between the proportion of semi-natural area at 1 to 2 km spatial scales and pollen beetle parasitism, and Grab et al. (2018) found a high rate of tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae) parasitism in strawberry fields within landscapes with a high proportion of semi-natural habitats at the 0.5 km spatial scale. Other studies, however, have reported that parasitoid responses to landscape complexity are species-specific. Zaller et al. (2009) indicated contrasting responses of stem weevil (*Ceutorhynchus* spp.; Coleoptera: Curculionidae) and rape pollen beetle parasitoids to roadside strips and hedges at the 0.25 and 0.5 km spatial scales. Other studies found no clear associations between landscape complexity and parasitism. For example, Costamagna et al. (2004) reported a lack of associations between parasitism of the true armyworm, *Pseudaletia unipuncta* (Haworth) (Lepidoptera: Noctuidae) and landscape complexity, although studies in previous years showed significant patterns (Menalled et al., 2003). Altogether, these studies suggest that semi-natural habitats in the landscape are not always associated with a higher rate of parasitism in agroecosystems.

Carryover effects based on host crop area in the landscape from previous years have been positively associated with the abundance of specialist herbivores and their parasitoids (Beduschi et al., 2015; Berger et al., 2018), but this has been poorly studied. Berger et al. (2018) reported a positive association between the proportion of previous year's oilseed crops and *Lygus rugulipennis* Poppius (Hemiptera: Miridae) abundance. Huseth et al. (2012) reported positive associations between distance to the previous year's potato fields and Colorado potato beetle *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) abundance in current potato

fields, with closer distances having higher infestation rate. However, studies of pollen beetle parasitism have reported inconsistent associations with the proportion of previous year's oilseed host crops: positive (Beduschi et al., 2015), neutral (Riggi et al., 2017), and negative (Rusch et al., 2011). Positive associations result from overwintering of herbivores in and around suitable host fields that allows herbivore population build-up (Boiteau et al., 2008; Huseth et al., 2012), overwintering of parasitoids within host crop fields (Beduschi et al., 2015), and spillover to recently planted host crops in the following year. These studies suggest that previous year's host crops may be important in explaining herbivore abundance and parasitism rates in agricultural landscapes and this effect vary among different species.

The cereal leaf beetle, *Oulema melanopus* L. (Coleoptera: Chrysomelidae) (CLB), is a globally important cereal specialist pest (Philips et al., 2011). In North America, yield losses up to 23% and 49% due to CLB injury have been reported on winter and spring wheat, respectively (Gallun et al., 1967; Webster et al., 1972). In southern Alberta, CLB produces one generation per year and is active between April and September with oviposition peaking in mid-May (Kher et al., 2011). Cereal leaf beetle larvae are present from May to July with a peak in mid-June, and complete four instars on the same cereal plant before dropping to the soil for pupation (Kher et al., 2011). Cereal leaf beetle overwinters as an adult in cereal field border grasses and nearby woodlands including tree bark woods, sparse woods and rolled leaves (Casagrande et al., 1977; Philips et al., 2011). Soon after the accidental introduction of CLB in the United States, a biological control program against its immature stages started in Michigan. *Tetrastichus julis* (Walker) (Hymenoptera: Eulophidae), a larval parasitoid of CLB was imported from Europe and established in several counties in Michigan, in 1973 (Logan et al., 1976). Currently, *T. julis* is recognized as the most efficient introduced larval parasitoid of CLB, with up to 60% and 33%

parasitism reported in Utah and Alberta, respectively (Dosdall et al., 2011; Dysart et al., 1973; Evans et al., 2006). In Canada, *T. julis* is active from early May to August, and peak parasitism occurs in mid-June (Kher et al., 2011). Unlike CLB that overwinters in semi-natural habitats surrounding cereal fields, *T. julis* overwinter as larvae inside CLB pupa within cereal fields (Dysart et al., 1973), and therefore cereal fields from the previous year can act as sources of *T. julis*. Understanding how landscape complexity influences CLB abundance and its parasitism is essential to implement a successful management strategy for CLB.

In this study, I investigated the direct and indirect effects of landscape complexity in the current and previous years on CLB abundance and its parasitism by *T. julis*. The objectives of this study were to investigate (1) the association between agricultural landscape complexity and the abundance of CLB and its parasitism, (2) the association between the proportion of cereal fields in the previous year on the current abundance of CLB and its parasitism, (3) the association between abundance of CLB and its parasitism. My hypothesis was that the percentage of crops and habitats in the landscape affect CLB abundance and its parasitism by *T. julis* in wheat fields. My predictions were: 1) given the resource concentration hypothesis, CLB abundance would be higher in landscapes with a high proportion of cereals, 2) given the natural enemy hypothesis, CLB parasitism would respond positively to the proportion of semi-natural habitats in the landscape, and 3) a higher proportion of cereals in landscapes in the previous year would increase the abundance and parasitism of CLB due to a spillover of individuals from nearby habitats.

3.3 Materials and methods

3.3.1 Study sites

During 2014 and 2015, 27 wheat fields were sampled each year, near the towns of Warner, Lethbridge, Taber, and Vauxhall in southern Alberta (Fig. 3). Landscape composition ranged from 2 - 77% of semi-natural habitats, a proxy for landscape complexity. The distance between these towns was 50 to 90 km, and the minimum distance between wheat fields was 4 km (except in 3 cases in which fields were separated by approximately 1 km). Except in four cases, fields sampled in 2015 did not overlap with those sampled in 2014, to prevent potential spatial correlation between years. Most fields were entire quarter sections (i.e. ~ 64.7 ha) and none were treated with insecticide in both years.

3.3.2 Cereal leaf beetle abundance and parasitism levels

CLB abundance and parasitism levels were assessed by collecting six sweep-net samples per field at least 50 m from a corner. Three of the samples were collected along a transect 20 m from the field border, and the other three samples were collected along a transect 100 m from the field border. Each sample comprised 50 × 180°-sweeps while walking. The contents of each sample were stored inside individual Ziploc plastic bags with 25% EtOH until sorting. Wheat fields were sampled during mid-June to coincide with peak CLB larvae and *T. julis* numbers. Cereal leaf beetle larvae were counted in each sample, and CLB abundance (CLB per 50 sweeps) was calculated as an average of the six samples per field. To determine percentage parasitism, up to 10 larvae from each sample were randomly selected and dissected under a microscope (10 x), for a minimum and maximum of 5 and 60 larvae per field, respectively. The mean percentage of parasitism was calculated for each of the six sampling points and the whole field. In 2014, 235 CLB larvae were reared from subsets of 10 random individuals from each field, and only *T. julis*

(n= 457) emerged. Thus, parasitism assessed by dissections of CLB larvae was attributed to this species.

3.3.3 Landscape quantification

I conducted detailed ground surveys of all crops and habitats within four circular sectors ranging from 0.5 to 2 km radius from the focal field, as recommended by Thies et al. (2003). I identified 26 land cover types and merged them into 12 categories for analysis based on their relative importance as hosts for CLB and their proportion in the landscape (Appendix A). Wheat was analyzed as a separate variable, but also merged with barley into the “major hosts” variable in alternative models. Although oat is a primary host of CLB, it is rarely grown in the study area (present in two out of 75 landscapes) and was therefore merged with corn and rye in the “minor hosts” category (Appendix A). All other crops that were not cereals were merged into a non-cereal crop category with the exception of canola and alfalfa in parasitism models; this crop remained a separate land-cover type due to its potential role in providing nectar to parasitoids (Idris & Grafius, 1997). Pasture was the most abundant semi-natural habitat in my study area and was analyzed as a separate variable. Field border grass and fallow field (fields that remained unseeded for one growing season) land cover types were merged into the grassland category. Trees and riparian vegetation were merged into a woodland category. Base geo-referenced maps were obtained from Alberta Soil Information Viewer website ([http://www1.agric.gov.ab.ca/\\$department/deptdocs.nsf/all/sag10372](http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/sag10372)). The proportion of different land cover types and landscape complexity metrics, including Simpson’s index of habitat diversity, richness, and evenness (Simpson, 1949), were estimated using digital maps made in ARC GIS 10 (ArcGIS, 2014) and using the Vegan package (Oksanen et al., 2015).

3.3.4 Data analysis

Mixed effect models were used to evaluate the effect of distance from the field border (20 m versus 100 m from the field border) on CLB abundance and parasitism, with sample location as a fixed factor nested in field, which was considered a random factor. Since no difference was found due to location (see results), only one estimate was used per field for further analyses. CLB abundance (average number of CLB/50 sweeps) and percentage parasitism were modeled with generalized linear models using negative binomial distributions to account for the over-dispersion observed in models using Poisson distributions and non-normality of residuals. The explanatory variables were the proportion of pasture, CLB major hosts (or wheat separately), non-cereal crops, CLB minor hosts, grassland, woodland, and habitat diversity (Appendix A). A categorical variable “year” and its interaction with other predictor variables was included in all models. Prior to performing model selection, Pearson correlation tests were used to detect potential multicollinearity among independent variables. Separate models were performed for each spatial scale (0.5, 1, 1.5, and 2 km radius). Variance Inflation Factor (VIF) values were calculated for the independent variables in the final models using the Car package (Fox & Weisberg, 2018), excluding variables with $VIF > 4$ to avoid multicollinearity (Crawley, 2013). Simplified models using a single semi-natural habitat variable (woodland + grassland + pasture) were constructed, but were not significant and were not explored further. The effect of cereal crops in the previous year on CLB abundance and percentage of parasitism was investigated using similar models. Only the major and minor host variables were included in these analyses since the proportion of semi-natural habitats in the landscape was generally similar between years. To avoid a biased estimation of parasitism, percent parasitism was analyzed in fields with >5 CLB larvae / field (2014, $n = 23$; 2015, $n = 16$). Stepwise AIC was used to determine the

best-fitted models (models with the lowest AIC value) using the Mass package (Venables & Ripley, 2003). Competing models ($\Delta i < 3.5$) are presented for each scale (Burnham et al., 2011). Pseudo-R squared values were estimated by comparing the null model that included only the intercept with models that included various independent variables, using the R Companion package (Mangiafico, 2017). Partial correlations were calculated to assess the association between dependent and independent variables using the rsq package (Agresti, 2012). All statistical analyses were performed in R (version 3.3.5, R Development Core Team, 2017).

3.4 Results

3.4.1 Cereal leaf beetle response to landscape composition in the current year

Average CLB larval abundance was higher in 2014 (25.2 ± 10.8 larvae/ sample, mean \pm SEM, $n = 27$ fields) than in 2015 (8.2 ± 2.33 larvae/ sample, $n = 27$ fields), resulting in significant overall and interacting effects of year of study with landscape variables in various models (Table 1, Fig. 4e). CLB abundance was similar at 20 and 100 m from the field border ($F_{1,57} = 0.59$, $P = 0.45$), and therefore, both locations were combined for further analyses. The model with the strongest support (model 6, Table 1) indicated positive associations between CLB abundance and proportion of wheat, minor hosts, woodland, and non-cereal crops at the 2 km scale (Table 1, Fig. 4a-4d). These four cover types had consistent positive associations with CLB abundance at various spatial scales (Table 1). The major hosts variable resulted in a similar model with considerable support at the 2 km radius scale (model 7, Table 1), but not at other scales. CLB abundance was negatively associated with the proportion of pastures at the 1.5 km and 2 km scales (models 5 & 8, Table 1) and positively to the proportion of grassland at the 0.5 km scale (model 1, Table 1). Habitat diversity showed a negative association with CLB abundance at the 0.5 km radius scale (model 1, Table 1). Habitat diversity was inconsistently correlated with the

proportion of semi-natural habitats ($r = -0.41, -0.03, 0.27, 0.46, P = 0.004, 0.04, 0.14, 0.005$) and crops ($r = +0.37, +0.02, -0.32, -0.54, P = 0.005, 0.98, 0.01, 0.001$) at the 0.5, 1, 1.5 and 2 km spatial scales, respectively. Percentage parasitism was negatively associated with CLB abundance at the 0.5, 1 and 1.5 km scales (Table 1).

3.4.2 Cereal leaf beetle response to cereal crops in the previous year

There were positive associations between the proportion of non-cereal crops in the study year and the proportion of wheat in the previous year at all scales ($r = 0.42, 0.46, 0.50, \text{ and } 0.45$, at the 0.5, 1, 1.5 and 2 km spatial scales, respectively; all $P < 0.01$). The proportion of major hosts during the previous year had consistent positive associations with CLB abundance at all scales (models 9-12, Table 1, Fig. 5a). In contrast, the proportion of minor hosts in 2013 was negatively associated with CLB abundance in 2014, but the proportion of minor hosts in 2014 was positively associated with CLB abundance in 2015 at all tested scales (models 9-12, Table 1, Fig. 5b).

3.4.3 Parasitism response to landscape complexity in the study year

CLB mean percentage parasitism was lower in 2015 (32.3 ± 5.39 , $n = 16$ fields) than in 2014 (55.7 ± 5.17 , $n = 23$ fields), which resulted in interacting effects of year of study with various landscape variables (models 1-6, Table 2, Fig. 6e). CLB parasitism was similar at 20 and 100 m from the field border ($F_{1, 42} = 0.01, P = 0.91$), and therefore, both locations were combined for all subsequent analyses. The model most supported by the data (model 4, Table 2) at the 2 km scale indicated no associations between the proportion of wheat, alfalfa, canola and CLB parasitism in 2014 (a high CLB year). However, the same model indicated positive associations between these three land cover types and CLB parasitism in 2015 and positive associations with the proportion of non-cereal crops in both years (Fig. 6). Similar associations were found at

smaller spatial scales (models 1-6, Table 2). CLB parasitism had positive associations with the proportion of major hosts and negative associations with the proportion of pasture at various scales only in 2015 (models 1, 3, 5 & 6, Table 2). CLB parasitism in 2015 was positively associated with CLB abundance at the 0.5 - 1.5 km spatial scales (models 1-2 & 3, Table 2).

3.4.4 Parasitism response to the landscape in the previous year

There was a non-significant positive trend of association between CLB major hosts in 2014 and CLB parasitism in 2015 (Pseudo- $r^2 = 0.18$, $P = 0.12$; DF = 34, Log-likelihood = -350.8).

3.5 Discussion

This is the first study on the effects of landscape complexity on CLB abundance and percent parasitism in agricultural landscapes. My results suggest both direct and indirect effects of landscape complexity on abundance of CLB. A direct effect was due to the positive response of CLB to the proportion of host crops (major and minor cereal hosts) in the landscape at multiple scales, both in the current and previous year, which supports the resource concentration hypothesis. The indirect effect results from increased parasitism in landscapes with a higher proportion of CLB major hosts, canola and alfalfa. These results highlight the need to study the functional role of different crops and habitats and their contributions to pest control services (Fahrig, 2017; Veres et al., 2013).

3.5.1 Direct effects of landscape complexity and parasitism on cereal leaf beetle abundance

The proportion of cereal crops in landscapes surrounding focal fields was positively associated with CLB abundance at 2 and 1.5 km scales, providing support for the resource concentration hypothesis. Other specialist herbivores, such as the crucifer flea beetle, *Phyllotreta cruciferae* (Goeze) (Root, 1973), and Colorado potato beetle, *Leptinotarsa decemlineata* (Say)

(Boiteau et al., 2008) (Coleoptera: Chrysomelidae), the Guatemalan potato moth, *Tecia solanivora* (Povolny) (Lepidoptera: Gelechiidae) (Poveda et al., 2012) and wheat stem sawfly (Rand et al., 2014), all show positive responses to the proportion of host plants in an agroecosystem, most likely due to higher chances of finding and colonizing host patches in agricultural landscapes (Root, 1973). For CLB, this positive association could be due to a combination of increased immigration to and decreased emigration from landscapes with high proportions of cereals. My results add to the body of research providing empirical support for the prediction of the resource concentration hypothesis that specialist herbivores will increase their abundance in landscapes dominated by their host plants.

My study is among the few studies (Beduschi et al., 2015; Berger et al., 2018; Riggi et al., 2017) investigating the carryover effect of the proportion of hosts in the landscape in the previous year on herbivore abundance. I found consistent positive associations between proportions of cereal crops at multiple scales in the previous year and CLB abundance in cereal fields in the current year. My result is consistent with Berger et al. (2018) who reported a positive association between the proportion of previous year's oilseed crops and the abundance of European tarnished plant bugs in oilseed fields. Similarly, Colorado potato beetle abundance in newly planted potato fields is explained by distance to potato fields in the previous year, because they overwinter in and around potato fields from the previous year (Boiteau et al., 2008; Huseth et al., 2012). In my study, the proportion of non-cereal crops in the current year and proportion of cereals in the previous year were highly correlated, suggesting that due to crop rotation, most of these non-cereal crops were cereals the year prior. This finding suggests that the association between non-cereal crops and CLB abundance in the current year may reflect a carryover effect of the proportion of cereals in the landscape in the previous year, rather than a

direct positive effect of non-cereal crops on CLB populations. Thus, caution should be taken when interpreting associations between herbivore abundance and annually rotated crops. The proportion of crop hosts in the landscape in the previous year could be a major factor determining the abundance of specialist herbivores in crops as was found in this study, and deserves further study in other insect pest systems.

The presence of overwintering habitats in agricultural landscapes can help to explain the abundance of herbivores in agricultural fields (Berger et al., 2018; Boiteau et al., 2008; Rusch et al., 2013b; Zaller et al., 2008). As predicted, a higher proportion of woodland in the landscape increased CLB abundance in wheat fields due to their role as overwintering sites (Casagrande et al., 1977). My result is in line with previous studies that reported a positive impact of woodland overwintering sites on the abundance of rape pollen beetle at multiple scales (Rusch et al., 2013b), and stem weevil (Zaller et al., 2008) and indicates the potential of semi-natural habitats to contribute to ecosystem disservices (i.e. increased pest problems) in some systems (Perez-Alvarez et al., 2018).

Cereal leaf beetle abundance was negatively associated with a higher proportion of pasture at various spatial scales in the landscape. Perennial grassy areas (e.g., pasture and grassy fallow) have been associated with reductions of specialist herbivore populations in crops through host crop dilution in agricultural landscapes (Huseth et al., 2012; Rusch et al., 2013a). My findings are consistent with this explanation, as I found negative correlations between the proportions of CLB major hosts and pasture, which resulted in lower proportion of major hosts in these landscapes. Similarly, Huseth et al. (2012) reported negative associations between Colorado potato beetle abundance in potato fields and proportion of grasslands surrounding the fields in a 200 m radius due to a potential disruption in the beetle dispersal from overwintering

habitats to recently planted potato fields. Alternatively, Rand et al. (2014) reported that the proportion of grasslands in the landscape did not affect wheat stem sawfly abundance or parasitism levels in Montana, and Zaller et al. (2008) reported no associations between the proportion of grasslands in the landscape and abundance of rape pollen beetle in oilseed crops. More studies are needed to determine the specific mechanism behind the negative response of CLB to the proportion of pasture.

The proportion of all semi-natural habitats combined (i. e. grassland, woodland, and pasture) was not associated with CLB abundance and parasitism. This finding is in line with the Tschumi et al. (2015) study, which found no associations between CLB density in wheat fields and the proportion of semi-natural habitats at the 0.75 km scale. However, when I tested semi-natural habitats separately, I found opposite effects on CLB abundance, suggesting caution when interpreting patterns resulting from merging all semi-natural variables into one category. In my study, the strongest association between landscape complexity and CLB abundance was found at the 2 km scale, suggesting that the 0.75 km scale tested in the Tschumi et al. (2015) study might have been too small to detect landscape effects. Further, I found a negative association between landscape diversity and CLB abundance at the 0.5 km scale, which was driven mostly by the diversity of crops at this scale. Landscape diversity was associated positively with the proportion of crops at smaller scales and the proportion of semi-natural habitats at the larger scales, suggesting the importance of scales in landscape-scale studies. I did not find any association between landscape diversity and CLB parasitism, suggesting a direct negative effect of landscape diversity on CLB rather than an indirect effect due to increased parasitism. Local diversity enhancements, such as flower strips, resulted in lower CLB abundance in neighboring wheat fields (presumably due to higher predation) in the study by Tschumi et al. (2015) but not in

another study (Pollier et al. 2019), further indicating inconsistent effects of semi-natural habitats at a small spatial scales. These results suggest that the role of semi-natural habitats can not always be generalized because habitats within this category might affect herbivores and natural enemies differently.

My findings indicate high impacts of *T. julis* on CLB populations, expanding previous studies conducted at local scales (Dysart et al., 1973; Evans et al., 2015; Evans et al., 2006) to the landscape scale. CLB parasitism remained above the 32% minimum threshold for successful classical biological control (Hawkins & Cornell, 1994). Because the impact of *T. julis* on CLB larval abundance happens the following year (i.e., parasitism does not decrease CLB abundance until the next generation), I hypothesize that this negative association is due to landscape-scale carryover effects of *T. julis* on CLB populations from previous years. The success of this parasitoid has been linked to its high specificity to CLB (Hervet et al., 2016), and high dispersal ability which resulted in no colonization lag between newly and previously planted wheat fields in disturbed agricultural landscapes (Evans et al., 2015). I conclude that CLB parasitism by *T. julis* has strong negative effects on CLB abundance in agricultural landscapes in southern Alberta.

3.5.2 Indirect effects of landscape complexity on cereal leaf beetle

The proportion of host crops in agricultural landscapes can indirectly influence herbivore parasitism rate due to a density-dependent response by parasitoids (Gunton et al., 2016). In the year with lower CLB abundance (2015), I observed a significant increase of parasitism associated with an increased proportion of major host crops at multiple scales. Contrary to our finding, Grab et al. (2018) reported negative associations between the proportion of crops and tarnished plant bug parasitism by the introduced parasitoid *Peristenus digoneutis* Loan

(Hymenoptera: Braconidae). The same authors concluded that the higher proportion of semi-natural habitat in the surrounding landscape was a key factor for explaining tarnished plant bug parasitism. The contrasting findings between Grab et al. (2018) and this study may be due to the parasitoid host specificity. While *T. julis* only attack CLB (Hervet et al., 2016), *P. digoneutis* can attack several species of *Lygus* and therefore have a wider range of accepted hosts (Mason et al., 2011), which enable the species to exploit resources and alternative hosts from semi-natural habitats. The presence of CLB in high numbers in cereal fields might have reduced *T. julis* searching time and increase parasitism (Stiling, 1987). Overall, my results are consistent with a density-dependent response of *T. julis* to CLB abundance, mediated by the amount of host crop available in the landscape.

Natural enemies can benefit from different crops in the landscape if those crops provide supplemental resources (e.g., pollen and nectar), as predicted by the supplementation hypothesis (Dunning et al., 1992). I found positive associations between CLB parasitism and proportion of cereals, alfalfa, and canola at multiple spatial scales. These positive associations can be due to carbohydrate resource availability for *T. julis*: honeydew produced by aphids in cereals and alfalfa, and nectar and pollen produced by flowering canola plants, which were flowering at the time of this study, but this needs to be tested in a future study. Given the proovigenic nature of *T. julis* (Dysart et al., 1973), I hypothesized that under low CLB populations, *T. julis* might have to spend more time locating its host, which results in higher dependency on carbohydrate resources to maximize its foraging ability and parasitism. This pattern is consistent with the positive association of CLB parasitism and these landscape habitats in the low CLB year (2015). Evans et al. (2010) reported increased *T. julis* longevity and 4 - 7 times higher CLB parasitism early in the season in cereal fields sprayed with sucrose. Cereals have been associated with increased natural

enemies in other systems. In western Canada, Samaranayake & Costamagna (2018) reported a positive association between cereals and predators in agricultural landscapes, and they attributed it to the presence of aphids and reduced insecticide applications in cereals. Although I did not quantify them, aphids were common in my samples, and pea aphids are ubiquitous in alfalfa in Western Canada (Uddin, 2005). Canola nectar improves diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae) parasitism by *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae) (Idris & Grafius, 1997). These findings suggest that CLB parasitism can be enhanced by alternative food resources present in crops (e.g., cereals, alfalfa and canola), which supports the landscape supplementation hypothesis.

Contrary to expectations, I found negative associations between the proportion of pasture at the 2 km scale and CLB parasitism, and no associations between CLB parasitism and other semi-natural habitats (woodland and grassland). Three possible reasons might explain these unexpected patterns. First, the high specificity of *T. julis* to CLB may limit its foraging in cereal fields where CLB is present (Hervet et al., 2016). Second, *T. julis* overwinters inside CLB pupae within cereal fields (Dysart et al., 1973). Unlike other systems, such as rape pollen beetle (Thies et al., 2003), a higher proportion of pasture, grasslands, or woodlands in the landscape would not enhance overwintering habitats for *T. julis*. Third, I found a negative correlation between the proportion of pasture and proportion of CLB major hosts in the landscape, which might explain lower CLB abundance in landscapes with a high proportion of pasture. Given the density-dependent response of *T. julis* to CLB (Kher et al., 2014), the negative effect of pasture on CLB parasitism might be due to the lower abundance of CLB in these fields. My findings are in line with a recent study by Karp et al. (2018) that summarized data from 31 countries and found a similar number of positive and negative associations between pest control and semi-natural

habitats in the landscape. Furthermore, although I did not account for the effect of predators in this study, positive effects of pastures on predators have been shown in other systems (Rusch et al., 2013a). Kheirodin et al. (2019) reported several species of predators consuming CLB under laboratory and field conditions, with up to 24.5 % predation on CLB eggs over 24 h in my study area, suggesting that predators can also be important mortality factors for CLB. Overall, my results suggest that different semi-natural habitats may have diverse roles in host-parasitoid systems and grouping them into one category may mask these roles.

My study is among the few (Beduschi et al., 2015; Riggi et al., 2017; Rusch et al., 2011) that have investigated the effect of the proportion of host crops in the previous year on current parasitism rates. I found that landscapes with a higher proportion of cereals in the previous year had no significant association with current levels of parasitism. This lack of association may be due to disturbances in cereal fields, such as tillage (regular or reduced), insecticides, and the high dispersal ability of the parasitoid. In my study area, tillage is seldom used (Awada et al., 2014), and insecticide applications are rare in cereals (Meers, 2018). Evans et al. (2015) reported a high dispersal ability of *T. julis* that enabled the species to locate CLB over distances up to 600 m in highly disturbed agricultural landscapes. Altogether, these findings suggest that the lack of association between parasitism and proportion of cereal fields in the previous year could be due to the high dispersal ability of *T. julis* that enable this species to locate its host over long distances and mask potential effects of overwintering sites.

In conclusion, my study suggests that the proportion of wheat and barley in the landscape in the current and previous years are positively associated with CLB abundance, supporting the resource concentration hypothesis. Cereal leaf beetle parasitism was also positively associated with the proportion of CLB major host crops, potentially due to a higher abundance of CLB in

these landscapes. Percentage parasitism was higher in landscapes with higher proportion of crops with a potential for providing carbohydrate resources, supporting the landscape supplementation hypothesis. My results suggest that habitat/crop diversification at the smaller landscape scale may be an important factor to reduce CLB numbers in southern Alberta. Altogether, my results suggest that designing agricultural landscapes with increased diversity can be an important habitat management strategy to control CLB, by reducing the proportion of CLB hosts and increasing the proportion of habitats supporting parasitoids.

Table 1. A summary of the generalized linear models with best support at various scales ($\Delta i < 3.5$ at each scale) evaluating associations between cereal leaf beetle (CLB) abundance (CLB/ 50 sweeps) and landscape variables in the current and previous years, percentage parasitism, and the year of the study in southern Alberta, Canada.

Model #	Radius (km)	Model	DF	Log-lik	AIC	Δi	Pseudo $-r^2$	P	Partial correlation
<u>Models with current year habitats</u>									
1	0.5 km	I – Y15*** + WA* + MNH** + NC** – PP** – SIM** + GR*	46	-360.8	378.8	0.00	0.34	0.002	-0.41 (Y); +0.38 (MNH); +0.38 (WA); +0.36 (NC) -0.31 (SIM); -0.29 (PP); +0.22 (GR);
2	1 km	I + MNH** – Y15*** – PP** + Y15:WA + TW ^{PS}	47	-362.9	378.9	0.00	0.32	0.002	+0.43 (MNH); -0.42 (Y); -0.30 (PP); +0.21 (Y:WA); +0.20 (TW)
3	1 km	I + MNH** – Y15** – PP* + WA + Y15:WA	45	-365.1	379.1	0.2	0.29	0.002	+0.39 (MNH) ; -0.39 (Y); -0.28 (PP); +0.20 (WA)
4	1.5 km	I – Y15** + MNH** + WA* + TW** – PP*	49	-360.3	376.9	0.00	0.32	0.001	-0.37 (Y); +0.35 (MNH); +0.33 (WA); +0.33 (TW); -0.25 (PP)
5	1.5 km	I – Y15** – PAS** + WA ^{PS} – PP*	49	-363.1	380.2	3.2	0.25	0.005	-0.40 (Y); -0.35 (PAS); +0.29 (WA); -0.24 (PP)
6	2 km	I + WA** + TW** + MNH** – Y15* + NC*	48	-358.7	372.7	0.00	0.37	0.0001	+0.39 (WA); +0.37 (TW); +0.32 (MNH); -0.30 (Y); +0.22 (NC)
7	2 km	I + WA** + MJH** + MNH* – Y15* + NC*	48	-360.4	374.4	1.7	0.35	0.0003	+0.40 (WA); +0.33 (MJH); +0.32 (MNH); -0.28 (Y); +0.26 (NC)
8	2 km	I – PAS*** – Y15** + WA**	50	-356.7	375.7	3.0	0.28	0.0005	-0.41 (PAS); -0.39 (Y); +0.35 (WA)
<u>Models with previous year cereal crops</u>									
9	0.5 km	I – Y14 ^{PS} + MJH ^{PS}	48	-358.6	366.6	-	0.15	0.016	-0.23 (Y); +0.22 (MJH)
10	1 km	I – Y14* + MJH ^{PS} + Y14:MNH* – Y13:MNH ^{PS}	46	-354.7	366.8	-	0.21	0.017	-0.30 (Y); -0.27 (Y:MNH); +0.25 (MJH)
11	1.5 km	I – Y14** + Y14:MNH** – Y13:MNH* + MJH*	46	-353.7	365.6	-	0.23	0.010	-0.34 (Y); +0.30 (Y:MNH); +0.24 (MJH)

12	2 km	I – Y14** + MJH** – Y13:MNH* + Y14:MNH*	46	-352.1	364.2	-	0.25	0.005	-0.35 (Y); +0.33 (MJH); +0.27 (Y:MNH)
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Notes: Model terms consist of intercept (I); landscape variables: barley (BA), canola (CA), field border and grasslands (GR), major hosts (MJH; wheat and barley), minor hosts (MNH; corn, oat and rye), non-cereal crops (NC; all annual crops that are not host of CLB), pasture (PAS), habitat diversity (SIM; Simpson diversity index), total wheat (TW; spring and winter wheat), wooded area (WA); the percentage parasitism of CLB (PP); the CLB abundance (CLB); and the study year (Y, which includes Y14 [2014] and Y15 [2015]). The colon symbol indicates interaction terms. The model reported in bold in the best-fitted model across all scales (smallest AIC across all models). Variable includes the degree of freedom (DF), Log-likelihood (Log-lik), and Akaike information criterion (AIC).^{PS} $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; other variables not significant (i.e. $P > 0.10$)

Table 2. A summary of the generalized linear models with best support at various scales (<3.5 Δi value difference at each scale) evaluating associations between mean cereal leaf beetle (CLB) parasitism (CLB parasitism/ field) and landscape variables, CLB abundance (CLB/ 50 sweeps), and the year of the study in southern Alberta, Canada.

Model #	Radius (Km)	Model	DF	Log-lik	AIC	Δi	Pseudo- r^2	P	Partial correlation
1	0.5 km	I – Y15*** + Y15:MJH*** – Y14:MJH + Y15:CA*** + Y14:CA + Y15:CLB*** – Y14:CLB	31	-356.8	374.8	0.00	0.47	0.008	- 0.59 (Y); + 0.56 (Y:MJH); + 0.50 (Y:CA); + 0.45 (Y:CLB);
2	1 km	I – Y15*** + Y15:CA*** – Y14:CA + Y15:TW** – Y14:TW + Y15:CLB** – Y14:CLB – Y15:MNH ^{PS} – Y14:MNH	28	-349.6	371.7	-	0.37	0.044	-0.52 (Y); +0.46 (Y:CA); +0.43 (Y:TW); +0.37 (Y:CLB); -0.25 (Y:MNH)
3	1.5 km	I – Y15*** + Y15:MJH** – Y14:MJH + Y15:CA** + Y14:CA + Y15:CLB* – Y14:CLB + Y15:ALF ^{PS} – Y14:ALF + NC	27	-345.8	369.8	-	0.43	0.019	-0.58 (Y); +0.45 (Y:MJH); +0.41 (Y:CA); +0.31 (Y:ALF); +0.28 (Y:CLB); +0.27 (NC)
4	2 km	I – Y15*** + Y15:TW*** – Y14:TW + Y15:ALF** – Y14:ALF + Y15:CA^{PS} + Y14:CA + NC^{PS}	32	-344.3	364.3	0.00	0.44	0.005	-0.60 (Y); +0.50 (Y:TW); +0.48 (Y:ALF); +0.32 (Y:CA); +0.27 (NC)
5	2 km	I – Y15: PAS* + Y14: PAS + Y15: ALF** – Y14: ALF – Y15	32	-351.6	365.6	0.89	0.32	0.013	+0.48 (Y: ALF); -0.32 (Y: PAS); -0.22 (Y);
6	2 km	I – Y15*** + Y15:MJH*** – Y14:MJH + Y15:ALF*** – Y14:ALF + Y15:CA ^{PS} + Y14:CA + NC ^{PS}	29	-347.3	367.3	3.00	0.46	0.003	-0.59 (Y); + 0.50 (Y:MJH); +0.46 (Y:ALF); +0.32 (Y:CA); +0.26 (NC)

Notes: Model terms consist of intercept (I); landscape variables: alfalfa (ALF), barley (BA), canola (CA), major hosts (MJH; wheat and barley), minor hosts (MNH; corn, oat and rye), non-cereal crops (NC; all annual crops that are not host of CLB), pasture (PAS), total wheat (TW; spring and winter wheat), the CLB abundance (CLB); and the study year (Y, which includes Y14 [2014] and Y15 [2015]). The colon symbol indicates interaction terms. The model reported in bold in the best-fitted model across all scales (smallest AIC across all models). For each scale, the model reported first is the model with the most support by the data, and subsequent models

are competing models ($\Delta i < 3.5$). Variable includes the degree of freedom (DF), Log-likelihood (Log-lik), and Akaike information criterion (AIC). ^{PS} $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; other variables not significant (i.e. $P > 0.10$)

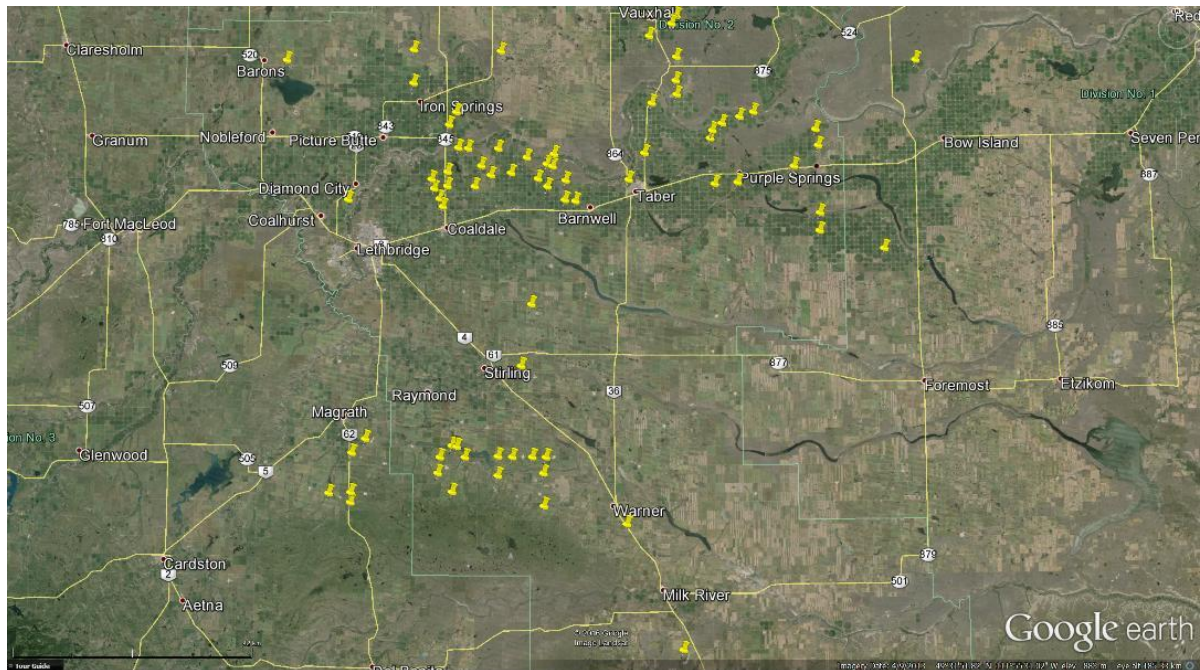


Figure 3. Location of the 75 wheat fields sampled (2014 and 2015 combined) during this study near Lethbridge, Alberta.

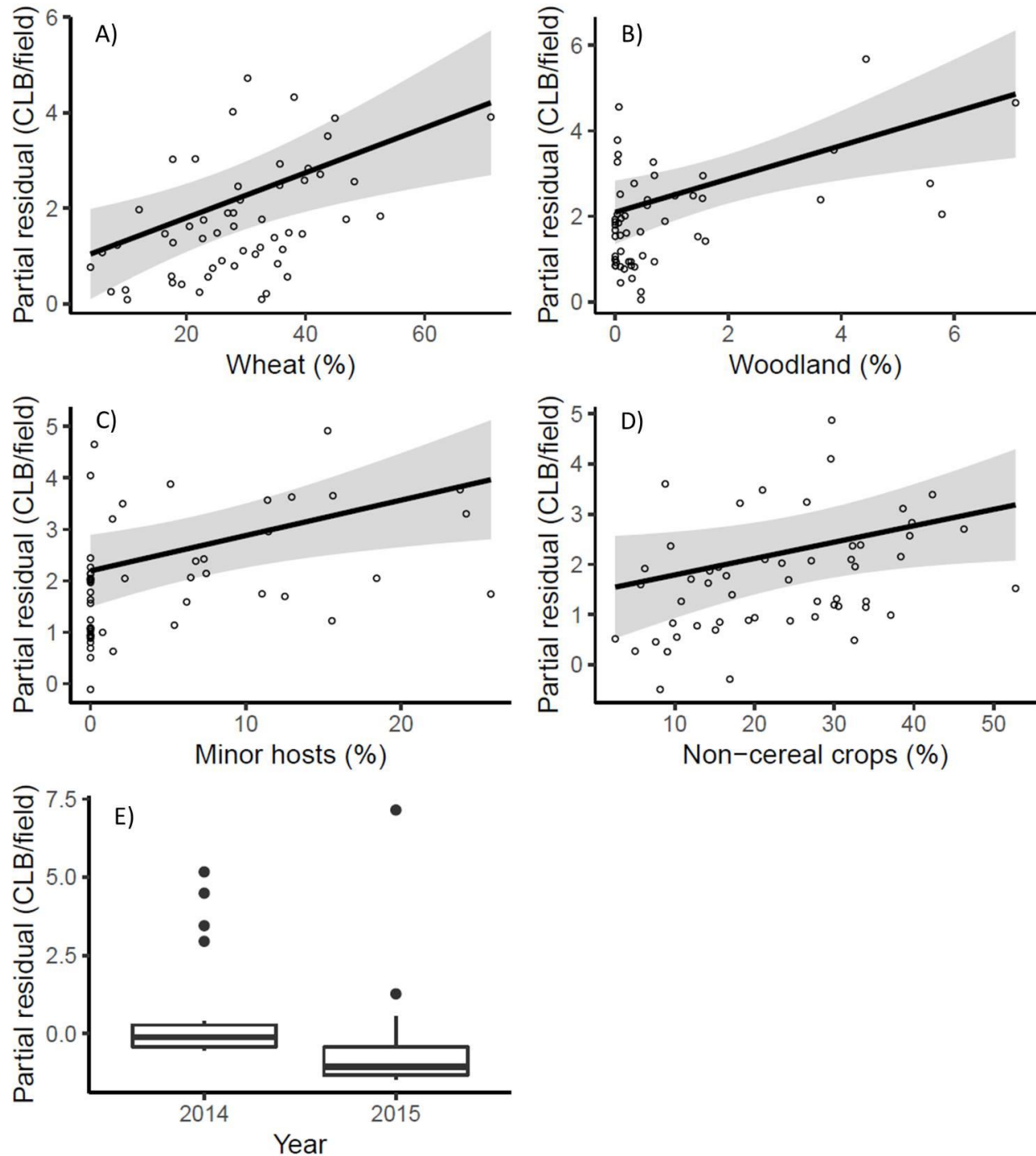


Figure 4. Average cereal leaf beetle (CLB) abundance (CLB/ 50 sweeps) in focal wheat fields in association with (A) % of total wheat, (B) % of woodland, (C) % of minor hosts (corn, oats, and rye), (D) % of non-cereal crops in the landscape at the 2 km scale; and (E) year of the study. The grey areas are 95% confidence intervals estimated by the Wald confidence interval test using

standard errors. Each point indicates the average CLB number (CLB/ 50 sweeps) per field ($n=54$). See Table 1 (model 6) for statistical details.

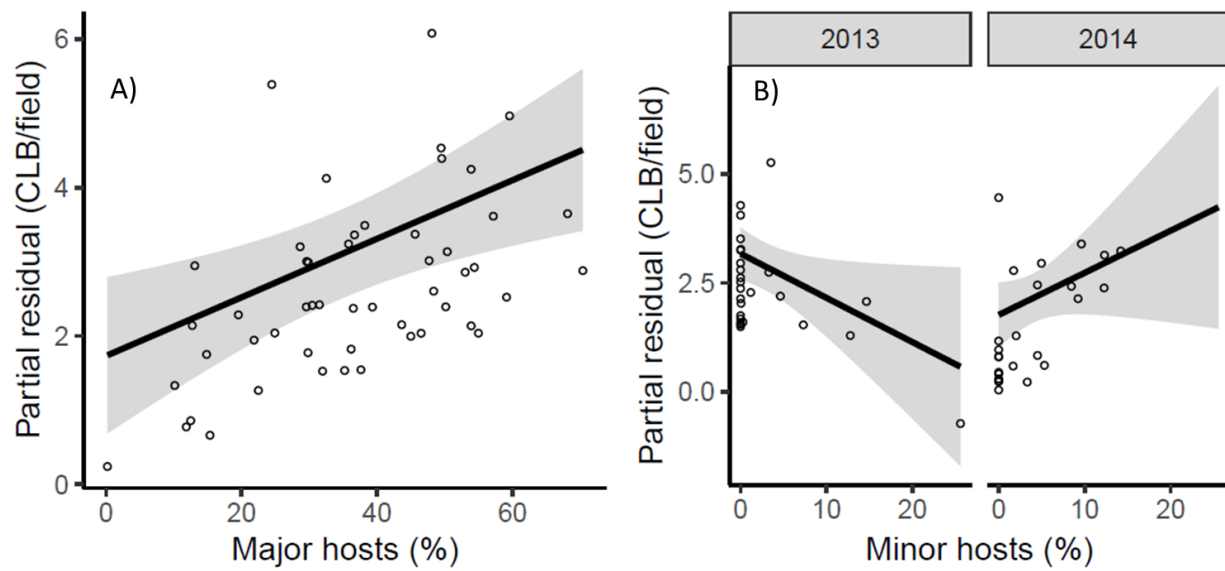


Figure 5. Average cereal leaf beetle (CLB) abundance (CLB/ 50 sweeps) in focal wheat fields in association with (A) % of major hosts (wheat and barley), (B) and % of minor hosts (corn, rye, and oats) in the previous year's landscapes, and its interaction with year. The grey areas are 95% confidence intervals estimated by the Wald confidence interval test using standard errors. Each point indicates the average CLB number (CLB/ 50 sweeps) per field (n= 50). See Table 1 (model 12) for statistical details.

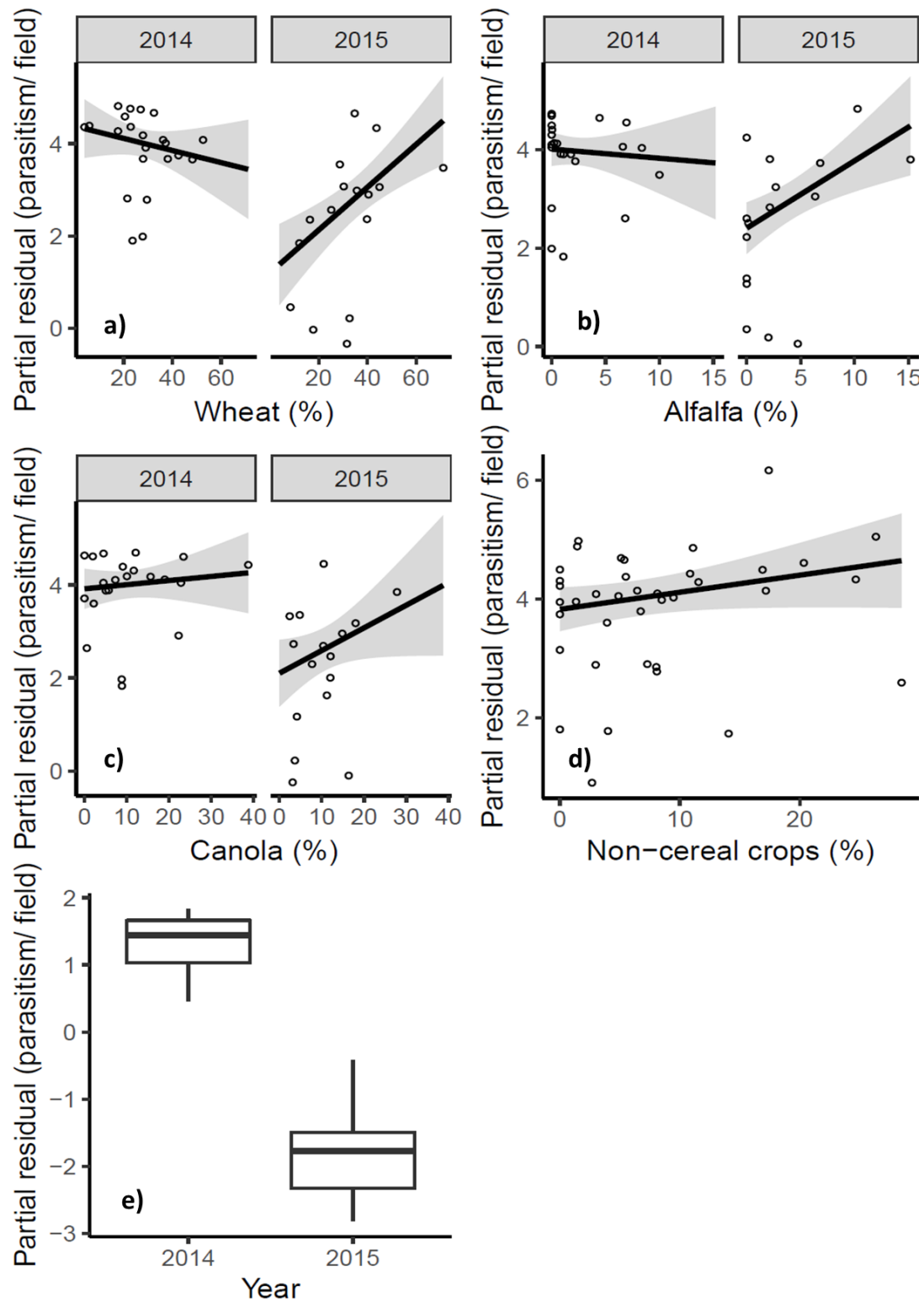


Figure 6. Average cereal leaf beetle (CLB) parasitism (parasitism/ field) in focal wheat fields in association with (A) % of CLB major hosts (wheat and barley), (B) % of alfalfa, (C) habitat

diversity (Simpson index), and (D) year of the study. The grey areas are 95% confidence intervals estimated by the Wald confidence interval test using standard errors. Each point indicates the average CLB parasitism per field (n= 39). See Table 2 (model 4) for statistical details.

CHAPTER 4

Laboratory and field tests of predation on the cereal leaf beetle, *Oulema melanopus* (Coleoptera: Chrysomelidae)

In this chapter, the potential predators of CLB were determined using various laboratory and field trials. This study suggests that various predator species contribute to CLB suppression in western Canada. Furthermore, the information reported in this study was the basis for the development of chapter 5 of this dissertation. This is the first study to report CLB predation by various common predators present in Western Canada.

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doi:10.1080/09583157.2019.1566437.

4.1 Abstract

The cereal leaf beetle (CLB), *Oulema melanopus* L. (Coleoptera: Chrysomelidae), is an invasive pest in North America recently reported in the Canadian Prairies. I performed a series of laboratory assays to identify potential predators and a field study to quantify predation of CLB eggs. In no-choice Petri dish assays, ground beetles (Carabidae), rove beetles (Staphylinidae), and several common lady beetle species (Coccinellidae) were the most consistent predators of eggs and larvae. *Nabis* spp. (Hemiptera: Nabidae), *Pardosa* spp. and *Pirata* spp. (Araneae: Lycosidae) consumed many larvae, but did not consume eggs. *Hippodamia* spp., *Coccinella septempunctata* (L.) (Coleoptera: Coccinellidae), and *Pterostichus melanarius* (Illiger) (Coleoptera: Carabidae) also fed on CLB eggs on potted plants when an alternative food source was available, *Sitobion avenae* (Fabricius) (Hemiptera: Aphididae). In my field study, I found an average of 24.5% of sentinel eggs disappeared over a 24 h period, likely due to predation. My results suggest that generalist predators may play an important role in the biological control of CLB, and warrant further study.

Keywords: Biological control, predation, lady beetles, alternative prey, carabid beetles.

4.2 Introduction

The cereal leaf beetle (CLB), *Oulema melanopus* L. (Coleoptera: Chrysomelidae), is an invasive pest in North America (Haynes & Gage, 1981), recently reported in western Canada (Dosdall et al., 2011; Kher et al., 2011). The species is native to Europe and feeds on a variety of important cereal crops including wheat, oats, and barley (Leibee & Horn, 1979). In Western Canada, CLB is present in British Columbia, Alberta, Saskatchewan, and Manitoba, following a steady pattern of geographical expansion (Dosdall et al., 2011). Cereal leaf beetle has caused crop losses ranging from 13% in winter wheat (Buntin et al., 2004) to 75% in barley (Webster & Smith, 1979), in the U.S.A. The species has one generation per year and overwinters as an adult (Gutierrez et al., 1974). In the spring, adults feed on wild grasses before colonizing cereal fields (Gutierrez et al., 1974). Female CLB lay eggs singly or in a small cluster on cereal leaves (McPherson, 1983a) from mid-May to mid-June in Western Canada (Kher et al., 2011). Eggs hatch within four to six days (Barton & Stehr, 1970) and the four larval stages typically take 10 to 14 days to complete (Guppy & Harcourt, 1978). The fourth instar larva enters a pre-pupal stage and drops into the soil to pupate. Adults emerge after 17 to 25 days (Philips et al., 2011). The larva is the most destructive stage; fourth instar larvae can consume up to 10 times its body weight (Calestru, 2008).

Studies of natural enemies of CLB have focused on the Palearctic parasitoid, *Tetrastichus julis* (Walker) (Hymenoptera: Eulophidae), introduced as a classical biological control agent in North America in 1969 (Stehr, 1970). In Canada, *T. julis* was first detected in British Columbia in 1988. It is highly efficient with up to 60% parasitism levels reported in the USA (Evans et al., 2015; Evans et al., 2006; Kher et al., 2014). Although *T. julis* greatly decreases CLB populations over time, it causes mortality at the pupal stage, allowing CLB

larvae to damage the plants within the field season (Dysart et al., 1973). Predators, on the other hand, can cause immediate mortality (Sunderland, 2002; Sunderland et al., 1997; Symondson et al., 2002), and contribute to the management of CLB by reducing plant damage within the field season. To date, no studies have investigated the role of generalist predators on the control of CLB, although this knowledge could be important, particularly for the management of CLB in areas without *T. julis*.

Several generalist predators contribute significantly to the control of other chrysomelid pests. Hazzard et al. (1991) reported egg predation rates of 37% and 58% for the first and second generations of *Leptinotarsa decemlineata* (Say), respectively, in western Massachusetts potato fields, USA, in 1987. They attributed egg predation to generalist predators such as ground beetles (Coleoptera: Carabidae), *Podisus* spp. (Hemiptera: Pentatomidae) and, particularly, *Coleomegilla maculata* (De Geer) (Coleoptera: Coccinellidae), the most common predator in their study fields. Wiebe & Obrycki (2004) reported an average of 26% predation on eggs of *Galerucella pusilla* Duftschmid in multi-site, 2-year field trials in Iowa. The same study reported 46% and 36% predation on sentinel larvae of *G. pusilla* on potted purple loosestrife plants for the first and second generations, respectively. In a field cage study, Montemayor & Cave (2012) reported that high-density treatments of *Podisus maculiventris* (Say, 1832) (Hemiptera: Pentatomidae) reduced overall *Microtheca ochroloma* Stål (Coleoptera: Chrysomelidae) survivorship by up to 39%. The authors concluded that a release rate of 4-10 first instar *P. maculiventris* per six turnip plants could control *M. ochroloma* in Florida. These studies suggest that generalist predators can be important mortality factors for chrysomelid eggs and larvae.

The objectives of my study were to (1) determine potential predators of CLB eggs and larvae in laboratory assays, (2) evaluate the impacts of promising predators on CLB in more

realistic arenas (i.e. potted wheat plants) and with alternative prey, and (3) estimate potential predation rates on CLB eggs under field conditions.

4.3 Material and Methods

To evaluate which predators would feed on CLB eggs and larvae, I first conducted no-choice tests in small arenas. Predator species that fed on CLB in the no-choice test were then placed in cages with potted plants, with CLB eggs and alternative prey (aphids) on wheat plants, to test their natural ability to predate on CLB eggs. Finally, to quantify the impact of natural predation in field conditions I assessed the rate of sentinel egg disappearance, putatively as a result of predation.

4.3.1 Insect culture

I collected CLB adults and potential predators in the spring using sweep nets from putative overwintering habitats (i.e., field roadside ditches and perennial grasslands near cereal fields) and cereal fields near Lethbridge, Alberta, Canada (49°46'54.59"N, 112°33'13.25W), in May 2014 and 2015. Approximately 250 CLB adult were maintained in each of 4 cages (46 cm width × 92 cm length × 46 cm height), under controlled conditions (21 ± 2 °C, 16L: 8D and RH = $21 \pm 3\%$). A potted wheat plant (CDC Go variety) at the boot stage (Zadoks stage 4; Zadoks et al., 1974) was placed inside each cage and checked them daily for eggs. I replaced damaged plants with fresh plants on a weekly basis and transferred plants containing CLB eggs into the larval cages, maintained under the same conditions as the adult cages.

I maintained all collected predators under controlled conditions (10 °C, 16L: 8D, and RH = $21 \pm 3\%$) in transparent plastic tubs (15 cm width × 25 cm length × 15 cm height) with perforated lids to allow airflow. I fed predators *ad libitum* with aphids, crickets, and flies,

selected according to predator size. Prior to use in experiments predators were held at room temperature (24 °C) and starved for 24 h. I identified all predators to genus (except Staphylinidae, identified only to family), and a subset of them to species (Acorn, 2007; Dondale & Redner, 1990; Dondale et al., 2003; Lindroth, 1961). I preserved voucher specimens from my 2015 trials in 95% ethanol, and deposited them in the Wallis-Roughley Museum of the Department of Entomology at the University of Manitoba.

4.3.2 Egg predation laboratory study

I selected predator taxa for the laboratory experiments based on their abundance in cereal fields during the oviposition period of CLB, and their previously reported role as predators of other chrysomelid species. I transferred ten CLB eggs (< 24 h old) onto excised wheat leaves on top of a small square piece of wet filter paper in a Petri dish (90 mm diameter × 15 mm height, Phoenix Biomedical Company., Bolton, Ontario, Canada). I then exposed ten CLB eggs for 24 h to individual adult predators in each replication (Table 3), under controlled conditions ($21 \pm 2^{\circ}\text{C}$, $\text{RH} = 21 \pm 3\%$). I then counted the remaining eggs under a microscope (Zeiss Stemi 2000-C, 40X) and assessed them for evidence of feeding damage (e.g., presence of holes on the eggs or part of eggs missing) in comparison with control eggs held under the same conditions, but without predators. In 2014, Petri dishes containing predators of the same species (or genus) were blocked by row, while in 2015 their arrangement was randomized. I did not detect any spatial bias due to the arrangement of the Petri dishes in the 2014 trial, thus I used both the 2014 and 2015 data for the analysis.

4.3.3 Larval predation laboratory study

I placed ten CLB larvae ($4 \times 4^{\text{th}}$, $3 \times 3^{\text{rd}}$, and $3 \times 2^{\text{nd}}$ instar larvae) in individual Petri dishes, on top of a piece of wheat leaf (6×1.5 cm) on a small square piece of wet filter paper. I confined an individual field-collected predator (Table 3) with the larvae for 48 h, under controlled conditions ($21 \pm 2^{\circ}\text{C}$, $\text{RH} = 21 \pm 3\%$) and counted the number of living larvae. I conducted two trials (2014 and 2015) with a total of 15 predator taxa (Table 3). The control treatment consisted of ten CLB larvae in Petri dishes without predators.

4.3.4 Microcosm alternative prey study (choice tests)

To confirm the predator status of species that fed on CLB eggs in the no-choice tests, I conducted further tests with predators that consumed eggs in the Petri dish experiment by using more realistic arenas. Using caged, potted wheat plants, I presented predators with CLB eggs and English grain aphids *Sitobion avenae* Fabricius (Hemiptera: Aphididae) as alternative prey. Cages were constructed of transparent Plexiglas (12 cm diameter \times 30 cm height) with three round holes (3 cm diameter) on each side covered by mesh to allow air flow. I closed the bottom of the cages by placing the cages over green plastic plates (Akro-Mils, Myers Ind., Akron, Ohio, USA). I placed an uninjured wheat plant (30 cm height, CDC Go variety, tillering stage Zadoks stage 3) recently transferred from a greenhouse in a pot (7.6 cm diameter \times 10.2 cm height) filled with a modified peat-based soil Cornell mix (Boodley & Sheldrake, 1977). I then haphazardly selected three leaves on each plant and placed three freshly laid CLB eggs (< 24 h) and three aphids (apterous adult *S. avenae*) on each leaf and one egg and one aphid on the stem of the plant using a brush, for a total of ten eggs and ten aphids per pot. I released one predator adult inside each cage and recorded the numbers of intact eggs and live aphids, after 24 h. I found most predators either on the soil or on the wheat plants at the end of each trial, indicating that they had

access to both the eggs and aphids (i.e. they did not fall from pots). I checked the remaining eggs under a microscope for evidence of feeding damage. I conducted this experiment under controlled conditions (21 ± 2 °C, RH = $21 \pm 3\%$, and 16L: 8D using artificial fluorescent light) over three consecutive days with three replicates for each predator taxa performed simultaneously each day, depending on predator availability (see Table 3 for details).

4.3.5 Egg predation field studies

I quantified the predation rate on CLB eggs in 10 wheat fields per year, in 2014 and 2015, using sentinel egg cards exposed to natural levels of predation during 24 h periods. I performed this experiment during the last week of May when CLB typically lay their eggs on cereal plants in southern Alberta, and winter wheat plants were at the tillering stage. Sentinel cards consisted of ten freshly laid eggs transferred with a brush on the non-sticky side of a piece of green masking tape and attached to a leaf of a wheat plant. My treatments were sentinel egg cards exposed to predators and controls protected with predator exclusion cages (Fig. 7a and b). Both treatments were in tomato cages (15 cm diameter \times 50 cm height). The open cage had no mesh and allowed predators to access the egg cards. The predator exclusion cage was completely covered by a white fine mesh (0.2 mm openings), buried into the soil. I removed any predators present on the focal plant and the surrounding soil surface before installing the cages. I set up each pair of open and exclusion cages in the same wheat row, 1 m apart, for a total of three pairs per field. Each pair of open and exclusion cages was considered a block and the distance between blocks was 20 m; all three replicate blocks were placed 100 m from the field edge. I placed a stretched plastic bag over the top of each cage to protect the egg cards from direct rainfall. After 24 h, I collected the egg cards from cereal fields and recorded the number of remaining eggs. I further verified the number of eggs in

the laboratory with a stereo microscope (Zeiss Stemi 2000-C, 40X) to assess feeding damage caused by predators. I added the number of damaged eggs to the number of missing eggs to calculate total egg mortality.

4.3.6 Data analysis

I conducted separate analyses for each year of the study. I used Kruskal-Wallis non-parametric tests to compare the effects of different predator taxa on the numbers of CLB eggs and larvae consumed in the no-choice experiments. I used non-parametric tests because the data were not normally distributed and no transformation stabilized the variance. After testing overall significance, I performed pairwise comparisons of treatments with Kruskal-Wallis tests and adjusted their p-values using the sequential Bonferroni method for multiple comparisons (Rice, 1989).

In the alternative prey study, I analyzed the arcsine-transformed proportion of the remaining CLB eggs and aphids with a split-plot ANOVA. Predator treatment was the whole-plot factor, prey species was the sub-plot factor, and date was included as a blocking factor, with no test to determine significance (Crawley, 2013). I then performed pairwise comparisons of predator treatments using pairwise t-tests adjusted for multiple comparisons using the sequential Bonferroni method (Rice, 1989). I conducted paired t-tests within each predator taxa to determine their preference for CLB eggs or English grain aphids. For the field study, I used a mixed effects model to test predation impacts on the average number of missing eggs on sentinel cards, with open and predator exclusion treatments as fixed factors nested within the field, which was treated as a random factor. All statistical analyses were conducted using R (version 3.3.3, R Development Core Team, 2017).

4.4 Results

4.4.1 Egg Predation laboratory study

Six (2014) and four (2015) predator taxa reduced the number of CLB eggs in Petri dishes relative to controls (2014: $\chi^2 = 74.4$, DF = 9, $P < 0.0001$; 2015: $\chi^2 = 64.0$, DF₁₀, $P < 0.0001$; Fig. 8a and b, respectively). The lady beetles *Hippodamia tredecimpunctata* (L.), *H. sinuata* Mulsant and *H. quinquesignata* (Kirby) consumed 80% to 90% of the eggs. *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), staphylinids and the carabids *Poecilus corvus* (LeConte), *Pterostichus lucublandus* (Say) and *Amara* spp. consumed 40% to 73 % of eggs and ranked as intermediate predators of CLB eggs. *Nabis* spp. and the spiders *Pardosa* spp., *Pirata* spp. (Araneae: *Lycosidae*) and *Tetragnatha laboriosa* Hentz (Araneae: *Tetragnathidae*) did not feed on eggs or did so only infrequently so that the number of remaining eggs in these treatments did not significantly differ from the control treatment. I found an average of zero and four remaining CLB eggs when exposed to *Pterostichus melanarius* (Illiger) (Coleoptera: Carabidae) and *Agonum* spp., respectively. However, due to the low number of replication (4 and 3 individuals, respectively), I excluded these species from the analysis (Sokal & Rohlf, 2012).

4.4.2 Larval predation laboratory study

Six (2014) and eight (2015) predator taxa predated large numbers of CLB larvae in Petri dishes (2014: $\chi^2 = 55.6$, DF = 9, $P < 0.0009$; 2015: $\chi^2 = 87.6$, DF = 12, $P < 0.0001$; Fig. 9a and b, respectively). *Nabis* spp., the carabid beetles *P. corvus*, *P. lucublandus* and *P. melanarius*, staphylinids and the lady beetles *H. sinuata* and *H. quinquesignata* (2014 trial), consumed high numbers of larvae (< 25% CLB larval survivorship). Spiders *Pirata* spp., and *Pardosa* spp., lady beetles *Hippodamia parenthesis* (Say) (Coleoptera: Coccinellidae), *H. tredecimpunctata* and *C. septempunctata* and the carabid beetle *Amara* spp. consumed intermediate numbers of larvae

(resulting in 26 - 75% CLB larval survivorship), and were significantly different from the control. I found low rates of mortality (< 25 %) of CLB larvae exposed to *T. laboriosa* and Linyphiidae spiders; these mortality rates did not differ from the control.

4.4.3 Microcosm alternative prey study

Overall, 50% and 37% of tested predators in 2014 and 2015 trials, respectively, consumed significantly a high number of CLB eggs in the presence of aphids as an alternative food source, but preference varied with predator taxon (Table 4, significant predator by prey interaction). *Coccinella septempunctata* and *H. parenthesis* consistently preferred aphids over CLB eggs (Fig. 10). *Hippodamia tredecimpunctata* showed the same trend, but it was only significant in 2014 (Fig. 10). Staphylinidae (only tested in the 2014 trial) removed more eggs than aphids, and *P. melanarius* fed only on CLB eggs, but these differences were only significant for *P. melanarius* in the 2014 trial. The lady beetles *H. sinuata* and *H. quinquesignata* removed high numbers of both prey consistently. Finally, *Amara* spp. and *P. corvus* did not remove any CLB eggs or aphids, and *P. lucublandus* only removed 2 % of the CLB eggs (Fig. 10).

4.4.4 Egg predation field study

The effect of field was not significant, indicating that predation was consistent among fields (2014: $F_{9,9} = 2.8$, $P = 0.08$; 2015: $F_{9,9} = 0.96$, $P = 0.51$; Fig. 11). At 24 h, the number of eggs remaining on sentinel cards was significantly lower in the open treatment (i.e. exposed to predation) compared to the control, in both years (2014: $F_{1,9} = 64.7$, $P < 0.001$; 2015: $F_{1,9} = 18.38$, $P = 0.002$; Fig. 11). The average percentage of missing eggs increased from 20% in 2014 to 29% in 2015.

4.5 Discussion

My results confirmed the potential of generalist predators commonly found in wheat fields to suppress immature stages of CLB. Out of 15 taxa tested, six taxa consumed a high number of eggs (< 25% CLB egg survivorship), and eight taxa caused high larval mortality (< 25% CLB larval survivorship). On average lady beetles consumed a high number of eggs, with most species except *H. parenthesis* differing from the control. My results are consistent with past studies that have reported lady beetle predation on immature stages of chrysomelids (Chang & Snyder, 2004; Sebolt & Landis, 2004; Shade et al., 1970). Shade et al. (1970) observed predation on CLB immature stages by various *Hippodamia* sp. lady beetles in Indiana cereal fields. Similarly, Sebolt & Landis (2004) reported high and intermediate predation on *G. californiensis* egg by *C. maculata* (85%) and *C. septempunctata* (38%), respectively, in a laboratory study. In a laboratory study, Chang & Snyder (2004) reported approximately 25% predation rates over 72 h on *L. decemlineata* eggs by *Hippodamia convergens* Guerin-Meneville (Coleoptera: Coccinellidae). However, an earlier study found no evidence of predation on *L. decemlineata* eggs or larvae by *C. septempunctata* in 24 h laboratory trials (Hilbeck & Kennedy, 1996). In my study, *C. septempunctata* consumed 40% of CLB eggs in no-choice tests in 24 h, and most *Hippodamia* species consumed a high number of eggs. The higher predation rate of *C. septempunctata* on *G. californiensis* and CLB eggs might be due to the smaller size of those eggs relative to *L. decemlineata* eggs (Capinera, 2001; Philips et al., 2011). In general, previous findings and my results confirm that the aphidophagous species of ladybeetles tested in my experiments have a wide range of accepted prey (Hodek et al., 2012) and can contribute to CLB suppression.

I found high rates of egg and larval predation by most carabids except by *P. lucublandus* in 2015. Similarly, Alvarez et al. (2013) reported a high predation rate on eggs and larvae of *L. decemlineata* by *P. melanarius*, suggesting that carabids can be important predators of immature chrysomelids. On average, under a no-choice condition in Petri dishes, lady beetles consumed fewer larvae than eggs. Although most predator taxa fed on eggs and larvae, nabid bugs and spiders (*Pirata* spp. and *Pardosa* spp.) only consumed larvae. This is consistent with Chang & Snyder (2004), who reported low rates of *L. decemlineata* egg predation by damsel bugs and spiders. Similarly, Olmstead & Denno (1993) reported that piercing-sucking predators such as *Nabis americanoferus* Carayon (Hemiptera: Nabidae) are more effective against *Charidotella bicolor* (Fabricius) and *Deloyala guttata* (Olivier) (Coleoptera: Chrysomelidae) larvae than mandibulate predators such as *C. septempunctata*. They attributed the difference to differential deterrent effects of the fecal shield of chrysomelids on these predators. Based on my results, some large mandibulate carabids can be added to the guild of predators that prefer larvae over eggs of chrysomelids. Similar to my findings, Groden et al. (1990) reported preference of *C. maculata* adults for *L. decemlineata* eggs and first instar larvae over third and fourth instar larvae in laboratory trials. These results and mine provide evidence that a diverse assemblage of predators help to ensure multiple life stages of a given pest are attacked.

Overall, predation rates on CLB eggs were lower in potted plants with alternative prey than in no-choice tests. Several factors can explain this difference. For instance, a lower tendency in carabids and staphylinids to climb the wheat plants and access the target prey may result in low attack rates on plants. Also, longer searching times were most likely required to find the prey on actual plants relative to Petri dish arenas. Finally, the presence of *S. avenae* as alternative prey may have indirectly reduced predation rates on CLB eggs. Most lady beetles

except *H. parenthesis* still removed a significant number of CLB eggs in the presence of alternative prey, but their consumption rates were slightly lower than in the no-choice trial. The majority of lady beetles consumed more aphids than CLB eggs, but *H. sinuata* and *H. quinquesignata* did not show a preference and consumed a high number of both prey. A similar pattern was reported for *C. maculata*; the presence of *Myzus persicae* (Sulzer) and *Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae) reduced predation on *L. decemlineata* first instars (Grodén et al., 1990). For *Coccinella transversoguttata* (Faldermann) (Coleoptera: Coccinellidae), feeding on its alternative prey, *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae), allowed it to increase its egg production and continue to kill many of its normal prey, *Acyrtosiphon pisum* (Harris) (Hemiptera: Aphididae) (Evans et al., 1999). In my study, most carabids and staphylinids did not show preferences, except *P. melanarius* in the 2014 trial, in which it preferred CLB eggs over aphids, confirming taxon-specific responses of predators to alternative prey. I note that these results suggest that *P. melanarius* was able to climb the wheat plants in my pots to reach CLB eggs, and this behavior was observed directly in a couple of instances (A. Kheirodin, personal observation). On average, I observed considerably lower CLB egg predation by other carabids and staphylinids when alternative prey were provided in the more realistic arenas relative to the simple Petri dish arena used in the no-prey choice study. However, the fact that these species did not feed on alternative prey suggests that this decline is due to a reduced capacity to climb the plants (e.g., searching behaviour) rather than the presence of alternative prey. Similarly, Koss & Snyder (2005) found taxon-specific responses by predators, where *Geocoris* spp. feed on *L. decemlineata* eggs and aphids at a similar rate, but *Nabis* spp. preferred aphids over *L. decemlineata* eggs and lowered the predation on *L. decemlineata* eggs. In general, similar to my study, they found lower predation of *L.*

decemlineata eggs by generalist predators in the presence of aphids. My results suggest that the presence of alternative prey in cereal fields can moderately reduce the predation on CLB eggs by some predators in the short term. However, more field studies are required to determine if alternative prey can help to sustain predator populations and enhanced the overall suppression of CLB in the long term.

In the field study, the average number of missing eggs was higher in open cages relative to predator exclusion cages, suggesting predation levels between 20% to 29% over 24 h. My results are consistent with previous studies that reported moderate to high predation on *L. decemlineata* eggs in Massachusetts (Hazzard et al., 1991) and high consumption of *L. decemlineata* eggs and small larvae by *C. maculata* in Michigan potato fields (Grodén et al. 1990). A cage study with sentinel egg cards by Chang & Snyder (2004) revealed an average of 7% *L. decemlineata* egg predation in Washington potato fields. The same study reported that damsel bugs, pirate bugs (Hemiptera: Anthocoridae), big-eyed bugs (Hemiptera: Geocoridae), and crab spiders (Araneae: Thomisidae) were dominant predators in potato fields. These results highlight the fact that predation rates are site-specific, as the diversity and abundance of key predators will vary considerably across sites and regions. Nechols et al. (1996) reported a 20% to 30% *Galerucella* spp. (Coleoptera: Chrysomelidae) field egg predation (during early and late summer, respectively), potentially by *C. maculata*. In a molecular study, Greenstone et al. (2010) found *L. decemlineata* DNA in 85% of *Perillus bioculatus* (Fabricius) (Hemiptera: Pentatomidae), 75% of *P. maculiventris* and only in 11% of field-collected *C. maculata* specimens tested. My study confirmed that some of the common predator species present in wheat fields in the study area during mid to late June, including lady beetles (*C. septempunctata*,

H. parenthesis, *H. sinuata*, and *H. tredecimpunctata*), *Nabis* spp. and carabids might cause significant egg and larval mortality in the field.

I conclude from my laboratory and field studies that generalist predators present in wheat fields have the potential to contribute to CLB suppression. In laboratory trials I observed predator preference for different life stages of CLB, suggesting the potential for complementary predation by different species. In the choice trials, I found that some taxa preferred CLB eggs over aphids, but I observed the opposite preference in some species. Field studies further supported the importance of predators on CLB egg mortality. Further studies, including molecular gut content analysis of field-collected predators, are needed to confirm the predator species that contribute to CLB suppression under field conditions. This information will be a key step to develop a more comprehensive conservation biological control program that takes into account the valuable service provided by generalist predators in the management of crop pests such as CLB.

Table 3. Total number of predator taxa tested in various laboratory predation trials on cereal leaf beetle (CLB) immature stages during 2014 and 2015. Laboratory trials include no-choice tests with CLB eggs and larvae in Petri-dishes, and tests in potted plants with CLB eggs and *Sitobion avenae* aphids as alternative prey.

Predator taxa	Number of replications					
	2014			2015		
	Egg	Larvae	Alt. prey	Egg	Larvae	Alt. prey
Coleoptera: Coccinellidae						
<i>Hippodamia sinuata</i> Mulsant	7	6	9	7	10	9
<i>Hippodamia tredecimpunctata</i>	10	10	9	10	9	9
<i>Coccinella septempunctata</i> L.	10	10	9	10	10	9
<i>Hippodamia quinquesignata</i> (10	10	9	--	--	--
<i>Hippodamia parenthesis</i>	10	10	9	10	8	9
Coleoptera: Staphylinidae	10	10	6	3*	--	9
Coleoptera: Carabidae						
<i>Poecilus corvus</i>	--	--	--	7	7	9
<i>Poecilus lucublandus</i>	--	--	--	7	7	9
<i>Amara</i> spp.	10	5	9	3*	--	--
<i>Pterostichus melanarius</i>	4*	--	6	3*	7	9
<i>Agonum</i> spp.	3*	--	--	--	--	--
Hemiptera: Nabidae						
<i>Nabis</i> spp.	10	10	--	10	6	--
Araneae: Lycosidae						
<i>Pirata</i> spp.	--	--	--	6	9	--
<i>Pardosa</i> spp.	--	--	--	10	10	--
Araneae: Tetragnatidae						
<i>Tetragnatha laboriosa</i>	10	10	--	10	10	--
Araneae: Linyphiidae	2*	--	--	--	7	--
No predator	10	10	9	10	10	9

NOTE: * Predator taxon not included in non-parametric test due to low replication. Alt. prey:

Alternative prey.

Egg: no-choice egg trial. Larvae: no-choice larvae trial.

Table 4. Results of the ANOVA for the effect of predator treatment (predator taxa, whole-plot treatment), prey species (cereal leaf beetle (CLB) eggs versus adult English grain aphid, sub-plot treatment) on CLB egg and aphid survivorship after 24 h.

	2014 Trial				2015 Trial		
Source of variation	DF	F	P value		DF	F	P value
Predator	8, 14	42.1	0.0001		8, 16	46.0	0.0001
Prey	1, 16	11.7	0.0035		1, 18	8.4	0.0094
Prey \times predator	8, 16	5.3	0.0022		8, 18	5.8	0.0009

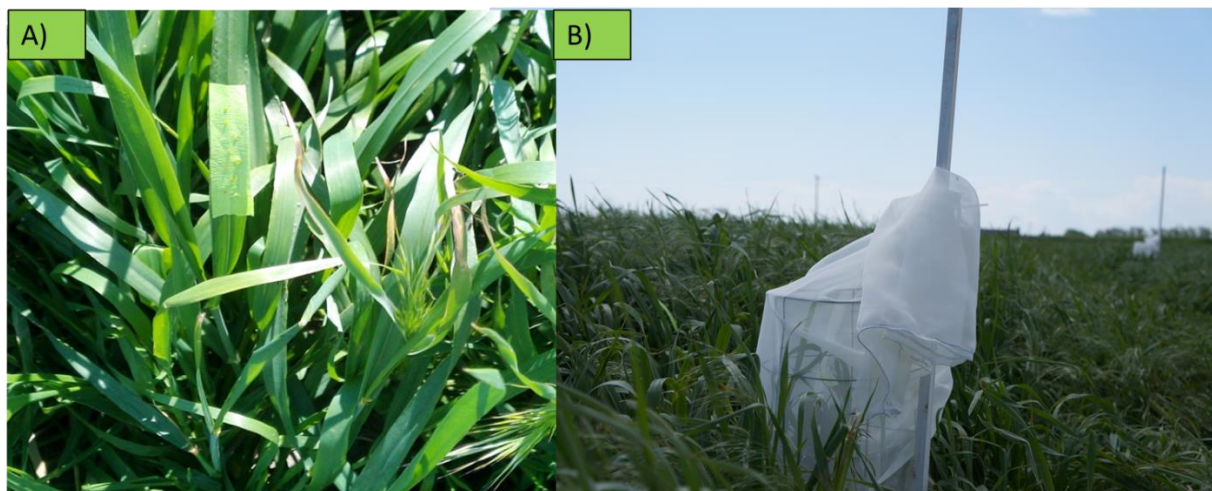


Figure 7. A) Sentinel egg cards with ten cereal leaf beetle (CLB) eggs in the open treatment (e.g. exposed to predation). B) Tomato cage covered by a fine mesh to protect the sentinel egg cards from predation in the control treatment.

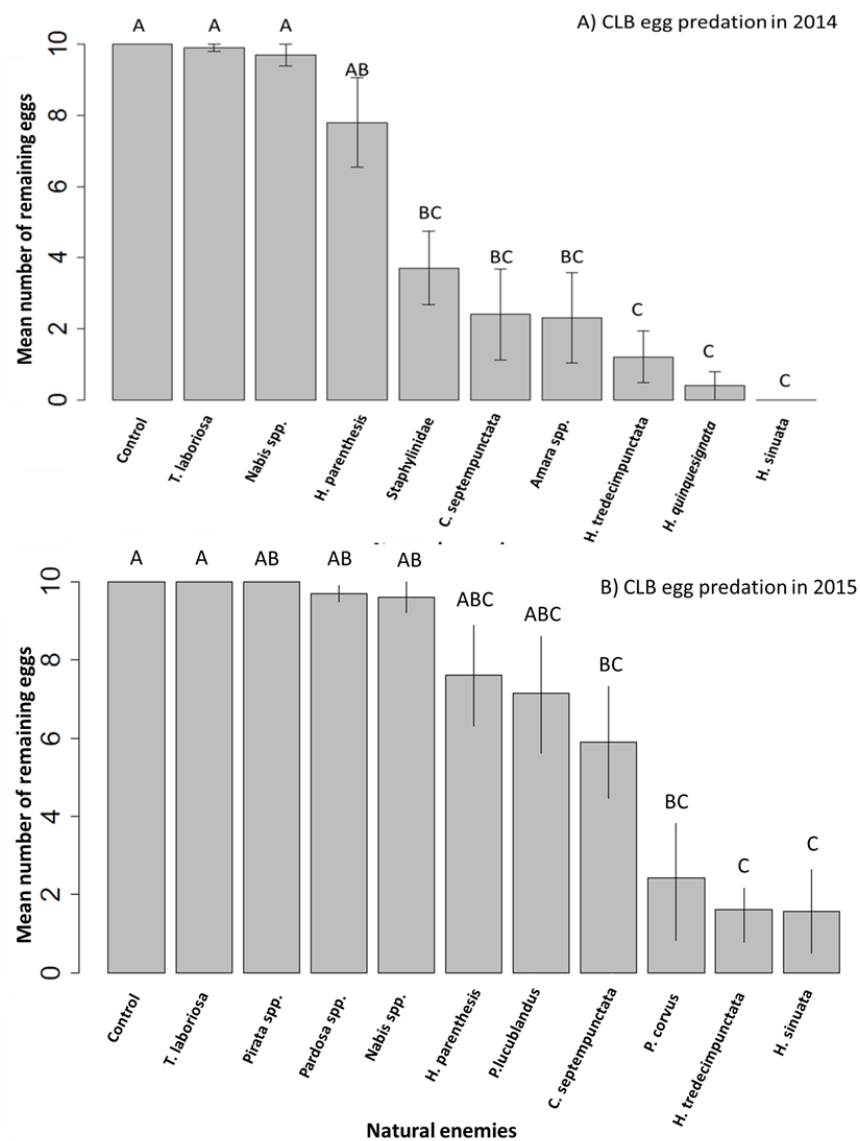


Figure 8. Mean number of remaining cereal leaf beetle (CLB) eggs \pm SE after exposure to different natural enemies (predator taxa) over 24 h in Petri dish laboratory tests conducted during A) 2014 and B) 2015. The letters indicate significant differences ($P < 0.05$) among the consumption rate of CLB eggs by various predator species using the sequential Bonferroni method. Bars followed by the same letter are not significantly different from one another.

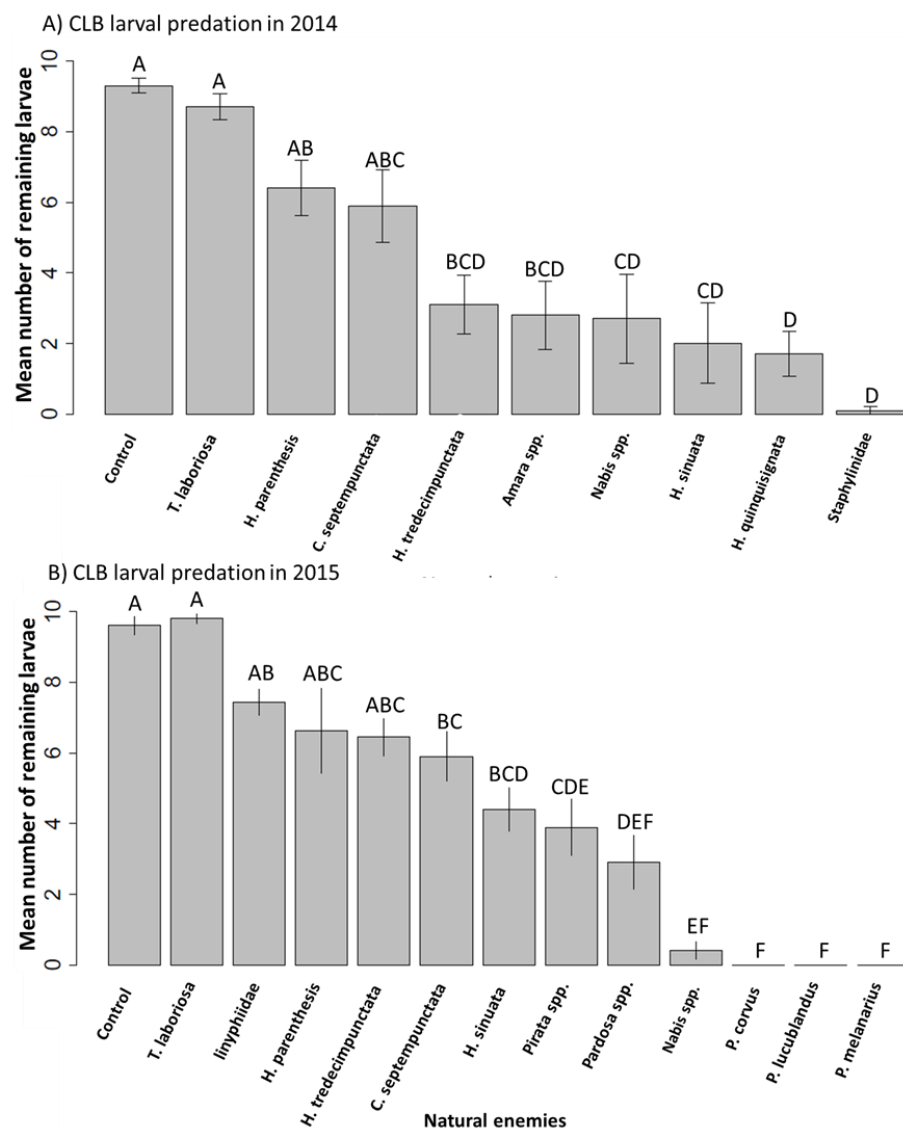


Figure 9. Mean number of remaining cereal leaf beetle (CLB) larvae \pm SE after exposure to various natural enemies (predator taxa) over 24 h in Petri dish laboratory tests conducted during A) 2014 and B) 2015. The letters indicate significant differences ($P < 0.05$) among the consumption rate of CLB eggs by various predator species using the sequential Bonferroni method. Bars followed by the same letter are not significantly different from one another.

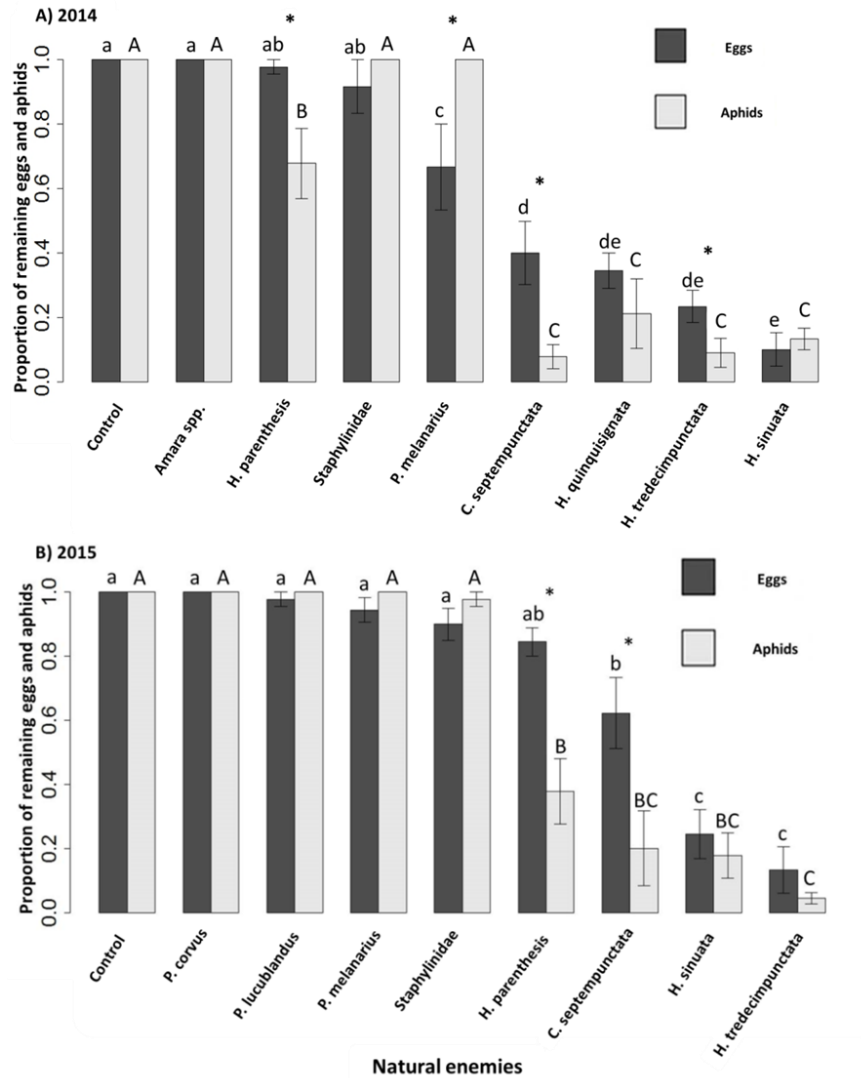


Figure 10. Mean proportion of remaining cereal leaf beetle eggs and aphids \pm SE exposed to different predator taxa in potted wheat plants after 24 h during A) 2014 and B) 2015. Significant differences ($P < 0.05$) between predator treatments are denoted in lower case letters for eggs and upper case letters for aphids, using the sequential Bonferroni method. Asterisks (*) indicate that the predator significantly prefers one prey over the other. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; other variables are not significant (i.e. $P > 0.10$). Bars followed by the same letter are not significantly different from one another

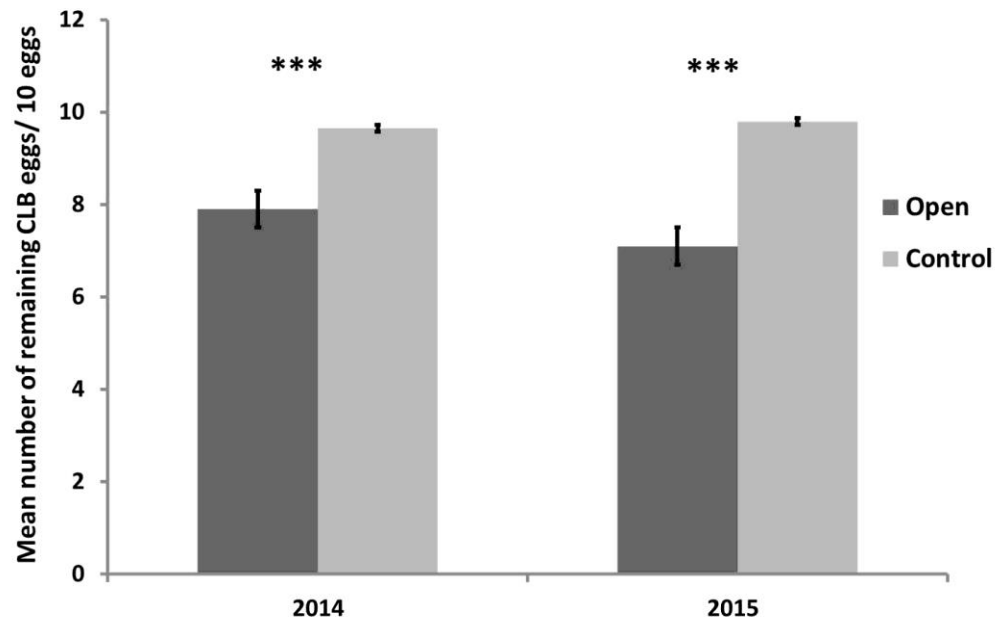


Figure 11. Mean number of remaining undamaged cereal leaf beetle (CLB) eggs on sentinel cards in open (i.e., exposed to ambient levels of predation) and control (protected by mesh cages) treatments \pm SE after 24 h of exposure in wheat fields in southern Alberta, Canada. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

CHAPTER 5

Consumption of cereal leaf beetle, *Oulema melanopus* L. (Coleoptera: Chrysomelidae) by generalist predators in wheat fields detected by molecular analysis

In this chapter, I developed a species-specific primer to detect CLB DNA in the gut content of predators. I used this new tool to provide empirical evidence that common generalist predators are feeding on CLB in cereals under field conditions. These results complement the findings of choice and no-choice laboratory experiments presented in chapter 4.

A slightly modified version of this Chapter has been accepted for publication in the *Journal of Entomologia Experimentalis et Applicata*.

5.1 Abstract

The cereal leaf beetle (CLB) *Oulema melanopus* L. (Coleoptera: Chrysomelidae) is a major pest of cereal crops that has recently been reported in western Canada. I developed a set of primers to detect CLB DNA in the gut of six common natural enemy predator taxa in wheat fields: lady beetles (20 positives out of 143 individuals), nabid bugs (73 positives out of 206 individuals), and wolf spiders (2 positives out of 25 individuals). *Nabis americanoferus* Carayon, 1961 (Hemiptera: Nabidae) and *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) were the most abundant predators in cereal fields, with 0.35 and 0.05 proportion of samples positive for CLB DNA, respectively. The prey DNA half-lives were used to adjust the estimates for *N. americanoferus* to 0.22, due to its longer DNA detectability relative to *C. septempunctata*. Overall, *Hippodamia parenthesis* (Say, 1824) (Coleoptera: Coccinellidae) had the highest proportion of positives at 0.43. There was a positive association between CLB abundance and proportion of *N. americanoferus* and *C. septempunctata* positives for CLB DNA. This study highlights the contribution of generalist predators to CLB mortality and their important role in conservation biological control for CLB. Furthermore, I provide a molecular tool that can be used to identify predators of CLB and predation frequency in agricultural fields.

Keywords: Molecular gut content analysis, cereal leaf beetle, half-lives, lady beetles, nabid bugs, wolf spiders.

5.2 Introduction

Generalist predators can be important mortality factors for agricultural insect pests and can contribute to IPM strategies for several economically important species (Symondson et al., 2002). Various methodologies have been used to determine key predators of specific pest taxa. These include laboratory choice and no-choice feeding trials (Grodén et al., 1990; Vankosky et al., 2011), field observations (Cloutier & Bauduin, 1995; Culliney, 1986; Greenstone, 1999), enzyme-linked immunosorbent assays (Stuart & Greenstone, 1990; Sunderland et al., 1987) and molecular gut content analyses (Greenstone et al., 2005; Harwood et al., 2004). The use of the polymerase chain reaction (PCR) techniques significantly increased the capability and accuracy of molecular techniques to detect predation events (King et al., 2008). In recent years, several species-specific primers have been developed to screen common predators of important agricultural pests using molecular gut content analysis, such as the Colorado potato beetle, *Leptinotarsa decemlineata* Say 1824 (Coleoptera: Chrysomelidae) (Greenstone et al., 2007), codling moth, *Cydia pomonella* (Linnaeus, 1758) (Lepidoptera: Tortricidae) (Unruh et al., 2016), flea beetles, *Phyllotreta* spp. (Coleoptera: Chrysomelidae) (Ekbom et al., 2014), cereal aphid, *Rhopalosiphum* spp. (Hemiptera: Aphididae) (Chen et al., 2000), soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae) (Harwood et al., 2007) and diamondback moth, *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae) (Ma et al., 2005).

The cereal leaf beetle (CLB) *Oulema melanopus* L. (Coleoptera: Chrysomelidae) was first reported in North America in Michigan in 1962 (Haynes & Gage, 1981), and has more recently been reported in Alberta, Canada in 2005 (Dosdall et al., 2011; Kher et al., 2011). The species originates from Europe and can cause 13 to 75% yield losses on winter wheat and barley, respectively (Buntin et al., 2004; Webster & Smith, 1979). Cereal leaf beetle has one generation

per year (Kher et al., 2011) and is typically active from mid-April to early September, with oviposition peaking in mid-May in southern Alberta (Kher et al., 2011; Kher et al., 2016). Both CLB larval and adult stages can cause damage to cereal plants through defoliation (Haynes & Gage, 1981). However, adult damage to cereal plants typically does not lead to a significant yield loss (Philips et al., 2011). Larval damage on the other hand, can result in a significant yield loss, and the fourth instar can cause up to 70% of the damage (Wilson et al., 1969). The immature stages of CLB (egg to fourth instar larvae) are not very mobile and usually complete their development on the same cereal plant (Kher et al., 2011). Given the low mobility of CLB immature stages and previous laboratory findings (Kheirodin et al., 2019), I hypothesized that foliar predators may be important mortality factors for CLB larvae in the field.

Several researchers have investigated the effect of parasitism on CLB control (Evans et al., 2015; Evans et al., 2006; Kher et al., 2014), but few studies evaluated predator impacts on CLB populations (Kheirodin et al., 2019; Shade et al., 1970). *Tetrastichus julis* (Walker, 1839) (Hymenoptera: Eulophidae) is a classical biological control agent imported from Europe, which significantly impacts CLB populations, with up to 60% parasitism reported in Utah (Evans et al., 2006). Even though *T. julis* is highly efficient for controlling CLB populations over time, it allows CLB larvae to continue feeding, thereby causing plant damage until pupation (Dysart et al., 1973). Thus, predators might play an important and complementary role in protecting cereal yields within the field season, due to their immediate impact on insect pests.

Generalist predators can be important mortality factors for Chrysomelidae beetles (Ekbohm et al., 2014; Greenstone et al., 2010; Shade et al., 1970). Greenstone et al. (2010) reported high predation (35-90%) of Colorado potato beetle, *L. decemlineata* by various taxa, including lady beetles (Coleoptera: Coccinellidae) and spined soldier bug, *Podisus maculiventris*

(Say) (Hemiptera: Pentatomidae) using molecular gut content analysis. Several important predator taxa have been associated with predation on chrysomelids, such as *Pardosa* spp. (Araneae: Lycosidae) (Ekbom et al., 2014), *P. maculiventris* (Hemiptera: Pentatomidae), *Coleomegilla maculata* (Degeer, 1775) (Coleoptera: Coccinellidae) (Giroux et al., 1995; Greenstone et al., 2007; Hilbeck et al., 1997) and *Pterostichus melanarius* (Illiger) (Coleoptera: Carabidae) (Alvarez et al., 2013). Based on field observations of CLB eggs and larvae predation, Shade et al. (1970) linked CLB immature stage mortality to the abundance of two lady beetles, *C. maculata* and *Hippodamia convergens* Guérin-Ménéville, 1842. Kheirodin et al. (2019a) also showed high CLB egg and larval predation by several common predators under laboratory conditions including: two species of Lycosidae spiders (Araneae), three species of *Hippodamia* and *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), four species of Carabidae (Coleoptera), one species of Staphylinidae (Coleoptera), and *Nabis* spp. (Hemiptera: Nabidae). However, a molecular gut content analysis of predators collected in cereal fields is necessary for predator species confirmation and frequency of predator species attack on CLB in the field.

The main purpose of this study was to determine which species of common generalist predators present in cereal fields feed on CLB. My goals were (1) design a set of specific primers to detect CLB DNA in the gut content of predators, (2) determine the predator species feeding on CLB in cereal fields using molecular gut content analysis, and (3) estimate the half-life digestion time for the most common predators found in cereal fields.

5.3 Materials and methods

5.3.1 Cereal leaf beetle (CLB) specific primer design

To design primers to specifically amplify CLB DNA, I targeted the cytochrome oxidase 1 (*COI*) barcoding region because it is easy to amplify, has been utilized before (Lunt et al., 1996), and has a large comparative sequence database in BOLD, The Barcode of Life Data System (Ratnasingham & Hebert, 2007). For *in silico* primer design and specificity testing, sequences were downloaded from the National Center for Biotechnology Information (NCBI) from taxa closely related to CLB (other Chrysomelidae) as well as common predator species present in Lethbridge cereal fields (Appendix 2). Taxa were selected based on their taxonomic relation to CLB. I obtained sequences from species within the chrysomelid subfamily Criocerinae (from NCBI nucleotide search engine) first, and then expanded my search to other chrysomelids that are common in western Canada (e.g., Colorado potato beetle, flea beetle, red turnip beetle). Further, common species in the Lethbridge area cereal fields and grasslands that are potential predators of CLB were included (Kheirodin et al., 2019). Sequences were checked and aligned in Bioedit (version 7.2.5, Hall, 1999) using the reading frame as a guide. Sequences of CLB and closely related species were visually inspected for regions that may be specific to the CLB *COI* sequences. Regions within sequences with the highest number of nucleotide mismatches between CLB and other non-target species were selected as potential sites to design CLB specific primers. Primer3 v.0.4.0 (Untergasser et al., 2012) was utilized to aid design for a pair of putatively specific primers for CLB. Given that each primer was designed with one IUPAC ambiguity code, there were four possible primer combinations. All combinations were tested against the non-redundant nucleotide database (NCBI) using Primer-BLAST (Ye et al., 2012) with the following search settings to further assess *in silico* specificity: targeting only organisms matching the

classes of Insecta, Arachnida, and prokaryotes; 0-2 total mismatches to CLB; four total mismatches to unintended targets; and ignoring targets with four or more mismatches to the primer. The four mismatches were selected to be more stringent as six led to many unrealistic hits to non-targets (Lefever et al., 2013). Because no arachnids had less than six total mismatches, and there were no prokaryotes in the primer blast, they were not included in the Appendix (3). All other parameters were set to default values.

5.3.2 Molecular Protocols

DNA was extracted from field caught CLB and predators (Table 5 & 6) using the DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer's protocol for tissue samples. Prior to DNA extraction the laboratory bench surface was carefully washed with 99% ethanol. Before DNA extraction, all specimens were washed using 95% ethanol and dried for 5 min. The head was removed from CLB larvae and the remainder of the body extracted. For predators, the legs, heads, and elytra were removed from specimens to minimize the possibility of DNA contamination within sweep net samples (e.g., contamination by CLB larval feces), and the remaining body parts (including the gut) were placed in 1.5 ml collection tubes for extraction. For spiders, the legs and prosoma were removed, and the opisthosoma (abdomen) was extracted (Macías-Hernández et al., 2018). An individual sterilized petri dish (60 mm diameter x 15 mm height, VWR) was used to process each predator sample (removing sample body parts) to avoid direct contact with the laboratory bench and minimize any probable contamination. The concentration of extracted samples was checked using a Nanodrop 8000 Spectrophotometer (Thermo Fisher Scientific). Polymerase chain reaction (PCR) conditions for the designed primers were optimized at four different concentrations of $MgCl_2$ (1.25 mM, 1.5 mM, 2 mM and 2.5 mM; New England Biolabs) in 25 μL reactions and four different annealing temperatures (52 °C,

54 °C, 56 °C and 58 °C). The strongest bands were observed with 2.5 mM of MgCl₂ and an annealing temperature of 56 °C. DNA was amplified using 0.2 mM of dNTPs (New England Biolabs), 400 nM of each primer (Integrated DNA Technologies), 2.5 mM of MgCl₂, 1 unit of *Taq* DNA polymerase (Qiagen), 1X *Taq* reaction buffer (10 mM Tris-HCl, 50 mM KCl and 1.5 mM MgCl₂, New England Biolabs), 0.11- 0.18 µg of sample DNA, and purified water (Thermo Fisher Scientific) to a final volume of 25 µL. The PCR reactions were carried out in a T100 Thermal Cycler (Bio-Rad) with initial denaturation at 95 °C for 1 min, followed by 34 cycles of 15 s denaturation at 95 °C, 15 s annealing at 56 °C, 45 s extension at 72 °C, and a final extension at 72 °C for 4 min. The PCR products were run on 1% agarose gel (Bio-Rad) containing 4 µL of Gel Red (Biotium) and visualized using a Gel DocTM EZ Gel documentation system (Thermo Fisher Scientific). Sequencing was completed at the University of Kentucky Health Care Genomics Centre, Lexington, KY.

5.3.3 Primer specificity and sensitivity testing

I also performed *in vivo* specificity testing by assessing if the primers amplified the extracted CLB but did not amplify any collected predators that were starved for 48h or non-target species (Table 5). The primers were tested against 4 – 6 specimens (depending on availability during tests) of 30 species from six different orders (Table 5). For these tests, insect specimens were collected from various crops and adjacent habitats in the Lethbridge area, using various methods (e.g., sweep nets, pitfall traps, etc.), and from lab colonies. Further, for predators screened for CLB DNA in their gut, all individuals of each predator species with positive results were sequenced (Appendix 4) to confirm the amplified DNA belonged to CLB. A subset of these sequences was uploaded to Genbank under Accession numbers MK277358-MK277369. The expected target COI gene fragment size was 286 bp. To test the sensitivity of

primers, extracted CLB DNA was diluted into five 10-fold serial dilutions (1:10 - 1:10000) by adding distilled water to 1 μ L of CLB DNA. For each dilution, five replications were performed.

5.3.4 Estimation of cereal leaf beetle predation in Alberta cereal fields

Samples of CLB and predators were collected in southern Alberta from 25 and 19 wheat fields, in 2014 and 2015, respectively. These cereal fields were selected after confirming the presence of CLB eggs or larvae through field observations. At each field, six samples were collected along two transects, 20 and 100 m from the field edge. The three sampling locations within each transect were 20 m apart. Each sample contained 50 sweeps (180 degrees), which was collected by walking 50 m parallel to the field border. Immediately after collection, all potential predators were separated using an aspirator and transferred to 250 ml vials with 95% EtOH to avoid DNA degradation and contamination. In the laboratory, each predator was transferred to a 25 ml vial to avoid the possibility of contamination. Predators were identified to species, genus or family, using regional keys (Acorn, 2007; Dondale & Redner, 1990; Dondale et al., 2003; Lindroth, 1961). Voucher specimens were deposited at the Wallis-Roughley Museum of Entomology at the University of Manitoba.

Depending on availability, I extracted DNA (as described above) from a maximum of ten randomly selected individuals of each potential predator species from each cereal field (Table 6), and the remaining intact individuals were preserved in 95% EtOH at -20 °C. I only processed predator species that showed predation potential against CLB in previous laboratory feeding trials (Kheirodin et al., 2019a, and Table 6). The only abundant predator species that was excluded from these tests was *Tetragnatha laboriosa* Hentz, 1850 (Araneae: Tetragnathidae), which did not feed on CLB immature stages under no-choice conditions (Kheirodin et al., 2019).

5.3.5 Half-life detectability

To estimate CLB DNA detectability in the gut of predators, a series of half-life detectability tests were performed at the University of Manitoba. About 500 CLB adults were collected with sweep nets from overwintering habitats (i.e., field roadside perennial grasslands) near Lethbridge, Alberta, Canada (49°46'54.59"N. 112°33'13.25W) to start a colony at the University of Manitoba. The colony was maintained in two cages (92 × 49 cm), under controlled conditions (21 ± 2 °C, 16L: 8D and RH = 21 ± 3%). Predators were collected from several cereal and alfalfa fields near Winnipeg and Carman, Manitoba, as they were not available in sufficient numbers in Alberta in 2016 (the year I estimated the half-life). Only *Nabis americanoferus* Carayon, 1961 (Hemiptera: Nabidae) and *C. septempunctata* were collected in enough numbers for half-life feeding trials. Predators were stored in transparent plastic tubs (15 × 25 cm) at 10 °C in a growth chamber and fed daily with water and ten English grain aphids, *Sitobion avenae* (Fabricius, 1794) (Hemiptera: Aphididae). All predators were starved for 48h prior to the feeding trials and only provided with water.

All feeding trials were performed in growth chambers, and using conditions based on average field temperatures (day: 16L, 24 °C; night: 8D, 19 °C). A standard protocol developed by Greenstone et al. (2010) was followed. In brief, a single laboratory reared CLB second instar larvae was used to standardize the amount of food that each predator consumed. Cereal leaf beetle larvae were selected for this study because it was the most abundant stage when predators were collected in Alberta (see results). For each interval, one predator was fed with one English grain aphid as a negative control. A specific number was assigned to each Petri dish and further grouped into 10 blocks (to eliminate any possible effect of light or location on digestion). Then a time interval was randomly assigned to each Petri dish within each block. The predators were

monitored until full consumption of CLB larvae occurred and the exact feeding time was marked on each Petri dish. Groups of ten (*C. septempunctata*) and six (*N. americoferus*) predators were removed at 0, 4, 8, 12, 16, 24 and 48 h after feeding, and stored in -20 °C in a 5 ml vial with 95% EtOH. Afterward, DNA extraction was performed, and predators were screened for the detectability of CLB DNA in their gut over the time series. I followed the method of Chen et al. (2000) to weight the relative importance of the proportion of positive individuals for CLB DNA. In brief, the shortest half-life (*C. septempunctata*) was set equal to one and was divided by the half-life of *N. americoferus* to calculate a detectability weighted value for *N. americoferus*. Then, the raw proportion of *N. americoferus* positive for CLB DNA was multiplied by its detectability weighted value to estimate the adjusted proportion of positives.

5.3.6 Statistical analysis

All statistical analyses were conducted using R (R version 3.3.3, R Development Core Team, 2017). A contingency table test was used to evaluate the overall differences in CLB proportion positives among tested predators. The proportion of predators with CLB DNA per field was calculated by dividing the number of positives by the total number of individuals tested per field. Then, the total proportion of positives per predator species was estimated by dividing the number of individuals tested positive for CLB DNA by its total number collected across all fields. Spearman correlation tests were used to assess associations between CLB field abundance and consumption of CLB by predators (proportion positives). Data from fields with at least four *N. americoferus* and *C. septempunctata* individuals in each field were used in this analysis. The variables were the average CLB larval abundance in each field (per 50 sweeps) and the proportion of predators that fed on CLB in these fields (estimated per field). Probit regression models were used to analyze CLB detectability half-life data. The response variable was the

presence or absence of CLB DNA in the gut content of predators fed with CLB at different time intervals. Finally, I ranked the predator species based on the proportion of CLB positive individuals sampled.

5.4 Results

5.4.1 Primer specificity and sensitivity

For *in silico* sensitivity testing, hits to the primer included several species of *Oulema* (with 0 - 4 mismatches), but also a few other species in other coleopteran families, such as Elateridae, Scirtidae, Ciidae, and Curculionidae, and one species of Tephritidae (Diptera) (Appendix 3). The designed primer pair: CLBCOIF, 5'-GCTTCGGWAATTGATTAGTTC -3' (56 °C melting temperature, 38% GC, 21 bp), and CLBCOIR, 5'-CGTATATTTYTTACTGTGGAGATGA -3' (57 °C melting temperature, 36% GC, 25 bp) successfully amplified CLB with a 286 bp fragment of COI DNA, but no other non-target species were found through *in silico* testing. There was little concern for other species of *Oulema* since *O. melanopus* is the only species reported in North America (Bezdek & Baselga, 2015; LeSage et al., 2007). All other taxa had at least four overall mismatches to the primer pair (Appendix 3). While the amplification of some non-targets was possible, none of these non-targets pose a major concern because the species are not present in North America or have biologies that would make predation by my field collected predators unlikely (e.g., Scirtidae are subaquatic marsh beetles; wireworms, spend most of their life underground and it is unlikely that the foliage predators tested feed on them).

The CLB DNA expected band size was 286 bp, and all positive results viewed through electrophoresis were at this length. Further confirmation of the specificity of my primer was

obtained by sequencing of the 95 positive samples of gut-screened predators with amplicons (Appendix 4). Ninety four of 95 sequences matched to CLB with a minimum of 98% identity (using NCBI default parameters for Megablast) and 36.8 % of sequences matched with 100% similarities, confirming that non-target amplification was very rare. The only non-target amplification was matched to *Leptopterna dolabrata* L. (Hemiptera: Miridae) with 99% similarities, which was detected from the gut of one *N. americoferus*. For further verification, DNA from *L. dolabrata* was screened against CLB primers, which resulted in amplification of the species DNA at low quality (faint bands). All other sequences were assigned to *O. melanopus* in the barcode of life database index number (BIN): AAK5928 (Appendix 4). The primers also did not amplify any of the predators-only DNA (Table 5), further suggesting specificity. The primer sensitivity test confirmed the amplification of CLB DNA in dilution concentrations as low as 1×10^{-2} CLB.

5.4.2 Field-collected predator screening

Out of 378 field-collected predators, 94 contained the CLB DNA in their gut as determined by CLB ~ 286 bp *COI* gene fragment amplification (Table 6). There were significant differences in the proportion of positives among predator species ($\chi^2 = 35.4$, DF = 6, $P < 0.01$). *Nabis americoferus* and *C. septempunctata* were the most commonly collected predators with 35% and 5% positive samples, respectively (Table 6). *Hippodamia parenthesis* (Say, 1824) (Coleoptera: Coccinellidae) had the highest incidence of positives with 43%. However, its abundance in cereal fields was lower than *C. septempunctata* and *Hippodamia sinuata* Mulsant, 1850. Other *Hippodamia* species had lower than 25% incidence of positives. Cereal leaf beetle DNA was detected in 13% of *Pardosa* spiders, but no individuals of *Pirata* spp. were positive for CLB DNA. Sweep net samples indicated that the populations of CLB were dominated by larvae

(Table 6), with a very small number of adults present (0.5 ± 1.1 adults/ 50 sweeps/ field). There was a positive correlation between CLB abundance (CLB larvae/ 50 sweeps/field) and the proportion of positives for *N. americoferus* ($r = 0.61$, $DF = 37$, $P < 0.01$) and a similar (but not significant) trend for *C. septempunctata* ($r = 0.43$, $DF = 6$, $P = 0.29$) per field. My best estimates of the predator importance ranking based on the raw incidences of positives are *H. parenthesis* > *N. americoferus* > *H. tredecimpunctata* > *H. sinuata* > *Pardosa* spp. > *C. septempunctata*.

5.4.3 Half-life adjustments

Only two predator species were abundant enough to test for half-life detectability: *N. americoferus* and *C. septempunctata*. The detectability of CLB DNA was longer in *N. americoferus* with 21.6 h relative to *C. septempunctata* with 13.8 h (Fig. 12). There were significant differences from zero in the slopes of probit models for *N. americoferus* and *C. septempunctata* (Slopes= -0.1055 and -0.0943; $\chi^2 = 29.27$ and 25.23, respectively, $DF = 1$, $P < 0.0001$). I allocated the weight of 1 and 0.63 to *C. septempunctata* and *N. americoferus*, respectively. Addition of the half-life values to the raw data reduced the relative importance of *N. americoferus* due to its longer detectability, from 35% to 22% in relation to *C. septempunctata* (Table 6).

5.5 Discussion

This is the first study to report field levels of CLB predation using molecular gut content analyses. I developed a pair of specific primers for the genus *Oulema*, which includes *O. melanopus*, a significant pest of cereals in North America. The primer targeted and amplified a 286 bp fragment of the barcoding region of the *COI* gene for CLB, which was confirmed by DNA sequencing for 94 out of 95 sequenced samples. A replicated PCR and gel electrophoresis assay was performed when there were multiple bands or a significant smear was observed in the

gel. All positive results were further sequenced to ensure that none of the positive PCR products were false positives. The NCBI data screening of the positive predators suggested that none of the potential non-target species that appeared in the primers *in silico* evaluation (Appendix 4) were amplified in this study. However, *L. dolabrata* was unexpectedly amplified using CLB primers. This species did not appear in the primer blast results as a potential non-target species. The further follow-up of sequencing *L. dolabrata* amplicons using the CLB primer confirmed the positive amplification. *Leptopterna dolabrata* was present in cereal fields at very low numbers, with only two individuals found in the 37 fields sampled. This species is an herbivore of forage grasses (e.g., alfalfa fields) and could be more abundant in field debris and grasslands close to cereal fields (Day, 1995; 2005). The one positive for this species could be due to its low abundance in the cereal fields, or a low preference of the predator species tested in this study for *L. dolabrata*. Moreover, the BLAST results suggested that the amplification of Elateridae as a target for amplification using primers for CLB DNA was not an issue in this study. While several species of Elateridae could be present in cereal fields, as wireworms are common agricultural pests, their immature stages are subterranean and feed on crop roots (Parker & Howard, 2001). Therefore, it is unlikely that predator species tested in this study encounter the immature stages of these species in cereal fields. Altogether, these results suggest high reliability of the primer pair in detecting CLB DNA in predators, but future studies using this primer pair in regions that *L. dolabrata* and other potential non-target species are present (most non-target species are not present in Canada) should consider sequencing their positive PCR products to ensure the amplification of *O. melanopus*.

This is the first field study to report CLB predation by six predator species commonly found in cereal fields: *H. parenthesis*, *N. americanoferus*, *H. tredecimpunctata*, *H. sinuata*,

Pardosa spp., and *C. septempunctata*. Cereal leaf beetle DNA was detected in 25.2% of field predators, suggesting CLB is an important food resource for predator populations in cereal fields. The overall percentage of positives found in this study is lower than the 46% levels reported for *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae) by Greenstone et al. (2010), but higher than the 15% reported by Ekbom et al. (2014) for flea beetles. The proportion of positives for CLB DNA was positively associated with CLB larval densities in cereal fields and showed that predators may switch their preference towards CLB. In my sampled fields, *N. americanoferus* and *Hippodamia* species had high incidences of positives and were abundant (*N. americanoferus* was three times more abundant than any other predator), suggesting they are the most important predators of CLB.

Kheirodin et al. (2019a) observed that up to 80% of CLB larvae (10 given larvae) were consumed daily by *N. americanoferus*, and up to 65 and 55% of CLB eggs and larvae were consumed by *Hippodamia* species, respectively. However, the proportion of positives observed in *Hippodamia* species for CLB DNA varied by species and between the laboratory no-choice trials (Kheirodin et al., 2019), and in this study. While, *H. parenthesis* consumed the lowest numbers of larvae compared to other *Hippodamia* species in laboratory no-choice assays (Kheirodin et al., 2019), it had the highest proportion of positives in the current study. On the contrary, while other *Hippodamia* species had a high rate of CLB eggs and larvae predation in the laboratory no-choice study, they had a low rate of positives in this study. Although I did not quantify the abundance of alternative prey in my samples, cereal aphid, and the rice leaf bug, *Trigonotylus caelestialium* (Kirkaldy) (Hemiptera: Miridae) were the most common potential alternative prey. Given the well documented preference of lady beetles for aphids (Grodén et al., 1990; Kheirodin et al., 2019; Koch et al., 2005; Musser & Shelton, 2003), it is expected that

some *Hippodamia* species fed on CLB at a lower rate in the field where aphids were present. Furthermore, Shade et al. (1970) observed predation on CLB immature stages by *H. tredecimpunctata*, *H. parenthesis*, and *H. convergens* Guérin-Ménéville 1842 in Indiana cereal fields. I conclude from the previous studies and mine that predation by foliar piercing-sucking (e.g., damsel bugs) and mandibulate predators (e.g., lady beetles) represents a significant mortality factor for chrysomelids and contribute to CLB suppression in western Canada. Foliar predators were the main focus of this study due to the high rate of predation on eggs and larvae shown in laboratory studies, but future studies should also assess field predation levels caused by ground predators, as some species were also important in laboratory tests (Kheirodin et al., 2019).

Half-life detectability was longer for CLB DNA (286 bp *COI* fragment) in *N. americanoferus* relative to *C. septempunctata*, resulting in a 64% higher chance of detecting positives. This result supports earlier studies that reported longer half-lives for hemipteran compared to coleopteran species. Greenstone et al. (2010) reported 60.5 h and 26.4 h detectability of half-lives for *Perillus bioculatus* (Fabricius, 1775) (Hemiptera: Pentatomidae) and *C. maculata* fed a single *L. decemlineata* egg, respectively. Similarly, Hosseini et al (2008) reported a longer half-life for *Nabis kinbergii* (Reuter, 1872) (Hemiptera: Nabidae) with about 36 h compared to *Hippodamia variegata* (Goeze, 1777) (Coleoptera: Coccinellidae) with 17.1 h, fed a single fourth-instar larva of *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae). Similar to previous findings, when I adjusted the raw proportion data with the weighted value, the importance of lady beetles increased relative to hemipteran predators due to their shorter detectability of half-lives. Future studies should focus on calculating half-lives for more species to better understand their relative importance. Altogether, I conclude that lady beetles such as *C.*

septempunctata have shorter detectability of half-lives relative to hemipterans such as *N. americanoferus*, and their proportion of positives should be treated with higher weight. However, due to their overall low abundances in wheat fields, their contribution might have been marginal relative to *N. americanoferus*, which was abundant and likely was the primary contributor to CLB control.

In this study, foliar predators had a higher incidence of predation relative to ground predators. While the overall proportion positive for foliar predators was 27.3%, only 7.2% of ground predators tested positive for CLB DNA. Kheirodin et al. (2019a) reported an average of 65% CLB larvae predation by wolf spiders *Pardosa* spp. and *Pirata* spp. However, here the CLB DNA was only amplified from 13% of field-collected *Pardosa* spp. and none from *Pirata* spp. (regardless of their longer detectability of DNA reported by Hosseini et al. (2008) for diamondback moth), suggesting these spiders may prefer other prey under field conditions or have a lower probability of encountering CLB. My result is in line with Lundgren et al. (2009) who reported that out of 1550 field-collected ground predators only 10.7% of individuals including Lycosidae spiders and carabids were positive for *Diabrotica virgifera* LeConte, 1868 (Coleoptera: Chrysomelidae) DNA. The same authors reported that Lycosidae, in particular, had an 18.6% frequency of predation and were among the best predators of *D. virgifera* immature stages. Similarly, Ekbom et al. (2014) reported that 19.4% of field-collected *Pardosa* spp. fed on *Phyllotreta* species (Chevrollet) (Coleoptera: Chrysomelidae). The sweep net samples taken in this study contained few spiders and carabid beetles and might have underestimated their relative contribution in CLB control. Therefore, caution should be used to interpret the relative importance of these predators in CLB control compared to foliar predators.

This study determined for the first time predation on CLB in the field by several common predator taxa present in cereal fields using molecular methods. My findings also confirm the reliability of laboratory feeding trials in the detection of potential predators of agricultural pests. My study was conducted when most CLB were at the larval stage and only a few adults were present; I also expect that there were very low numbers of CLB eggs in the wheat fields when I collected the samples. Therefore, future research should focus on early season sampling to determine egg predators of CLB, which can reduce early colonization of CLB in cereal fields. The primers were sensitive, but not across all dilutions, suggesting that future studies using this primer set should ensure its sensitivity for detection of CLB eggs in the guts of predators. However, the detectability of the CLB DNA would depend upon the number of eggs consumed and the time since feeding. The previous laboratory study (Kheirodin et al., 2019), suggested that various generalist predators are capable of consuming at least 10 CLB eggs daily. Future studies should consider performing half-life detectability trials for one and three CLB eggs (CLB lay eggs singly or in a cluster of one to three eggs, (Helgesen & Haynes, 1972)), to understand better the chance of detecting CLB eggs in the gut of predators. My study provides a useful tool for monitoring predator impacts in this system, with the ultimate goal of incorporating predators into integrated pest management of CLB.

Table 5. Predator taxa, target and non-target species tested for primer specificity against the cereal leaf beetle (CLB) CLBCOIF and CLBCOIR specific primers. The “Gel Result” provides information on whether CLB primer amplified DNA (+) or not (-) for the insects listed.

Taxa tested	# Tested	Gel result	Location
Starved predator taxa			
Araneae			
Lycosidae			
<i>Pardosa</i> spp.	4	-	Lethbridge, Ab
<i>Pirata</i> spp.	4	-	Taber, Ab
Tetragnathidae			
<i>Tetragnatha laboriosa</i> Hentz, 1850	4	-	Coaldale, Ab
Linyphiidae	4	-	Lethbridge, Ab
Insecta			
Coccinellidae			
<i>Coccinella septempunctata</i> Linnaeus, 1758	10	-	Lethbridge, Ab
<i>Hippodamia tredecimpunctata</i> (Linnaeus, 1758)	4	-	Lethbridge, Ab
<i>Hippodamia sinuata</i> Mulsant, 1850	4	-	Taber, Ab
<i>Hippodamia quinquesignata</i> (Kirby, 1837)	4	-	Taber, Ab
<i>Hippodamia parenthesis</i> (Say, 1824)	4	-	Lethbridge, Ab
Carabidae			
<i>Poecilus corvus</i> (LeConte, 1837)	4	-	Coaldale, Ab
<i>Poecilus lucublandus</i> (Say, 1823)	4	-	Coaldale, Ab
<i>Amara aenea</i> (DeGeer, 1774)	4	-	Coaldale, Ab
<i>Pterostichus melanarius</i> (Illiger, 1798)	4	-	Coaldale, Ab
<i>Agonum</i> spp.	4	-	Coaldale, Ab
Staphylinidae	4	-	Lethbridge, Ab
Nabidae			
<i>Nabis americanoferus</i> Carayon, 1961	10	-	Lethbridge, Ab
Reduviidae			
<i>Reduvius</i> spp.	4	-	Lethbridge, Ab
Syrphidae			
<i>Platycheirus</i> spp.	5	-	Coaldale, Ab
Target species			
Chrysomelidae			
<i>Oulema melanopus</i> (Linnaeus, 1758)	10	+	Lethbridge, Ab
Non-target species			
Chrysomelidae			
<i>Leptinotarsa decemlineata</i> (Say, 1824)	4	-	Winnipeg, Mb
<i>Entomoscelis americana</i> Brown, 1942	4	-	Winnipeg, Mb
<i>Phyllotreta striolata</i> (Fabricius, 1801)	6	-	Winnipeg, Mb
<i>Phyllotreta cruciferae</i> (Goeze, 1777)	6	-	Winnipeg, Mb
Miridae			
<i>Trigonotylus caelestialium</i> (Kirkaldy, 1902)	10	-	Coaldale, Ab
Aphididae			
<i>Sitobion avenae</i> (Fabricius, 1794)	10	-	Taber, Ab
Tipulidae			
<i>Tipula</i> spp.	4	-	Taber, Ab
Cecidomyiidae			
<i>Sitodiplosis mosellana</i> (Gehin, 1857)	5	-	Winnipeg, Mb
Eulophidae			
<i>Tetrastichus julis</i> (Walker, 1839)	4	-	Lethbridge, Ab

Ichneumonidae	4	-	Lethbridge, Ab
Thripidae			
<i>Limothrips denticornis</i> (Haliday, 1836)	4	-	Taber, Ab

Table 6. The result of the molecular gut content analysis of predators to determine if they have consumed cereal leaf beetle (CLB). Predators were collected in 41 wheat fields near Lethbridge, Alberta, in 2014 and 2015 during the late field season in July. “Total # of positives” represents individual predators containing CLB DNA in their gut, confirmed by polymerase chain reaction for CLB cytochrome oxidase 1 DNA region.

Species	Total # Screened ¹	Total # Positives	Proportion of positives	Adjusted proportion ²	Fields ³	CLB/50 sweeps ⁴
<i>Nabis americoferus</i>	206	73	0.35	0.22	39/44	16.4 ± 38.2
<i>Coccinella septempunctata</i>	72	4	0.05	0.05	25/44	17.4 ± 45.6
<i>Hippodamia tredecimpunctata</i>	13	3	0.23	--	6/44	6.6 ± 7.8
<i>Hippodamia parenthesis</i>	16	7	0.43	--	10/44	4.7 ± 6.1
<i>Hippodamia sinuata</i>	41	6	0.14	--	13/44	4.9 ± 7.5
<i>Pardosa</i> spp.	15	2	0.13	--	8/44	48.8 ± 53.1
<i>Pirata</i> spp.	10	0	0	--	6/44	9.2 ± 8.7
<i>Poecilus</i> spp.	2	0	0	--	1/44	4.5 ± 0

¹ Number of predators collected per species in wheat fields using a sweep net in 2014 & 2015.

² Proportion of positives after multiplying the raw proportion with the weighted value of each predator.

³ Number of fields with the presence of the particular predator species/ total number of fields sampled.

⁴ Average number of CLB larvae/50sweeps (± SD) found in fields with the presence of the listed predator species.

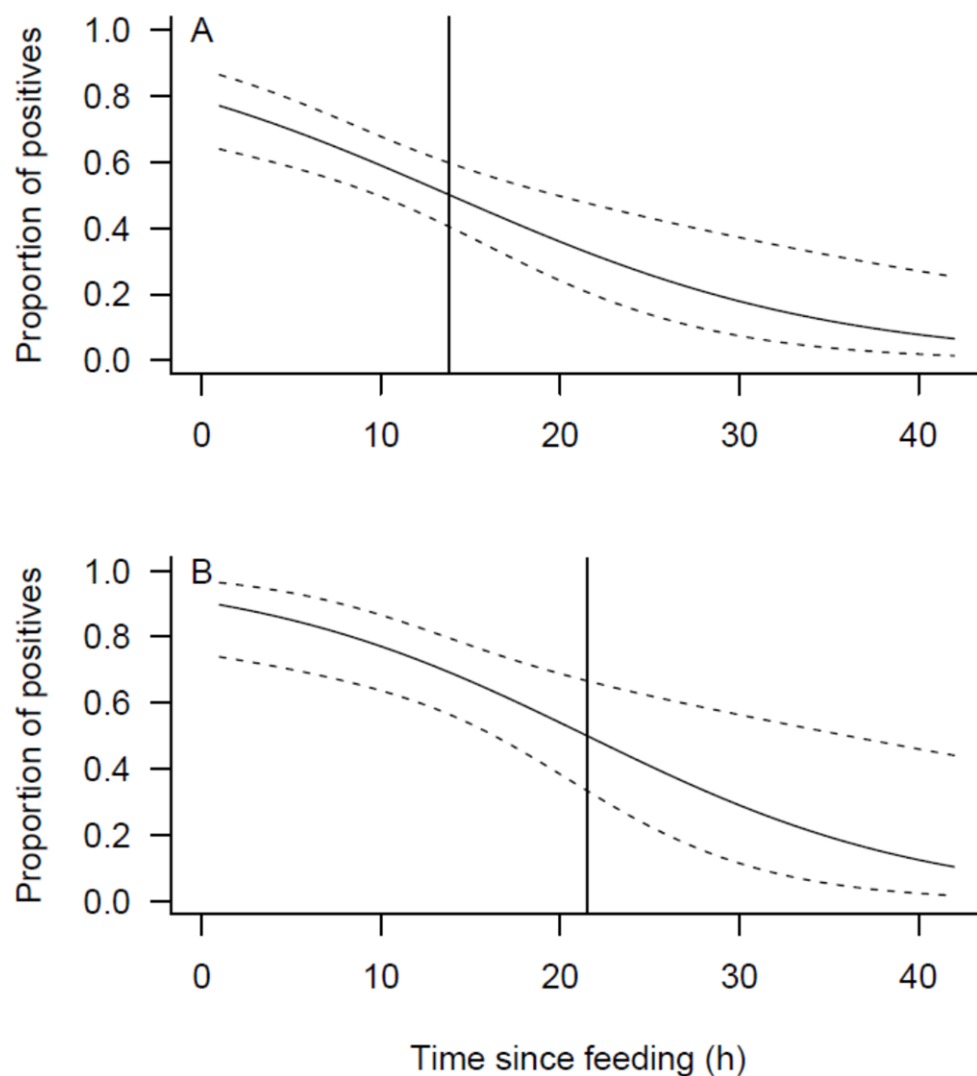


Figure 12. Proportion of positives of *Coccinella septempunctata* (A) and *Nabis americanoferus* (B) that fed on cereal leaf beetle (CLB) larvae in the laboratory up to 48 h. The solid horizontal line indicates the fitted probit model and dotted lines indicates the 95 percent confident intervals. The vertical line indicates the half-life detectability of DNA for each species.

CHAPTER 6

General discussion and conclusion

Chapter 6

General Discussion

In this dissertation, I have investigated various ecological factors affecting abundance of cereal leaf beetle (CLB), *Oulema melanopus* L. (Coleoptera: Chrysomelidae), in wheat fields. It is widely accepted that the abundance of herbivores and their natural enemies in agroecosystems are influenced by the complexity of the landscape (Karp et al., 2018). Therefore, it is important to understand how CLB and its natural enemies respond to the proportion of various habitats (crops and semi-natural habitat) around cereal fields, to provide recommendations for its sustainable management in western Canada. To date, the effect of landscape structure on CLB abundance and its parasitism by *Tetrastichus julis* (Walker) (Hymenoptera: Eulophidae) has not been evaluated. Filling this knowledge gap is important for CLB management because the success of biological control may depend on landscape complexity (Bianchi et al., 2006).

The cereal leaf beetle is an invasive pest recently found in western Canada and expanding its range in Alberta, Saskatchewan and Manitoba (Dosdall et al., 2011). The species was first found in Michigan in 1962, and since then it has continued to expand its geographical distribution regardless of the implementation of various management techniques (chemical and biological) (Haynes & Gage, 1981). However, classical biological control has been very successful in suppressing CLB populations in agricultural field crops (Evans et al., 2015; Evans et al., 2006) including southern Alberta (Dosdall et al., 2011). Several parasitoid species were imported from Europe, and among them, *T. julis* is the most effective (Dysart et al., 1973).

The extensive focus on controlling CLB using *T. julis* has resulted in neglecting the study of the role of generalist predators. However, generalist predators can contribute significantly to the control of chrysomelids in agricultural field crops (Greenstone et al., 2010; Hazzard et al.,

1991). In this dissertation, I investigated the relative contribution of natural enemies including generalist predators and a parasitoid wasp *T. julis* in CLB suppression (Chapter 3-5), to understand better the role of natural enemies in CLB management.

The density of herbivores in agricultural fields can affect their predation and parasitism rates due to potential density-dependent responses (Gunton et al., 2016). The natural enemy response to the density of their hosts depends on their host specificity, dispersal potential, and hosts' mobility (Stiling, 1987). Three responses are common: lack of response to host density, negative density-dependent response, and positive density-dependent response (Gunton et al., 2016). Several natural enemies, including *T. julis* (Kher et al., 2014), positively respond to the density of their host in agricultural landscapes (Gunton et al., 2016). Therefore, understanding how natural enemies respond to the density of CLB in agricultural fields can have important implication for the success of CLB biological control. In this dissertation, I evaluated the relationship between CLB abundance in cereal fields and biological control rates by the parasitoid *T. julis*, and the generalist predators *Nabis americanoferus* Carayon, 1961 (Hemiptera: Nabidae), and *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), in order to understand its effects on the success of biological control.

My objectives were to determine (1) how landscape complexity affects the abundance of CLB and its percentage parasitism by *T. julis* (Chapter 3), (2) how the proportion of suitable hosts in the current and previous years affects CLB abundance and parasitism (Chapter 3), (3) the relationship between natural field abundance of CLB and its control by natural enemies in wheat fields (Chapter 3), (4) what predator species feed on CLB immature stages under laboratory conditions (Chapter 4), and (5) the identity of the foliar predator species attacking

CLB immature stages and their frequency of predation in cereal fields using molecular gut content analysis (Chapter 5).

6.1 Summary of the thesis chapters

In Chapter three, I examined the effect of landscape structure on the abundance of CLB and its parasitism by *T. julis*. Landscapes with a high proportion of cereal and non-cereal crops had high CLB abundance in cereal fields. Similarly, the proportion of suitable cereal host crops in the previous year was positively associated with current CLB abundance in cereal fields. On the contrary, higher habitat diversity and a high proportion of pasture (a major semi-natural habitat) negatively influenced CLB abundance. Cereal leaf beetle parasitism also had positive associations with the proportion of wheat and barley at various scales, and negatively responded to the proportion of pasture in the landscape. This similarity was potentially due to the positive association between CLB abundance and its parasitism, suggesting positive density-dependent response. Cereal leaf beetle parasitism was positively associated with the proportion of canola and alfalfa at 0.5 to 2 km scales, likely due to provision of carbohydrate resources for *T. julis* that boosted its parasitism potential. In general, my results suggest that habitat diversity at the landscape level (0.5 km scales) is an important factor explaining CLB numbers in cereal fields through its direct negative effect on CLB numbers likely due to dilution of CLB primary hosts in these landscapes. By contrast, agricultural intensity resulting in high proportion of host crops in the landscape is responsible for high CLB infestation in cereal fields, contributing to a build-up of CLB populations from year to year.

In chapter 4, I identified potential predators of CLB immature stages under laboratory no-choice and choice experiments. Several predator species commonly found in cereal fields in southern Alberta consumed CLB immature stages under no-choice assays. These included lady

beetle species, carabids, and staphylinids, which all consumed CLB eggs and larvae. Under choice assays with the addition of wheat plants and English grain aphid as an alternative prey, lady beetles continued to feed on CLB eggs, but at a reduced rate. Ground predators, however, had an overall low rate of predation in this set up, likely due to their reluctance to climb the plants to eat CLB eggs. The predation potential varied significantly among predator species and CLB life stages. For instance, while wolf spiders and *Nabis* spp. did not consume CLB eggs, they consumed a high number of CLB larvae. In the field trials using sentinel egg cards and predator exclusion cages, I found an average of 24.5% egg disappearance per day likely due to predation. This study demonstrates that the assemblage of generalist predators present in cereal fields may increase overall mortality of CLB and may play an important role in the sustainable management of this pest.

In chapter 5, I identified several predator species attacking CLB immature stages in cereal fields using molecular gut content analysis. I designed a highly specific set of primers to screen field-collected predators for evidence of predation on CLB. Cereal leaf beetle DNA was detected from the gut of most predator species that showed predation potential under laboratory conditions (chapter 4), confirming that predation on CLB was not an artifact of experimental conditions. *Hippodamia* species were among the best predators of CLB along with *N. americoferus* which was the most abundant foliar predator species in cereal fields. The most abundant lady beetle species was *Coccinella septempunctata*, but it had the lowest rate of positives (0.05 proportion of positives), suggesting that this invasive species has a low contribution to CLB mortality. *Hippodamia parenthesis* (Say, 1824) (Coleoptera: Coccinellidae) and *N. americoferus* had the highest proportion of positives at 0.43 and 0.35, respectively. However, when the proportion of positives for *N. americoferus* was adjusted using its DNA half-

life detectability, it dropped to 0.22 due to its longer DNA detectability relative to *C. septempunctata*. The proportion of positives for CLB DNA in *N. americanoferus* and *C. septempunctata* was positively associated with CLB larval abundance in cereal fields, indicating positive density-dependent responses. The result of this study confirmed the contribution of several common generalist predator species in CLB management and further indicates that CLB is an important food resource for these predator species in cereal fields.

6.2. General conclusions and recommendations

6.2.1 Factors affecting cereal leaf beetle abundance in cereal fields within agricultural landscapes

The results of my studies indicate that both CLB and its parasitoid are affected by landscape complexity around cereal fields. I concluded that the presence of canola and alfalfa in the surrounding landscape can help to elevate parasitism, most likely through the provision of alternative food resources, such as nectar and aphid honeydew, which supported the landscape supplementation hypothesis. I concluded that diversity of crop and semi-natural habitats at the landscape level is an important factor negatively affecting CLB numbers in wheat fields in the Lethbridge area, likely due to dilution of CLB primary hosts in these landscapes. On the contrary, the proportion of cereal hosts in the landscape is the key element increasing CLB abundance in cereal fields, which provides support for the resource concentration hypothesis (Root, 1973; Tscharntke et al., 2012). Therefore, my results suggest that designing agricultural landscapes in a way that cereal fields would be near habitats providing these alternative food resources, such as flower strips or flowering canola fields, could contribute to increase CLB parasitism. Further, my results also suggest that diverse crop rotation regimes (cereal and non-

cereal crops) that may reduce the overall proportion of cereals within agricultural landscapes will help to disrupt CLB meta-population build-up in these landscapes.

Along with landscape complexity and *T. julis* parasitism (Chapter 3), the predation on CLB eggs early in the season (Chapter 4) and larvae late in the season (Chapter 5) is another important factor affecting CLB abundance in cereal fields. Early season predation on CLB eggs can affect colonization of CLB in cereal fields, which may complement the effect of *T. julis* on the population of CLB on the previous year. The late-season predation on CLB larvae, however, may disrupt the parasitism through consumption of already parasitized larvae (intraguild predation), which can reduce *T. julis* density in the next season. Alternatively, predators might avoid already parasitized larvae as shown in other host-parasitoid systems (Roger et al., 2001), which can result in complementary effects between predators and *T. julis* on CLB suppression. The avoidance behaviour can be due to the lower quality of parasitized prey relative to healthy prey as shown for parasitized aphids (Bilu & Coll, 2009). Furthermore, *T. julis* may parasitize fewer CLB larvae in the patches that generalist predators are present or have been active recently as shown in other parasitoid-predator-pest systems (Almohamad & Hance, 2014; Taylor et al., 1998). Therefore, apart from direct potential effect of predators on *T. julis*, predators might alter *T. julis* parasitism indirectly due to change in its oviposition behaviour as shown for pea aphid (Taylor et al., 1998), mealybug (Chong & Oetting, 2007) and green peach aphid parasitoids (Almohamad & Hance, 2014). This behavioural change may result in lower parasitism pressure on patches with generalist predators, which can affect parasitoid distribution in the patches and result in an increase or decrease of the overall biological control rate. Overall, the significant contribution of natural enemies, including *T. julis* (Chapter 3) and generalist predators (Chapter 4 & 5) to CLB control, demonstrated in my studies suggest that scouting cereal fields prior to

spraying insecticides is necessary to avoid unnecessary applications that may disrupt the control provided by these natural enemies. My study showed that natural enemies have strong potential in suppressing CLB populations in western Canada cereal fields, and suggests habitat management strategies should be directed in a way to maximize their action in agricultural landscapes. In particular, habitat management strategies could focus on increasing the area of canola and alfalfa in landscapes currently dominated by wheat, which may enhance the parasitism potential and reduce CLB abundance in cereal fields.

Besides landscape complexity and natural enemy effects on CLB abundance, abiotic factors may also affect CLB abundance and its parasitism. Cereal leaf beetle abundance and its parasitism were lower in 2015, in which the average summer temperature was higher and precipitation was lower than in 2014 (Worldweatheronline, 2014; 2015). However, I cannot entirely rule out that lower CLB abundance in 2015 was due to a high CLB parasitism in 2014, or due to the interaction between parasitism and weather conditions. Evans et al. (2013) reported lower CLB parasitism in warm springs due to delay in CLB larval phenology that resulted in a phenological mismatch between CLB and adult *T. julis* activity. My results also suggest that the efficacy of *T. julis* in CLB control might be reduced in warm years, and hence the contribution of generalist predators might be more crucial in warm years to compensate for reduced parasitism levels and complement *T. julis* to provide high levels of biological control. A long term data series is needed to understand weather effects on this system, similar to a 15-year study of lygus bugs (Varis, 1995).

6.3 Future directions

In this dissertation, I highlighted the importance of landscape complexity and parasitism (Chapter 3), and predation by several generalist predators on herbivore abundance in agricultural

fields (Chapter 4-5). My results highlight the importance of diversification at the landscape level in reducing CLB numbers in cereal fields. Given the importance of diversification, future studies should consider investigating the effect of local-scale diversity (within field diversification) on CLB abundance and parasitism. This is particularly important because previous meta-analyses (Letourneau et al., 2011; Poveda et al., 2008) suggest a strong positive association between local-scale diversity and natural enemy abundance, which resulted in lower herbivore pressure in agricultural fields. The within-habitat diversity includes practices such as increased vegetation diversity, cover cropping, trap cropping and addition of flowering strips within or around agricultural fields, which can affect herbivore abundance and crop yields (Letourneau et al., 2011). Previous studies reported inconsistent effects of local-scale diversity (addition of flower strips) on CLB numbers in cereal fields, ranging from no effect (Pollier et al., 2019) to negative effects (Tschumi et al., 2016; Tschumi et al., 2015). Tschumi et al. (2015) further investigated whether the effect of the flower strips on CLB abundance at the local scale depends on the proportion of semi-natural habitats within a 750 m radius scale, and found no interaction between these two scales. Their results suggest that CLB reduction due to the addition of flower strips was due to a strong positive effect on several generalist predators, regardless of the proportion of semi-natural habitats in the surrounding landscape. By contrast, my study suggests that landscape effects on CLB abundance and parasitism are important, but are best detected at larger scales (1.5 to 2 km). Therefore, it is important that future studies investigate the interaction between landscape complexity at large scales and local scale diversity on CLB abundance. According to the intermediate landscape complexity hypothesis, local scale diversification is often most successful in landscapes that have intermediate levels of complexity, and less successful in highly complex or highly simplified landscapes (Tscharntke et al., 2012).

Therefore, I predict that the local scale diversity would have a stronger effect in CLB control in wheat fields surrounded by landscapes with intermediate levels of complexity. Furthermore, similar to the effect of landscape-scale diversification, I predict that crop diversification (e.g., polyculture) at the field scale would result in lower CLB numbers in cereal fields following the resource concentration hypothesis (Root, 1973). Finally, following the natural enemy hypothesis (Root, 1973), I predict that the addition of flower strips within cereal fields would increase overall rate of CLB biological control by its specific parasitoid and generalist predators, due to provision of nectar and pollen for these natural enemies that boost their longevity and fitness. The integration of local and landscape level diversity along with several abiotic and biotic factors, and management regimes, will remain a long-term challenge to maintain an economically and environmentally sustainable cropping system.

In Chapter five, I confirmed the contribution of several generalist predators to CLB control late in the season. Future studies should consider investigating the effect of predators early in the season using molecular gut content analysis, to understand better the overall impact of predators in CLB control. The fact that predators feed on CLB larvae in cereal fields opens a new window for future research, to investigate the potential interaction between predators and *T. julis* and its consequence for CLB biological control. This study will be particularly important because it will determine whether the combined effect of predators and *T. julis* is synergistic, additive or non-additive (Ferguson & Stiling, 1996), resulting in increased biological control or alternatively, a disruption of CLB parasitism. My studies (Chapter 4 & 5) are the first to indicate a significant contribution of several generalist predators in CLB control along with *T. julis* (Chapter 3). As a result of these findings, future studies should consider developing a dynamic action threshold for CLB. The dynamic action threshold is a relatively recent approach, in which

the effect of natural enemies is integrated into the economic threshold. Future studies should conduct cage experiments with cereal plants that contain a varying number of CLB, predators and *T. julis* (presence of one group, presence of both groups) to understand how CLB abundance, crop damage and yield differ between these treatments. Several factors should be investigated, such as interactions between predators and parasitoids, and the effect of the guild of natural enemies on CLB abundance and crop yield. Then, it would be possible to correlate the density of these natural enemies with CLB abundance and crop yield and estimate their effect in protecting cereal crop yields. In general, the dynamic action threshold would take *T. julis* and generalist predators into account to reduce insecticide sprays that can disrupt natural biological control. Such judicious use of insecticides over the long term may lead to a more resilient agroecosystem with greater inherent resistance to pest damage from a more vigorous community of natural enemies.

Appendix 1.

Mean and range percentage of different land-cover types in 2 km radius in the 54 agricultural landscapes studied in southern Alberta, Canada, during field experiments in 2014 and 2015.

Major land-cover types were used in generalized linear models to investigate the response of cereal leaf beetle (CLB) and its parasitoid, *Tetrastichus julis* to landscape complexity.

Major habitat types	Major land-cover types	Diversity land-cover types	% area	
			Mean	Range
Crops		Total	60.20	20.33 – 93.64
	Major hosts ¹ (Wheat) ² (Barley) ²	Total	32.64	3.90 – 71.11
		Wheat	28.38	3.90 – 71.11
		Barley	4.26	0.00 – 23.67
	Non-cereal crops ³ (Canola) ⁴	Total	22.69	5.19 – 52.73
		Canola	9.839	0 – 38.74
		Potato	5.110	0.00 – 27.06
		Beet	1.065	0.00 – 9.163
		Bean	1.926	0.00 – 17.53
		Flax	0.604	0.00 – 6.95
		Hemp	0.164	0.00 – 4.67
		Peas	3.546	0.00 – 28.48
		Sunflower	0.192	0.00 – 7.29
	Minor hosts ⁵	Total	4.732	0.00 – 25.77
		Corn	3.932	0.00 – 25.77
		Oat	0.202	0.00 – 4.83
		Rye	0.485	0.00 – 18.42
	Alfalfa ⁶	Alfalfa	0.549	0.00 – 15.19
Semi-natural habitats		Total	32.89	5.01 – 79.02
	Pasture ⁷	Total	26.94	0.12 – 75.78
		Native Pasture	24.33	0.12 – 75.78
		Cultivated pasture ⁸	2.612	0.00 – 18.00
	Grassland ⁹	Total	6.945	0.80 – 23.80
		Field border grass	1.422	0.00 – 6.212
		Grass	5.392	0.80 – 23.80
		Fallow	0.131	0.00 – 4.97
	Woodland ¹⁰	Total	0.898	0.00 – 7.08
		Trees	0.246	0.00 – 1.53
		Riparian vegetation	0.652	0.00 – 6.97
Other habitats		Total	4.156	0.37 – 28.46
	Water ¹¹	Water	2.631	0.00 – 27.61
	-----	Wetland ¹²	0.224	0.00 – 8.11
	-----	Urban ¹³	1.743	0.37 – 4.60
	-----	Unknown ¹⁴	0.661	0.00 – 9.44 ¹⁵
		Total	100.00	

¹ Major cereal host of CLB (wheat and barley).

² Wheat and barley were also separately included in various CLB and CLB parasitism models, due to inconsistent associations of barley and year of the study.

³ Non-cereal crops include crops that are not hosts of CLB.

⁴ Canola was also separately tested in CLB parasitism models since it was a potential source of nectar for parasitoids.

⁵ Minor-hosts include cereals that are not primary host of CLB (crops that are not prominent cereals in Alberta agricultural landscapes, or are not CLB preferred oviposition hosts).

⁶ Alfalfa fields within agricultural landscapes.

⁷ Native pastures include large natural grasslands used for animal production (cattle, horse, and sheep).

⁸ Cultivated pasture includes forage grass such as brome grass (*Bromus* spp.), canary grass (*Phalaris canariensis* L.).

⁹ Grasslands includes field border grass, grassy area in the corner of fields and parks.

¹⁰ Woodland includes hedgerows and riparian.

¹¹ Water includes all area with waters such as river and lakes.

¹² Wetland includes natural areas seasonally with water and various aquatic plants. Due to the low proportion this landscape variable excluded from the models.

¹³ Urban area in the landscape includes roads, buildings, train tracks. Due to lack of proper biological function associated with this variable, I excluded this variable from the analysis.

¹⁴ Fields where it was not possible to identify the vegetation cover due to the lack of public access.

¹⁵ This value is due to one large agricultural field that did not have public access to ground proof.

Appendix 2. The identity of various insect non-target and predator species with their associated NCBI Accessions # used to design specific primers for the cereal leaf beetle, *Oulema melanopus*.

Target species	NCBI Accession #	Order tested	Family tested	Non-target and predator species tested	NCBI Accession #
Chrysomelidae <i>Oulema melanopus</i>	KP406719-KP406720 KF656330-KR490936 KF656072-KF656078	Coleoptera	Chrysomelidae	<i>Phyllotreta vittula</i>	JX243022, JX243023
	<i>Phyllotreta striolata</i>			KJ967011, KM441436	
	<i>Phyllotreta cruciferae</i>			KU188455, KU188456	
	<i>Oulema duftschmidi</i>			KF655856, EU498311	
	<i>Oulema mauroi</i>			KP406717-KP406721	
	<i>Leptinotarsa decemlineata</i>			KP406718	
	<i>Crioceris quinquepunctata</i>			DQ649096, DQ649101	
	<i>Crioceris duodecimpunctata</i>			KC123334, KC123333	
	<i>Crioceris asparagi</i>			JF775787, JF775786	
	<i>Lema bitaeniata</i>			JF775779, KF654214	
	<i>Lema foveipennis</i>			DQ001935	
	<i>Lema fulvipes</i>			DQ001937	
	<i>Lema hamate</i>			DQ001929	
	<i>Lema immaculipennis</i>			DQ001938	
	<i>Lema insularis</i>			DQ001939	
	<i>Lema lichenis</i>			DQ001941	
	<i>Lema obliterate</i>			DQ155990	
	<i>Lema regularis</i>			DQ001940	
	<i>Lema trilinea</i>			DQ001948	
	<i>Lema trivitta</i>			DQ001945, AB862373	
	<i>Lilioceris merdigera</i>			DQ001944	
	<i>Neolema approximata</i>			AM283149	
				DQ001933	
			Coccinellidae	<i>Coccinella septempunctata</i>	HQ711975-HQ711981
		<i>Hippodamia tredecimpunctata</i>		KJ965869, KJ965750	
		<i>Hippodamia parenthesis</i>		JF296304- JF296308	
		Carabidae	<i>Pterostichus melanarius</i>	KM451324-KM447944	
			<i>Amara aenea</i>	KJ966903-KM441787	
Hemiptera		Nabidae	<i>Nabis americoferus</i>	KR043334- KR043239	
Araneae		Lycosidae	<i>Pardosa laura</i>	AB564726, AY836141	

Appendix 3. A list of potential non-target insect hits of CLBCO1F and CLBCO1R primers generated by primer-blast in NCBI.

Order	Family	Subfamily	Genus	Species	No. Total Mismatches ¹	Distribution	Notes ²
Coleoptera	Chrysomelidae	Criocerinae	<i>Oulema</i>	<i>melanopus</i>	0-2	Europe, Canada	In BIN ³ BOLD:AAK5928, <i>Oulema melanopus</i>
Coleoptera	Chrysomelidae	Criocerinae	<i>not listed</i>	<i>sp.</i>	0-1	Canada	In BIN ³ BOLD:AAK5928, <i>Oulema melanopus</i>
Coleoptera	not listed	not listed	<i>not listed</i>	<i>sp.</i>	0	Canada	No concern ⁴
Coleoptera	Chrysomelidae	Criocerinae	<i>Oulema</i>	<i>duftschmidi</i>	0-4	Europe	No concern
Coleoptera	Chrysomelidae	Criocerinae	<i>Oulema</i>	<i>erichsonii</i>	0-3	Europe	No concern
Coleoptera	Chrysomelidae	Criocerinae	<i>Oulema</i>	<i>mauroi</i>	0-2	Europe	No concern
Coleoptera	Chrysomelidae	Criocerinae	<i>Oulema</i>	<i>sp.</i>	0-4	Europe	No concern
Coleoptera	Ciidae	Ciinae	<i>Ceracis</i>	<i>nigropunctatus</i>	4	Brazil	No concern
Coleoptera	Curculionidae	Cryptorhynchinae	<i>Trigonopterus</i>	<i>myops</i>	4	Melanesia	No concern
Coleoptera	Elateridae	Elaterinae	<i>Dalopius</i>	<i>sp.</i>	4	Europe, Russia N.America	Low concern ⁵
Coleoptera	Elateridae	Prosterninae	<i>Eanus</i>	<i>decoratus</i>	4	Canada	Low concern
Coleoptera	Scirtidae	Scirtinae	<i>Contacyphon</i>	<i>sp.</i>	4	E. Canada	In BIN BOLD:AAG7259, ⁶ <i>Contacyphon obscurus</i>
Coleoptera	Scirtidae	Scirtinae	Contacyphon	obscurus	4	E. Canada	In BIN BOLD:AAG7259, <i>Contacyphon obscurus</i>
Coleoptera	Scirtidae	Scirtinae	<i>not listed</i>	<i>sp.</i>	4	-	No concern
Diptera	Tephritidae	Tephritinae	<i>Eurosta</i>	<i>solidaginis</i>	4	Canada, USA	Golden Rod Gall fly ⁷

1. Count includes mismatches on both primers.
2. Primer combination: GCTTCGGTAATTGATTAGTTC with CGTATATTTCTTACTGTGGAGATGA only yielded hits to *Oulema*. Combination GCTTCGGTAATTGATTAGTTC with CGTATATTTTTTACTGTGGAGATGA only yielded hits to *Oulema*.
3. BIN*: Barcode Index Numbers (BIN): Clustered barcode sequences that create OTUs (operational taxonomic units) reflective of species groupings.
4. Species with a low possibility to be amplified by my designed primer in Canada due to distribution range of these species.

5. Species with possibility to be amplified as a non-target of primer. However, the biology of wireworms (subterranean) makes amplification unlikely.
6. *Contacyphon* sp. are marsh beetles, larvae typically associated with stagnant or flowing water and adults are riparian on decomposing vegetation (sub-aquatic).
7. While I can't rule this species out completely, there are no studies suggesting the predators listed in this study impact the goldenrod gall fly (Abrahamson et al., 1989) .

Appendix 4. The result of BOLD blasts against the COI species database for sequences of predatory species screened for cereal leaf beetle (CLB: *Oulema melanopus*) DNA.

Query ID	Best ID ¹	Top %	Low %	BIN	Avg. distance	Distance to NN ²	NN BIN	NN ID
AKH01	<i>Oulema melanopus</i>	100	100	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH02	<i>Oulema melanopus</i>	100	100	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH03	<i>Oulema melanopus</i>	99.59	99.59	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH04	<i>Oulema melanopus</i>	99.6	99.6	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH05	<i>Oulema melanopus</i>	100	100	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH06	<i>Oulema melanopus</i>	100	100	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH07	<i>Oulema melanopus</i>	99.6	99.6	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH08	<i>Oulema melanopus</i>	100	100	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH09	<i>Oulema melanopus</i>	100	100	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH10	<i>Oulema melanopus</i>	100	100	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH11	<i>Oulema melanopus</i>	100	100	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH12	<i>Oulema melanopus</i>	100	100	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH13	<i>Oulema melanopus</i>	99.6	99.6	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH14	<i>Oulema melanopus</i>	99.4	99.4	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH15	<i>Oulema melanopus</i>	99.6	99.6	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH16	<i>Oulema melanopus</i>	99.6	99.6	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH17	<i>Oulema melanopus</i>	99.6	99.6	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH18	<i>Oulema melanopus</i>	99.6	99.6	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH19	<i>Oulema melanopus</i>	99.6	99.6	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH20	<i>Oulema melanopus</i>	100	100	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH21	<i>Oulema melanopus</i>	99.6	99.6	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH22	<i>Oulema melanopus</i>	99.6	99.6	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH23	<i>Oulema melanopus</i>	100	100	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH24	<i>Oulema melanopus</i>	100	100	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH25	<i>Oulema melanopus</i>	99.6	99.6	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH26	<i>Oulema melanopus</i>	99.6	99.6	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH27	<i>Oulema melanopus</i>	99.6	99.6	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH28	<i>Oulema melanopus</i>	99.6	99.6	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH29	<i>Oulema melanopus</i>	99.6	99.6	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH30	<i>Oulema melanopus</i>	99.6	99.6	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH31	<i>Oulema melanopus</i>	99.6	99.6	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH32	<i>Oulema melanopus</i>	99.2	99.2	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH33	<i>Oulema melanopus</i>	98.8	98.8	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>

AKH72	<i>Oulema melanopus</i>	100	100	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH73	<i>Oulema melanopus</i>	99.6	99.6	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH74	<i>Oulema melanopus</i>	99.6	99.6	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH75	<i>Oulema melanopus</i>	99.5	99.5	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH76	<i>Oulema melanopus</i>	99.6	99.6	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH77	<i>Oulema melanopus</i>	100	100	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH78	<i>Oulema melanopus</i>	99.6	99.6	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH79	<i>Oulema melanopus</i>	99.6	99.3	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH80	<i>Oulema melanopus</i>	100	100	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH81	<i>Oulema melanopus</i>	100	100	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH82	<i>Oulema melanopus</i>	100	100	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH83	<i>Oulema melanopus</i>	100	100	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH84	<i>Oulema melanopus</i>	100	100	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH85	<i>Oulema melanopus</i>	99.6	99.6	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH86	<i>Oulema melanopus</i>	99.6	99.6	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH87	<i>Leptopterna dolabrata</i>	99.24	87.12	BOLD:AAB5081	0.42% (p-dist)	2.09% (p-dist)	BOLD:ZMBN042	<i>Leptopterna ferrugata</i>
AKH88	<i>Oulema melanopus</i>	100	100	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH89	<i>Oulema melanopus</i>	100	100	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH90	<i>Oulema melanopus</i>	99.6	99.6	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH91	<i>Oulema melanopus</i>	99.6	99.6	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH92	<i>Oulema melanopus</i>	99.6	99.6	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH93	<i>Oulema melanopus</i>	99.6	99.6	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH94	<i>Oulema melanopus</i>	99.6	99.6	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>
AKH95	<i>Oulema melanopus</i>	99.6	99.6	BOLD:AAK5928	0.25% (p-dist)	2.89% (p-dist)	BOLD:ABA8948	<i>Oulema duftschmidi</i>

1. Best prediction for the matches of the Sanger-sequenced amplicons retrieved from the BOLD reference database.
2. The distance to nearest neighbour BIN, typically representing a different species

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