ASSESSING THE IMPACTS OF NATURAL ENEMIES AND CANOPY STRUCTURE ON ORANGE WHEAT BLOSSOM MIDGE, *SITODIPLOSIS MOSELLANA* (DIPTERA: CECIDOMYIIDAE), IN THE PEACE RIVER REGION OF ALBERTA

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To my parents,

who have long supported my passion for science and entomology.

ABSTRACT

Orange wheat blossom midge, *Sitodiplosis mosellana*, is an invasive, economically important pest in spring wheat in the Canadian Prairies. In the Peace River region of Alberta, little is known about this pest and its natural enemies. Impacts of crop type and canopy structure on assemblages of carabid beetles and, in turn, their impact on wheat midge populations were assessed in a field plot trial. Few effects of canopy structure and crop type influenced carabid assemblages, though high plant densities in wheat resulted in lower carabid activity density and species richness in 2017. A survey was conducted where wheat midge larvae were collected throughout the region and reared to assess wheat midge parasitoid distribution and diversity. All parasitoids recovered were identified as *Macroglenes penetrans*, with the exception of a single individual in the genus *Inostemma*. Future research should investigate impacts of specific carabid species on wheat midge populations in this region.

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LIST OF ABBREVIATIONS

AAFC	Agriculture and Agri-Food Canada
AI ha	Active ingredient per hectare
AB	Alberta
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
BC	British Columbia
CAD	Canadian dollars
cv.	Cultivar
ºC	Degrees celsius
cm	Centimetre
DD	Degree days
EI	Ecoclimatic indices
kg	Kilograms
km	Kilometres
L3	Third larval instar
m	Metres
mm	Millimetres
RO	Reverse osmosis
S	Species richness
sp.	Species (Singular)
VB	Varietal blends
WCCP	Western Committee on Crop Pests

GENERAL INTRODUCTION

Objectives

This thesis seeks to investigate the impacts of canopy structure and natural enemies on the orange wheat blossom midge, *Sitodiplosis mosellana* in the Peace River region of Alberta. It incorporates five main objectives:

1. Assess the effects of crop type and canopy structure on carabid assemblages in this region. This will help determine if predatory species likely to feed on wheat midge larvae may become more or less dominant in the carabid assemblage.

2. Examine the effect of plant density on wheat midge populations. Seeding rates chosen influence the plant density in the field and, in turn, wheat midge density.

3. Characterize the diversity of carabid assemblages found in the region. The Peace River region has unique growing conditions compared to the rest of the Canadian Prairies which likely influences the species make up of its carabid assemblages.

4. Determine whether carabid assemblages have a significant effect on wheat midge populations. Some level of control may be provided by dominant carabid species in this region for wheat midge populations.

5. Determine the geographic distribution, species diversity, and percentage parasitism of parasitoids of wheat midge in the region. Wheat midge parasitoids have been reported in the region but little is known about the species diversity and their impact on wheat midge populations in this area.

CHAPTER 1: LITERATURE REVIEW – INTEGRATED PEST MANAGEMENT STRATEGIES AND PATTERNS OF ESTABLISHMENT OF ORANGE WHEAT BLOSSOM MIDGE IN DIFFERENT REGIONS

1.1. Introduction

The orange wheat blossom midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae) is an invasive species accidentally introduced to North America in the 1800s (Elliott *et al.*, 2009; Fitch, 1856; Knodel, 2007). Since its introduction to Canada, wheat midge has become an important economic pest in spring wheat (*Triticum aestivum* L.) throughout the Canadian Prairies (Elliott *et al*. 2009). Damage from wheat midge can be devastating to wheat production. During an outbreak in 1983, crop yields in Saskatchewan, Canada were reduced by approximately 30%, resulting in an estimated loss of $65 million CAD (adjusted to 2018 dollars) (Olfert *et al.*, 1985). Since the major outbreaks that occurred in the prairies in the 1980s, wheat midge has continued to be a major pest in Canada and worldwide. This literature review will describe the general morphology and biology of *Sitodiplosis mosellana*. It will investigate current management strategies used to combat this pest. Finally, it will explore the geographical spread and establishment of this pest in Canada and worldwide.

1.2. Biology

Adult and larval stages of the wheat midge are characterized by their bright orange colouration (Olfert *et al.*, 1985). The adult stage measures approximately 3 mm in length (Olfert *et al.*, 1985). There are records of wheat midge feeding on many members of the grass family including barley (*Hordeum vulgare* L.), rye (*Secale cereale* L.), and couch grass (*Elymus repens* L.), but it is widely known as a pest of major economic importance in wheat (Borkent, 1989). This insect has one generation each year (W. N. Cheng *et al.*, 2009). Adults lay their eggs in late June or early July on wheat florets (Fig. 1.1) (Olfert *et al*. 1985). Flights of wheat midge begin at dusk and continue throughout the night (Pivnick & Labbe, 1993). The adult female inserts its ovipositor between the glume and glumella, placing eggs on the young kernel. Affolter (1990) observed that eggs are sometimes laid on the beard and rachis, but often suffer high mortality due to desiccation. Eggs hatch within five days of being laid (Olfert *et al*. 1985).

Figure 1.1. Female wheat midge ovipositing on wheat in early July near Girouxville, AB. Image courtesy of Shelby Dufton and Amanda Jorgensen (AAFC-Beaverlodge).

Damage by wheat midge occurs in the larval stage, when it feeds on the kernel of the wheat (Fig. 1.2) (Elliott *et al.*, 2009). The larva feeds for two to three weeks, causing shrivelling and distortion of the kernels (Olfert *et al*. 1985, Elliott *et al*. olfert). This leads to a reduction in yield, grade, quality of grain, and results in poor meal baking quality (Affolter, 1990; Elliott *et al.*, 2009). Damage caused by wheat midge larvae can also facilitate secondary attacks by fungal diseases including *Fusarium graminearium* and *Septoria nodorum* (Oakley *et al.*, 1994). Areas damaged by larval feeding are found to be a highly suitable medium for both pathogenic and saprophytic fungi (Oakley *et al.*, 1994). Kernels are often shrivelled or cracked, deformed, or underdeveloped (Affolter, 1990). These damaged kernels are of reduced weight and are often lost during the cleaning of the grain (Dexter *et al.*, 1987).

Figure 1.2. Wheat midge larva feeding on a shrivelled wheat kernel. Image used with permission from Amanda Jorgensen (AAFC-Beaverlodge).

Mature, third instar, larvae drop to the soil from the wheat head then form overwintering cocoons in the soil (Olfert *et al.*, 1985) (Fig. 1.3). Movement of the larva from the kernel in the head of the host plant to the soil is triggered by moisture or rainfall events (Affolter, 1990; Olfert *et al.*, 1985). As soon as they fall to the soil surface, larvae tunnel to a depth of approximately 10 cm using a spoon-like structure called the *spatula sternalis* (Affolter, 1990). Once buried, the larvae spin a spherical cocoon and enter an obligatory diapause (Affolter, 1990). This diapause was experimentally shown to be broken by a combination of all three of the following conditions: a cold period of at least 120 days at low temperature (below 10 ºC), an undefined period at normal temperature (above 0 ºC), and a 5 week period during which soil moisture is extremely high (Affolter 1990). These conditions are usually met the following spring, when the larva returns to the soil surface and pupates, emerging as an adult fly in late June or early July (Fig. 1.3) (Elliott *et al.*, 2009).

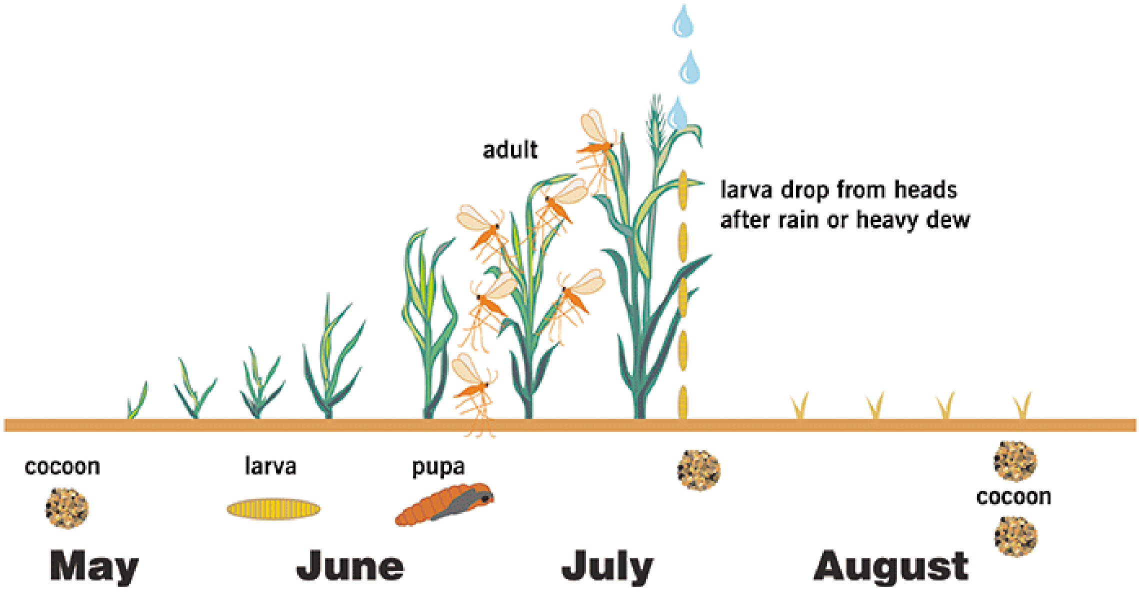

Figure 1.3. The wheat midge lifecycle throughout the growing season. Image used with permission from Extension Entomology, North Dakota State University

Wheat midge larvae have been shown to persist in their overwintering cocoons for up to 13 years, which reduces inbreeding within populations (Barnes, 1952). This "superdiapause" can occur when conditions to break diapause are not met in a given year (Affolter, 1990). Thus, flights of adult wheat midge may comprise multiple generations (Barnes, 1956). This larval persistence and the unpredictable emergence of adults make it difficult to forecast wheat midge densities from year to year (Barnes, 1952).

1.3. Wheat Midge Control

Control for wheat midge relies on an extensive system of integrated pest management. Cultural controls, in which the environment is modified, include the use of different seeding rates, tillage regimes, and resistant wheat cultivars (Elliott *et al.*, 2011b). To date, two types of resistance in wheat have been investigated for wheat midge: antibiotic resistance and antixenotic resistance (Lamb *et al.*, 2000a; McKenzie *et al.*, 2002). Currently, only the antibiotic resistance is well understood and has been used to breed tolerant varieties of wheat using the gene, *Sm1* (Vera et al., 2013). Chemical controls include the spraying of foliar insecticides during pest outbreaks (Elliott, 1988a; Elliott & Mann, 1997; Olfert *et al.*, 2009). Two insecticides, chlorpyrifos and dimethoate, are registered and used for wheat midge control in Canada (Alberta Agriculture and Forestry, 2018). There has also been research into the use of natural enemies as a management strategy for wheat midge. Any organism that kills, decreases the reproductive potential of, or otherwise reduces the population of another organism, can be classified as a natural enemy. Natural enemies of wheat midge include predaceous beetles and parasitoid wasps (Affolter, 1990; Basedow, 1973; Floate, 1990; Reeher, 1945). A final component of wheat midge management has been the development and use of forecasting models. These models predict emergence events for this pest based on biological and environmental data (Basedow, 1977a, 1980; Elliott *et al.*, 2009; Jacquemin *et al.*, 2014; Jacquemin *et al.*, 2008; Olfert *et al.*, 2016). Control strategies will be discussed in further detail in the following sections.

1.3.1. Cultural Control

Different management strategies have been investigated and reported for wheat midge in terms of cultural control. These include altering the agro-ecosystem as well as the crop itself. Researchers in Saskatchewan found that larval populations were 1.4-3.0 times higher in early-seeded wheat than in late-seeded wheat and 1.4-2.0 times higher in zero-till wheat than in tilled wheat (Elliott *et al.*, 2002). Adult emergence was 1.4-2.0 times higher in early-seeded wheat compared to late-seeded wheat and 1.5-2.0 times higher in systems without tillage (Elliott *et al.*, 2002). The lowest emergence of adult wheat midge and their parasitoids was in late-seeded systems using high tillage (Elliott *et al.*, 2002). Elliott *et al.* (2002) recommended avoiding wheat production with zero-till to reduce the buildup of wheat midge populations. Advanced heading can be achieved by using high seeding rates and early planting to avoid coinciding with populations of adult wheat midge in mid to late July (Elliott *et al.*, 2011b). The buildup of wheat midge populations can be discouraged by avoiding continuous cropping of wheat in the same field (Elliott *et al.*, 2011b). Elliott *et al.* (2002) recommended that canola, flax, or legumes should be grown in the Canadian Prairies if larval populations exceed 1200 larvae per square metre (Elliott *et al.*, 2011b).

1.3.2. Antibiotic Resistance

Current control of wheat midge includes the development of resistant wheat cultivars (Chavalle *et al* 2015). An association between dead first-instar larvae and resistant cultivars was the first indication of some form of antibiotic resistance for wheat midge (Barker & McKenzie, 1996). In 2002, while trying to confer that resistance to spring wheat, it was discovered that resistance to wheat midge was based on a single gene called *Sm1* (McKenzie *et al.*, 2002). The *Sm1* gene increases the production of phenolic acid in response to feeding damage (Ding *et al.*, 2000). Phenolic acid causes larvae to leave the kernel they are feeding on and die of starvation (Ding *et al.*, 2000). This gene was used to breed wheat midge tolerant cultivars of spring wheat in two different ways:

directly selecting for a response to the pest and indirectly with molecular markers linked to the *Sm1* gene (Vera *et al.*, 2013). This was then followed by a bioassay to confirm the resistance (Vera *et al.*, 2013).

The first midge tolerant varieties of wheat became commercially available in 2010 (Vera *et al.*, 2013). These included the varietal blends AC® Unity VB, AC® Goodeve VB, and AC® Glencross VB (Vera *et al.*, 2013). In 2011, AC® Fieldstar VB and AC® Glencross VB became available for commercial production (Elliott *et al.*, 2011b). There are currently 27 wheat midge tolerant varieties available commercially in Canada (Table 1.1) (Midge Tolerant Stewardship Team, 2018).

Table 1.1. List of wheat midge tolerant varieties currently available in Canada and the year they became commercially available (Midge Tolerant Stewardship Team, 2018).

Wheat class	Variety	Commercially available
Canadian Western Red Spring	AC® Unity VB	2010
Canadian Western Red Spring	AC® Goodeve VB	2010
Canadian Western Red Spring	AC® Fieldstar VB	2011
Canadian Western Red Spring	AC® Shaw VB	2012
Canadian Western Red Spring	CDC Utmost VB	2012
Canadian Western Red Spring	AC® Vesper VB	2013
Canadian Western Red Spring	CDC Titanium VB	2016
Canadian Western Red Spring	CDC Hughes VB	2018
Canadian Western Red Spring	CDC Landmark VB	2018
Canadian Western Red Spring	AAC Prevail VB	2018
Canadian Western Red Spring	AAC Jatharia VB	2017
Canadian Western Red Spring	AAC Cameron VB	2016
Canadian Western Red Spring	Syn479 VB	2018
Canadian Western Extra Strong	AC® Glencross VB	2010
Canadian Prairie Spring Red	AC® Conquer VB	2012
Canadian Prairie Spring Red	AC® Enchant VB	2014
Canadian Prairie Spring Red	AAC Tenacious VB	2018
Canadian Prairie Spring Red	AAC Foray VB	2016
Durum	AAC Marchwell VB	2016
Durum	CDC Carbide VB	2017
Canada Western Special Purpose	KWS® Charing VB	2018
Canada Western Special Purpose	KWS® Sparrow VB	2018
Canada Western Special Purpose	AAC Awesome VB	2019
Soft White Spring	AAC Indus VB	2018
Soft White Spring	AC® Sadash VB	2013
Soft White Spring	AAC Chiffon VB	2016
Soft White Spring	AAC Paramount VB	2016

Blends of different cultivars or varietal blends (VB) released since 2010 have a ratio of 9:1 of midge tolerant wheat to susceptible wheat (Vera *et al.*, 2013). The refuge provided by the 10% susceptible seed ensures that wheat midge adults are still able to oviposit (Vera *et al.*, 2013). Subsequent larvae are able to develop and a new generation of adults are available to mate and reproduce with whatever resistant individuals might survive (Vera *et al.*, 2013). In this way, the maintenance of a 10% susceptible seed reduces the likelihood of selection for resistance to the *Sm1* gene (Vera *et al.*, 2013). The susceptible portion of the cultivar additionally provides a refuge for parasitoids by maintaining a small population of wheat midge for them to attack (Doane *et al.*, 2013). In spite of this refuge, there are concerns about wheat midge populations developing resistance to the *Sm1* gene. If a wide area was planted solely with resistant cultivars, adults from populations in susceptible wheat would be less likely to immigrate and introduce avirulence alleles (Chavalle *et al.*, 2017). This would increase the local frequency of the virulence allele and increase resistance within the wheat midge population. (Chavalle *et al.*, 2017).

1.3.3. Antixenotic Resistance

Although antibiotic resistance to wheat midge is well understood and has been implemented to help manage this pest, antixenosis of wheat lines is also a viable option. Antixenosis resistance occurs when plant processes cause insect behaviour to be altered to avoid the plant as a food or oviposition site (Kogan & Ortman, 1978). Lines of antixenotic wheat were found to reduce the density of wheat midge eggs in the early 2000s (Lamb *et al.*, 2000a; Lamb *et al.*, 2002b). The mechanism conferring this resistance is not well understood, but could be valuable alone or in combination with the *Sm1* gene (Fox *et al.*, 2009; Gharalari *et al.*, 2009a). More than one gene is involved in the inheritance of oviposition deterrence, and environment may have a significant influence on phenotypic expression (Gharalari *et al.*, 2009a; Lamb *et al.*, 2002b). There is no evidence for linkage between the antibiosis gene *Sm1* and the oviposition deterrence gene (Gharalari *et al.*, 2009a). Rather than morphological traits, it is hypothesized that volatiles emitted by the wheat spikes and surface chemicals may be detected and used by female wheat midge when selecting oviposition sites (Ganehiarachchi & Harris, 2009; Gharalari *et al.*, 2009b; Gharalari *et al.*, 2011; Lamb *et al.*, 2001). Further investigations into these volatiles, antixenotic resistance, and their use in wheat midge control are needed.

1.3.4. Chemical Control

Foliar insecticides are the main form of chemical control used to manage wheat midge. Chlorpyrifos, dimethoate, methoxychlor, and permethrin were insecticides granted emergency registration in Canada for wheat midge control in 1984 (Elliott, 1988a). In Canada, application of foliar insecticides is recommended once the economic threshold of one adult wheat midge per four to five wheat heads has been exceeded (Olfert *et al.*, 2009). Insecticides should only be applied if the economic threshold is reached when wheat is still susceptible to wheat midge (Zadoks stages 50-59) which is when the wheat head emerges from the boot (Elliott & Mann, 1996; Zadoks *et al.*, 1974). Between heading and anthesis (flowering) (Zadoks stage 60), damage from wheat midge declines 35- to 240-fold (Elliott & Mann, 1996). Insecticidal sprays are applied uniformly over the heads to ensure contact control of eggs and young larvae (Elliott, 1988b). Elliott (1988b) found that excellent control was provided by an aerial application of dimethoate or chlorpyrifos in wheat. Aerial applications of these insecticides were applied when 55-60% of heads were emerged from the flag leaf and provided kernel protection, yield improvement and economic returns (Elliott, 1988b). Elliott (1988b) found that waiting approximately 6 days after midge oviposition began allowed for an extended application window and improved spray coverage. Despite these benefits, Elliott (1988b) cautioned that these insecticides could potentially have a negative impact on parasitoids of wheat midge. Insecticidal sprays can be less effective due to the protection the larva receives when it is concealed on the kernel inside the wheat head (Elliott, 1988b; McKenzie *et al.*, 2002). Orienting the nozzles on ground sprayers 40 degrees forward rather than downward and applying sprays at higher volumes can help improve the deposit uniformity on the wheat heads (Elliott, 1988a, 1988b). Methoxychlor and permethrin were delisted due to environmental concerns (Floate *et al.*, 2002). Currently, dimethoate and chlorpyrifos are the only active ingredients registered for the control of wheat midge in Canada (Alberta Agriculture and Forestry, 2018).

1.3.5. Natural Enemies

1.3.5.1. Generalist Predators

Wheat midge are vulnerable to predation when larvae drop down to the soil at the end of the season to overwinter (Basedow, 1973) and when they pupate on the soil surface (Floate *et al.*, 1990). Floate *et al.* (1990) showed fourteen species of carabid beetles fed on wheat midge larvae under laboratory conditions. In the absence of alternative prey, *Bembidion quadrimaculatum* L., *Bembidion obscurellum* Motschulsky, *Agonum placidum* Say, and *Pterostichus corvus* LeConte respectively killed 9, 15, 43, and 48 larvae per beetle per day (Floate *et al.*, 1990). In the field, daily predation was estimated to range from <1 to 86 wheat midge larvae per square metre (Floate *et al.*, 1990). Basedow (1973) found that carabid species *Pterostichus vulgaris* L., *Agonum dorsale* Pontoppidan, and *Lorocera pilicornis* Fabricius consumed an average of 3.3, 3.3, and 3.0 wheat midge larvae per individual per day under laboratory conditions, respectively. Spiders may also consume egg, larval, and adult stages of wheat midge (Barnes, 1955; Basedow, 1973). Holland and Thomas (2000) conducted a study that manipulated invertebrate polyphagous predator populations using exclusion techniques to assess predation impacts on wheat midge in fields in the United Kingdom. They found there was little impact on wheat midge larval numbers before oviposition occurred but indicated that predators might help reduce larval populations returning to the soil (Holland & Thomas, 2000).

1.3.5.2. Parasitoids

More specialized natural enemies are also important to consider in the management of wheat midge. Parasitoids are important beneficial insects in agriculture, laying their eggs on, or inside hosts, where they develop and eventually kill their host (Wheeler, 1914). The parasitoid complex for wheat midge and a closely-related, co-occurring species, *Contarinia tritici* Kirby (Diptera: Cecidomyiidae), includes 27 recorded species, with eight of those species specific to wheat midge (Affolter, 1990). The four main ovo-larval endoparasitoids of wheat midge include: *Macroglenes* *penetrans* Kirby (Hymenoptera: Pteromalidae), *Platygaster tuberosula* Kieffer, *Euxestonotus error* Fitch, and *Inostemma mosellanae* Flight (Hymenoptera: Platygastridae) (Chavalle *et al.*, 2015a; Elliott *et al.*, 2011a; Olfert *et al.*, 2003). Of these species, only *M. penetrans*, *P. tuberosula*, and *E. error* have been reported in North America. These species oviposit into the eggs of their hosts (Chavalle *et al.*, 2015a; Olfert *et al.*, 2003). The host larva develops until the adult parasitoid is ready to emerge, resulting in the death of the host (Doane *et al*. 1989).

Macroglenes penetrans is one of the most prolific parasitoids of the wheat midge and is the most studied. Originating from Europe, it has been postulated that *M. penetrans* was introduced into North America with its host in the 1800s (Affolter, 1990; Mason *et al.*, 2017). *Macroglenes penetrans* was initially reported in Manitoba in the 1950s during an outbreak of wheat midge and again in Saskatchewan during an outbreak of major economic importance in the 1980s (Mason *et al.*, 2017). This chalcidoid wasp is a metallic, blue-green colour and ranges in size from 1.3-1.9 mm. Males have prominent rust-bicoloured eyes and females have black eyes (Affolter, 1990). The species is univoltine and is well synchronized with its host (Affolter, 1990). Beginning in late May or early June in Europe and early July in Canada, *M. penetrans* emerges over a 6-week period (Affolter, 1990; Doane & Olfert, 2008). During this period, it deposits its eggs into those of its host which hatch within 5-12 days (Affolter, 1990). The adult female searches for eggs by inserting its ovipositor between the glume and glumella and moving it like a drill (Affolter, 1990). Once a target is found, the female wasp lays a single egg in the wheat midge host (Affolter, 1990). Affolter (1990) observed that mean fecundity per female was 205 ± 20 (SD) eggs based on dissections of gravid females. *Macroglenes* *penetrans* overwinters as a second instar larva. The following spring, when diapause is broken, the parasitoid larva will consume its host and moult into a non-feeding third-instar larva before it pupates on the soil surface (Affolter, 1990).

Euxestonotus error is a palearctic species that likely also followed the wheat midge into Canada from Europe, where the parasitoid is widely distributed (Affolter, 1990; Mason *et al.*, 2017). The first record of *E. error* in North America was reported in New York state in 1861 by Fitch (Gahan, 1933). This species is approximately 1-1.2 mm long and is black with a shiny, flat scutellum (Affolter, 1990). *Euxestonotus error* demonstrates a degree of host specificity to the wheat midge (Affolter, 1990). It is univoltine and well synchronized with its host, attacking the egg and completing development in the final larval stage of the host (Affolter, 1990). *Euxestonotus error* explores wheat heads during the day and evening by vibrating its antennae over the search area (Affolter, 1990). Affolter (1990) hypothesized that hosts were likely detected using olfaction. After identifying a host in the wheat head, the female wasp searches the area with its ovipositor and lays a single egg (Affolter, 1990). *Euxestonotus error* has a high mean fecundity at 477 ± 35 (SD) eggs based on dissections of gravid females (Affolter, 1990). This parasitoid overwinters as a first- or second-instar larva, completing its development in May by devouring its host and pupating on the soil surface (Affolter, 1990).

Platygaster tuberosula also originates from Europe, but unlike *M. penetrans* and *E. error,* this parasitoid species did not follow its host into North America on its own. This species was introduced to help control the wheat midge in Canada (Doane *et al.*, 2001). *Platygaster tuberosula* was released as a biocontrol agent in Saskatchewan in

1993 and 1994 (Doane *et al.*, 2001; Olfert *et al.*, 2003). This parasitoid is shiny, black and 1.5-2 mm in length (Johansson, 1936). It is faintly hairy with an angled body that tapers towards the posterior end of the abdomen (Johansson, 1936). Since its introduction, this parasitoid has become established near its release site in Saskatchewan, but has not been reported outside of the province (Knodel & Ganehiarachchi, 2016; Olfert *et al.*, 2003; Shanower, 2005; Thompson & Reddy, 2016).

Parasitoids play a major role in the integrated pest management of wheat midge in Canada. In Saskatchewan, average combined rates of parasitism by *M. penetrans* and *P. tuberosula* were observed at 64% in 2001 (Olfert *et al.*, 2003). Parasitism rates of *M. penetrans* were recorded at 35% in Montana in 2004, but no incidences of *P. tuberosula* were recorded (Shanower, 2005). Olfert *et al.* (2009) estimated the value of parasitoid populations in Saskatchewan to be in excess of $298.5 million CAD (adjusted to 2018 dollars) during the 1990s. Strategies can be applied to help conserve populations of parasitoids, including the appropriate timing of insecticide applications. Applications of insecticides prior to parasitoid emergence would help to reduce the number of wasps affected (Elliott & Mann, 1997). Applying insecticides at the lowest recommended rate would additionally help to reduce the residual toxicity of the spray to the wasps (Elliott & Mann, 1997).

1.3.6. Forecasting Models

Numerous studies have been conducted in Germany, Finland, the United States, the United Kingdom, and Canada to determine the most accurate model to forecast the emergence of wheat midge. Many models are based on the accumulations of degree-days (DD). A degree-day (or growing degree-day) is used to measure the amount of heat accumulation above a specific base temperature over a period of 24 hours (Herms, 2004). These accumulations are used to predict insect development from termination of diapause to adult emergence (Herms, 2004). Two major categories of model have been used to describe wheat midge emergence: phenological models and bioclimatic models. Phenological models are developed by monitoring phenological events from year to year and noting the number of degree-days that have accumulated since a specific start date (Herms, 2004). Some of the phenological models developed to predict wheat midge emergence additionally include precipitation as a factor. Bioclimatic models incorporate multiple environmental parameters and can be used to predict the establishment and distribution of insect pests (Olfert *et al.*, 2016).

1.3.6.1. Phenological Models

A number of models have been developed to help predict aspects of wheat midge phenology. Models developed for Germany predict that the end of diapause occurs within five weeks following 220 DD above 3 ºC from January 1 following a rainfall event of >5 L/m^2 (Basedow, 1977a, 1980; Basedow & Gillich, 1982). In Finland, where the predictive power of the German models was poor, a model was developed predicting adult emergence following an accumulation of 370-400 DD above 5 ºC from January 1 (Husberg & Kurppa, 1988; Kurppa, 1989b). In the United States, in Montana, 450 DD and 1300 DD above 4.5 ºC from March 1 must occur before larvae emerge from their cocoons and pupate and before 10% female adult emergence, respectively (Knodel &

Ganehiarachchi, 2016). Two models were developed for Canada in the 2000s. It was found that 306 DD above 9 ºC from March 1 were needed for 50% adult emergence in the laboratory (Wise & Lamb, 2004). Emergence of 10% adults in Saskatchewan occurred after 693 DD above 5 ºC following March 1 (Elliott *et al.*, 2009). The emergence model for the United Kingdom was based on days rather than degree days (Oakley, 1994; Oakley *et al.*, 1998). It indicates that diapause ends when soil temperatures rise above 13 ºC following a period of heavy rain fall, with adults emerging 3 weeks after such an event (Oakley, 1994; Oakley *et al.*, 1998).

Direct comparisons of different phenological models developed for wheat midge often identify unreliable, approximate, and contradictory results, which suggests consideration of other factors is needed (Jacquemin *et al.*, 2014). It was found that emergence could not be predicted solely as a response to temperature accumulation. In Belgium, Jacquemin *et al.* (2014) developed their own model based on definite rainfall events and adult emergence. They incorporated 'waves of emergence' or emergence events triggered by rainfall events into their model (Jacquemin *et al.*, 2014). The model developed included three separate phases: a temperature accumulation of 250 DD above 3 ºC beginning January 1, a phase that lasts until mean daily temperature rises above 13 ºC and a rainfall event occurs, and then an accumulation of 160 DD above 7 ºC after the event (Jacquemin *et al.*, 2014). Following these conditions, adult wheat midge should emerge (Jacquemin *et al.*, 2014).

1.3.6.2. Bioclimatic Models

Bioclimatic models can be utilized to predict the establishment of an insect species in regions where it is not yet present. Olfert *et al*. (2016) proposed a bioclimatic simulation model (ecological niche model) to predict the distribution and extent of wheat midge into new areas. This model was developed using CLIMEX® software (Hearne Scientific Software, South Yarra, Australia) which includes temperature, diapause, light, moisture, heat stress, cold stress, wet stress, and dry stress as parameters (Olfert *et al.*, 2016). Using CLIMEX®, ecoclimatic indices (EI) are derived which define locations suitable for potential distribution and also estimate relative abundances of the pest (Olfert *et al.*, 2016). The bioclimatic model for wheat midge was originally developed and compared with observed distributions and abundances of wheat midge in North America (Olfert *et al.*, 2016). The distributions for Europe, Asia, and North Africa were then used to validate the model further (Olfert *et al.*, 2016). This model has been used to predict the movement and range expansion of wheat midge due to climate change (Olfert *et al.*, 2016). In North America, areas in the northern Canadian Prairies, northern British Columbia, and isolated areas of Alaska would have potential for wheat midge outbreaks by 2030 (Olfert *et al.*, 2016).

1.3.6.3. Forecast Maps

In the Canadian Prairies, one of the tools utilized to predict and control wheat midge is the forecast maps published by the Prairie Pest Monitoring Network, a collaborative effort that includes researchers from Agriculture and Agri-Food Canada, Manitoba Agriculture, Saskatchewan Ministry of Agriculture, Alberta Agriculture & Forestry, and university researchers (PPMN, 2014-2018). These maps are released to the public annually and are created using the bioclimatic model developed by Olfert *et al.* (2016) (Fig. 1.4) (PPMN, 2014-2018). The maps incorporate data collected from surveys throughout the Canadian Prairies in British Columbia, Alberta, and Saskatchewan provinces each year (Fig. 1.4) (PPMN, 2014-2018). These maps report some of the most reliable predictions of wheat midge outbreaks each year and allow growers to plan accordingly (Fig. 1.4) (PPMN, 2014-2018).

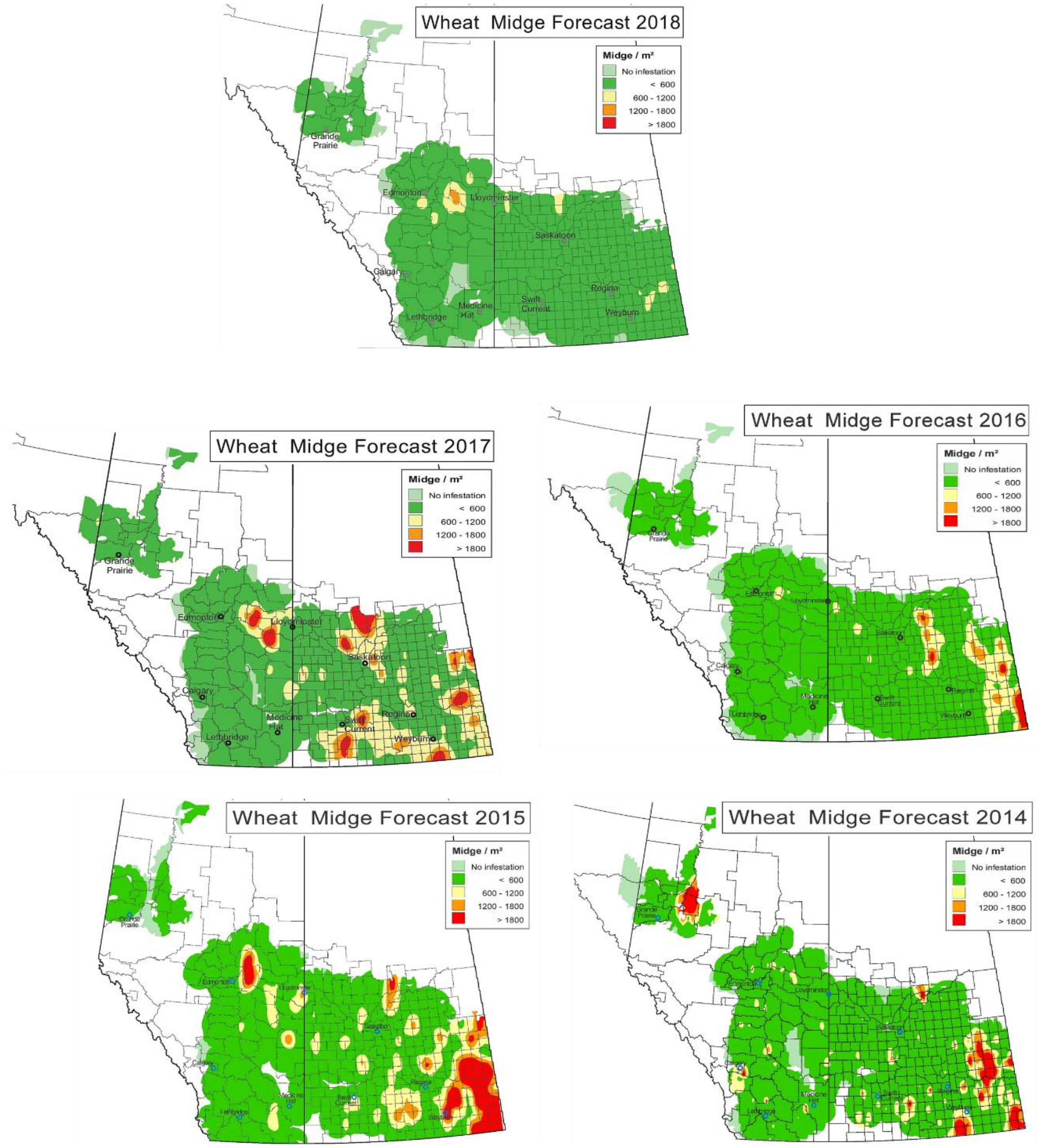

Figure 1.4. Forecast maps for the wheat midge throughout Alberta and Saskatchewan from 2014 to 2018. Image used with permission from Owen Olfert, Agriculture & Agri-Food Canada. Pale green = no infestation, green = <600 midge / m^2, yellow = 600 – 1,200 midge / m^2, orange = 1,200 – 1,800 midge / m^2, red = 1,800 midge / m^2.

1.4. Historical Presence and Management of Wheat Midge

The wheat midge is believed to be Palearctic in origin (Affolter, 1990; Doane *et al.*, 2013; Olfert *et al.*, 2009). Its current distribution overlaps the occurrence of wheat production, especially between the 42nd and 62nd parallels (Affolter, 1990; Olfert *et al.*, 2016). This species is widely distributed and has been recorded in 30 countries: Algeria, Austria, Belgium, Bulgaria, Canada, China, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Israel, Italy, Japan, Luxembourg, Montenegro, the Netherlands, Norway, Poland, Romania, Russia, Serbia, Slovakia, Sweden, Switzerland, Ukraine, United Kingdom, and the United States (European and Mediterranean Plant Protection Organization Global Database, 2018; Fauna Europaea, 2018). The wheat midge is currently a major economic pest in Asia, Europe, and North America. The following sections will explore the establishment of wheat midge in these regions and discuss current and potential future methods of control.

1.4.1. Asia

The earliest report of wheat midge in the literature describes its presence in China in the 1310s (Duan *et al.*, 2013). The two largest outbreaks of wheat midge in China occurred in the northern part of the country in the 1950s and again in the 1980s, which resulted in estimated losses of one billion kg of wheat each year (Miao *et al.*, 2013). Wheat production occurs mainly in the north of China in the provinces of Henan and Hebei (Duan *et al.*, 2013). In 2007, 233 million ha of wheat were affected by wheat midge (Wu *et al.*, 2009). Wheat midge populations have continued to spread further east and north in China each year as weather conditions and crop distributions have changed (Miao *et al.*, 2013; Wu *et al.*, 2009).

In Japan, the earliest report of damage from wheat midge was in 1935 and was said to be drastic (Katayama *et al.*, 1987). Outbreaks were also reported for 1949 and 1952 (Katayama *et al.*, 1987). Due to the decline of wheat production in Japan, the wheat midge was not seen again until 30 years later in 1981 in Kyoto Prefecture (Katayama *et al.*, 1987). Katayama *et al.* (1987) found that with the mechanization of the harvesting process, wheat midge populations increased from year-to-year even when there were poor environmental conditions for the pest.

1.4.2. Europe

Wheat midge is prolific throughout much of Europe, where it is predominantly a pest in winter wheat (Berzonsky *et al.*, 2003). Outbreaks of the pest were recorded as early as the late 1890s as well as in the 1930s, 1950s, and the late 1970s in Sweden and Central Europe (Kurppa, 1989a). The first record in Finland, in the 1930s, was reported on rye (Hukkinen & Vappula, 1936; Kurppa, 1989b), with subsequent outbreaks in 1983, 1985, 1987, and 1989 (Helenius & Kurppa, 1989; Kurppa & Husberg, 1989). Yield losses as high as 30-40% were reported in the country in 1983 (Kurppa & Husberg, 1989). In Denmark, heavy losses due to wheat midge were first reported in 1855 (Skuhrava *et al.*, 2006). Later outbreaks occurred in 1894-1895, 1903, 1905, and 1936 (Skuhrava *et al.*, 2006). It is considered a minor pest in Denmark (Skuhrava *et al.*, 2006). Wheat midge was reported in Italy as early as 1953 (Skuhrava & Skuhravy, 1994). High numbers of wheat midge were reported in the Netherlands in 1968 and 1969 and again in 1980 and 1984 (Daamen & Stol, 1993).

The earliest record of wheat midge in the United Kingdom was in 1741 in England (Webster, 1891). The earliest outbreaks of wheat midge in the United Kingdom were recorded in 1916, 1920, and 1926 (Oakley, 1994). The most prevalent outbreaks of the pest in the UK occurred in 1993 (Oakley, 1994) and in 2004 (Bruce *et al.*, 2007). In 1993, losses due to wheat midge damage were reported to exceed $102.1 million CAD (adjusted to 2018 dollars) (Oakley, 1994). Crop losses in 2004 surpassed $174.6 million CAD (adjusted to 2018 dollars) despite applications of insecticides (Oakley *et al.*, 2005).

Original control recommendations in Europe included applying insecticidal spray after wheat heads emerge from the flag leaf if an economic threshold of one female per one to three wheat heads was exceeded (Basedow & Schutte, 1973; Lescar, 1984; Oakley, 1981; Olsson, 1980). European growers are now advised against non-targeted or unnecessary spraying for wheat midge, citing negative impacts on important natural enemies like *M. penetrans* and predators such as spiders and dance flies, *Platypalpus* spp. (Hybotidae) (Bruce & Smart, 2009). Prior to 2009, midge-tolerant varieties of wheat were not commercially available with resistance limited to varieties of feed wheat (Bruce & Smart, 2009). Many areas of the United Kingdom continue to grow susceptible varieties of wheat to meet market demands for higher quality (Bruce & Smart, 2009).

Studies in the United Kingdom show that adult catches in pheromone traps reliably indicate periods of wheat midge flight activity and are strongly correlated with infestation levels (Bruce *et al.*, 2007). Occasionally, however, this correlation is confounded by movement of females between fields (Bruce & Smart, 2009). The most effective formulations for use in pheromone traps were found to be polyethylene vials loaded with 5 mg of 2,7-nonadiyl dibutyrate and rubber septa loaded with 1 mg of 2,7-nonadiyl dibutyrate (Bruce *et al.*, 2007).

In Germany, the earliest incidence of wheat midge occurred in 1930 (Barnes, 1932). Outbreaks of the pest were reported by Basedow in 1977 (Basedow, 1977b). In 1973, the economic damage threshold of 13 larvae per wheat head was developed in Germany (Basedow & Schutte, 1973). It was also recommended that chemical control could be considered profitable at densities of one ovipositing female midge per three heads of wheat (Basedow & Schutte, 1973). Basedow and Schutte (1973) found that, in terms of insecticides as control methods for wheat midge, parathion was ineffective but that both malathion and methoxychlor were very effective. In field experiments conducted in Germany, high wheat midge mortality rates were attributed to epigeal predaceous arthropods (Basedow, 1973). Basedow (1973) suggested that, given these implications, large-scale use of broad-spectrum insecticides might increase pest populations by driving down predator populations. Researchers in Germany recommend following integrated pest management strategies including cultural control, monitoring tools, agronomic practices, plant resistance and biocontrol for wheat midge (Gaafar *et al.*, 2011). Gaafar *et al.* (2011) recommend following the practice of monitoring the presence of wheat midge from the time the wheat head emerges until it flowers as recommended by Lamb *et al.* (2001).

Serious outbreaks of wheat midge occurred in Belgium in the early 2000s (Jacquemin *et al.*, 2008). The effectiveness of insecticides against wheat midge was assessed in winter wheat in 2012 and 2013 in Belgium (Chavalle *et al.*, 2015b). Researchers found insecticide treatments led to increases in yield and, in particular, found that chlorpyrifos provided an effective control against wheat midge (Chavalle *et al.*, 2015b). However, they cautioned against the protection offered by chlorpyrifos due to its harsh effects on beneficial parasitoids like *Macroglenes penetrans* (Chavalle *et al.*, 2015b).

1.4.3. North America

1.4.3.1. United States

The wheat midge was first introduced and became a pest in North America in the early 1800s (Felt, 1912). It was recorded first in 1820 in northwestern Vermont and a major pest in the state by 1828 (Fitch, 1856). However, Fitch (1865) believed, based on anecdotal evidence, that wheat midge was brought to Quebec in unthreshed wheat from England. It then made its way along the St. Lawrence and Chambly rivers until it reached Vermont (Fitch, 1856). By 1830, larvae were found in fields in New York where it became a pest by 1832 (Fitch, 1856). Initially, growers attempted to mow and dry wheat for hay while it was still green to destroy the larvae (Fitch, 1856). This was met with little success and crops were “decimated” over the next few years, resulting in few growers willing to grow the grain (Fitch, 1856). In 1834, wheat midge became a problem in New Hampshire and the state of Maine and reached injurious levels in Montreal (Fitch, 1856).

The wheat midge was declared “the most terrible pest ever encountered by wheat growers” by 1854, when it reached such destructive levels that many fields were not harvested (Fitch, 1856). Fitch (1856) conservatively estimated a loss of $372.15 million

CAD (adjusted to 2018 dollars) for wheat producers in New York State during in 1854. It was during this year that the wheat midge reached western Pennsylvania, Ohio, and eastern Indiana (Fitch, 1856). One control strategy encouraged at the time included attaching a sieve to winnowing machines or fanning mills to separate out wheat midge larvae to be destroyed later (Fitch, 1856). Although many of the earliest records of wheat midge include a variety of identifications and different names, Felt (1921) concluded that there was little doubt that these records referred to *S. mosellana*.

In the 1990s an outbreak of wheat midge in North Dakota resulted in a loss of $58.7 million CAD (adjusted to 2018 dollars) in gross revenue (Knodel & Ganehiarachchi, 2016). Since 1995, wheat midge has been detected in all counties in North Dakota east and north of the Missouri River (Knodel & Ganehiarachchi, 2016). Knodel and Ganehiarachchi (2016) cite that one of the most useful methods of cultural control for the wheat midge in North Dakota is early planting. Seeding early causes "incoincidence," where peak wheat midge emergence occurs after the wheat heads emerge and flower, minimizing wheat midge infestation (Basedow & Schutte, 1974; Knodel & Ganehiarachchi, 2016). Crop rotations that include soybean, sunflower, flax, peas, lentil, chickpeas, oats, or corn are recommended to reduce the opportunities for wheat midge reproduction from year to year (Knodel & Ganehiarachchi, 2016). Chemical control should be used when the economic threshold of one wheat midge per four or five heads has been reached and should be applied after four days if 30 percent of the wheat is heading, immediately if 70 percent of wheat is at heading to flowering, or immediately if 30 to 60 percent of wheat heads are flowering (though only a reduced level of control will be achieved) (Knodel & Ganehiarachchi, 2016). Knodel and Ganehiarachchi (2016) recommend using the resistant cultivar Egan, which incorporates the *Sm1* gene, a hard red spring wheat cultivar developed by the Montana Agricultural Experiment Station. *Macroglenes penetrans* is cited as one of the most important natural enemies in North Dakota with an average parasitism rate of 22 percent (Knodel & Ganehiarachchi, 2016).

First reports of minor damage from wheat midge in Montana were reported in the 1990s (Shrestha & Reddy, 2017). Surveys for wheat midge in Montana occurred in 2000 to 2004 and found the pest to occur in the northern and central parts of the state (Shanower, 2005). An outbreak with losses over $2.1 million CAD (adjusted to 2018 dollars) was recorded for Flathead County alone in 2006 (Stougaard *et al.*, 2014). The range of this pest seems to be expanding to the north, central, and eastern parts of the state within the last decade (Shrestha & Reddy, 2017).

Control strategies in Montana include applications of insecticides at peak adult emergence (Thompson & Reddy, 2016). However, strong winds often coincide with the period when insecticide spraying is necessary in this state, which may cause unsuitable conditions for application (Thompson & Reddy, 2016). Chlorpyrifos or lambda-cyhalothrin are the two insecticides most commonly used for wheat midge control in Montana (Thompson & Reddy, 2016). Most recently, efforts have been made to research botanical pesticides like jasmonic acid, a natural plant hormone (Shrestha & Reddy, 2017). Shrestha and Reddy found that the biopesticide product jasmonic acid and the insect pathogenic nematode *Steinernema feltiae* with 1% Barricade® polymer gel were able to reduce larval populations of wheat midge, increase grain yield in spring wheat, and reduce kernel damage levels compared to a control treatment of water (Shrestha & Reddy, 2017).

Other control efforts have been made in Montana using natural enemies of the wheat midge. Following a report indicating 52% parasitism of wheat midge larvae by *M. penetrans* in the state, efforts have been made to expand the prevalence of this parasitoid (Shanower, 2005; Thompson & Reddy, 2016). Introductions were focused in areas where the incidence of the parasitoid was not yet recorded like areas around Flathead, Montana (cited in Thompson and Reddy (2016) as a personal communication with B. Stougaard). The initial attempt saw little success and a second attempt was made in 2014, in Flathead and Pondera counties (Thompson & Reddy, 2016).

The wheat midge was reported in the past in southern parts of Washington state in the mid-1900s but never made it into the eastern part of the state (Reeher, 1945). Borkent (1989) suggests that this may be due to separate introductions of the wheat midge into the western and eastern areas of North America.

1.4.3.2. Canada

The wheat midge has been reported in Canada since the early 1800s and now occurs from coast to coast. The first record of wheat midge in Canada occurred in Quebec in 1819 (Sanderson, 1915). As described by J.W. Dawson, "this destructive little creature ha[d]…extended its ravages" to Nova Scotia by 1850 (Dawson, 1850). It was found in every part of the province, causing an almost "total abandonment of wheat culture" in some cases (Dawson, 1850). Recommendations at the time included cutting the wheat early and collecting larvae from the chaff in order to reduce the following years' populations (Dawson, 1850). The pest made its way to western Canada by 1902, when it was first reported in Manitoba (Fletcher, 1902).

The earliest record found in the literature of wheat midge in British Columbia comes from a report from the Dominion Experimental Farms in 1914 (Hewitt, 1914). The insect was reported as being “prevalent” in Agassiz, B.C. (Hewitt, 1914). Severe outbreaks were seen in crops of spring wheat in 1905-1908 in the Lower Fraser Valley (Hewitt, 1914). The wheat midge was not considered a noteworthy pest until the 1950s (Allen, 1955). Major outbreaks occurred in northeastern Saskatchewan and northwestern Manitoba in the 1980s (Doane *et al.*, 2013; Olfert *et al.*, 1985). These economically significant outbreaks spread throughout most of Manitoba and Saskatchewan by the 1990s (Elliott *et al.*, 2011b). Most of the wheat-growing area in western Canada is now infested by wheat midge (Olfert *et al.*, 2009).

Most recently, the wheat midge has made its way to the Peace River region of northern Alberta. Outbreaks of the pest were first reported in the region in 2011 although it was likely in the region before at non-economic levels (WCCP, 2011, 2013). The first samples of wheat midge larvae were found near the communities of Rocky Lane and High Prairie, AB on CDC Teal and CDC Alsask varieties in 2011 (Otani, 2011). Infestations were particularly high in the northeastern portions of the region, especially in 2014 (Alberta Agriculture and Forestry, 2014) (Figure 1.4). Currently, the Peace River region is still planted mostly to wheat midge susceptible varieties of spring wheat although some movement towards wheat midge tolerant varieties has been observed (Otani, 2016, personal communication).

1.5. Conclusions

The wheat midge is a widespread and economically important wheat pest and it is important to consider a full and comprehensive strategy for its control. Historical pest management strategies for wheat midge have included attempting to mow and dry wheat while it was still green and sieving out wheat midge in winnowing machines, but these did not prove to be very effective. Large outbreaks in the 1970s, 80s, and 90s caused a push for a more thorough understanding of wheat midge and its control. Most of the literature agrees that the inclusion of crop rotation, tillage, and early seeding at high seeding rates are important cultural controls that help avoid the buildup of wheat midge populations.

Since their introduction in 2011, cultivars with the antibiotic resistance conferred by the *Sm1* gene have come to the forefront of wheat midge control. As of 2015, antibiotic-resistant cultivars make up approximately 18% of the wheat acres grown in western Canada (Midge Tolerant Stewardship Team, 2015). Even with the incorporation of a portion of susceptible plants as a refuge, the use of these cultivars should be closely monitored for a breakdown of the resistance they convey. In areas where wheat midge outbreaks are forecasted to occur, tolerant cultivars should be sown.

When growing susceptible cultivars of wheat, chlorpyrifos and dimethoate are the insecticides registered in Canada for the control of wheat midge. These foliar-applied, broad spectrum insecticides should only be applied once the recommended threshold of one adult midge in four to five wheat heads is reached and only during stages when wheat is vulnerable. There are many benefits to using chemical control in the short term including kernel protection, improvements in yield, and better economic returns (Elliott, 1988b). However, monitoring for this pest can be difficult and must occur at dusk, when wheat midge adults are flying (Pivnick & Labbe, 1993).

The use of the chemical control could have long term implications for natural enemies of the wheat midge. Insecticide applications applied at an inopportune time could end up reducing or eliminating natural enemies instead of wheat midge. Elliott (1988b) found delaying insecticide applications by 6 days allowed producers more time to reassess the necessity of using foliar-applied broad spectrum insecticides based on midge populations, increased the window of time for applying these insecticides, improved spray coverage, and reduced residual requirements. This delay does not decrease the protection and control and could minimize the impact of insecticides on adult parasitoids (Elliott, 1988b). However, applications of insecticide can still be detrimental to parasitoid and predator populations. Elliott and Mann (1997) found that reducing the rate of application of chlorpyrifos from 0.40 kg to 0.24 kg AI ha^{-1} did not lower the amount of control and protection provided by the insecticide. This reduction would help decrease the cost of control and reduce harmful effects on parasitoids and other natural enemies (Elliott & Mann, 1997). Floate *et al.* (1989) found that soil residue remained toxic to carabid beetles for up to 16 days after chlorpyrifos applications.

Natural enemies have been, and continue to be, a very important aspect of wheat midge control. There is evidence that predatory arthropods present in wheat agricultural systems provide some measure of control. Midge larvae on the soil surface are consumed by ground beetles and spiders (Basedow, 1973; Floate *et al.*, 1990). Further investigations examining the role of these predators should be undertaken to determine their impacts on wheat midge populations. Parasitoids also have an important place in wheat midge control. Since its introduction in Saskatchewan, *Platygaster tuberosula* has not been found in any areas surveyed beyond that province (Olfert *et al.*, 2003; Shanower, 2005). Further follow up surveys should be undertaken to determine the distribution of this parasitoid. *Macroglenes penetrans*, however, is found in most areas throughout North America in conjunction with wheat midge populations (Knodel & Ganehiarachchi, 2016; Olfert *et al.*, 2003; Shanower, 2005; Thompson & Reddy, 2016).

One of the most effective and practical tools used in wheat midge control today is the forecast maps developed using the bioclimatic models based in the CLIMEX® software as well as parameters relating to host plants, wheat midge, and parasitism rates (Olfert *et al.*, 2016). These tools allow producers to make pre-emptive control decisions based on the predicted risk of outbreaks for the year. This can be crucial in determining where and which wheat cultivar to plant. These models will also be crucial in determining if and where the wheat midge will expand its range in the future.

1.6. Future Directions

When considering economically serious field crop pests in North America, few have multiple integrated pest management strategies developed to help manage them. Wheat midge has, arguably, one of the most developed set of strategies for its control. However, no single form of wheat midge control (cultural, chemical, or biological) has completely mitigated this pest’s impact on wheat production. Therefore, as long as wheat midge continues to be a major pest across the Canadian Prairies, further integrated pest management strategies should continue to be explored. As the exact mechanism of resistance is yet to be determined, antixenosis in wheat lines is one aspect of wheat midge control that should be investigated. Biopesticides might be another strategy that might provide effective control into the future.

CHAPTER 2: EFFECTS OF CANOPY STRUCTURE AND CROP TYPE ON ORANGE WHEAT BLOSSOM MIDGE AND GROUND BEETLE ASSEMBLAGES IN THE PEACE RIVER REGION OF ALBERTA, CANADA

2.1. Abstract

Orange wheat blossom midge, *Sitodiplosis mosellana* Géhin (Diptera: Cecidomyiidae), is an invasive, economically important pest of wheat (*Triticum* spp.) first detected in the Peace River region of northern Alberta, Canada in 2011. One prominent group of natural enemies of this pest is carabid beetles (Coleoptera: Carabidae), about which little is known for the region. A field plot experiment was seeded in Beaverlodge, AB to investigate relationships between host plant species and seeding rate on subsequent wheat midge infestations and carabid assemblages. The experiment consisted of four replicates of 16 spatially paired treatments. Treatments included two wheat varieties (*Triticum aestivum* cv. AC Stettler and CDC Utmost), canola (*Brassica napus* cv. Invigor L120), peas (*Pisum sativum* cv. CDC Meadow), and lambsquarters (*Chenopodium album* L.). Seeding rates were manipulated in wheat and canola treatments. Wheat midge densities were determined by dissecting wheat heads collected from field plots. Weekly pitfall trap collections (May 24 to August 15, 2016 and May 30 to September 4, 2017) were used to evaluate carabid communities and activity densities. Fifty-eight species of carabid were identified (2016: $n = 4,511$ beetles, 2017: n = 3,284). The three numerically dominant species were *Poecilus lucublandus* Say, *Amara thoracica* Hayward, and *Carabus taedatus* Fabricius in 2016 and *P. lucublandus, Amara obesa* Say, and *Amara littoralis* Dejean in 2017. No effect of crop type on carabid assemblages was observed. High plant densities in paired wheat treatments resulted in lower carabid activity density and species

abundance. Wheat midge larval density was not affected by carabid activity density either year of the study. This is the first study to characterize carabid assemblages in agricultural ecosystems in this region. Future studies assessing impacts of *Poecilus lucublandus* on wheat midge populations could prove beneficial in the control of this pest.

2.2. Introduction

Orange wheat blossom midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae), is a serious economic pest of spring wheat, *Triticum aestivum* L., in North America. In Canada, adults lay their eggs on wheat heads over a period of five to six weeks beginning in late June to early July (Olfert *et al.*, 1985). Neonate larvae emerge from eggs within four to seven days and move into the florets, where they begin to feed on the developing kernel (Doane & Olfert, 2008; Elliott *et al.*, 2009). Larval feeding results in shrivelled, cracked, and distorted kernels (Dexter *et al.*, 1987). This damage can cause yield loss and reduce the grade of the grain depending on the population density, spatial distribution, and the timing of oviposition relative to heading and anthesis (Dexter *et al.*, 1987; Elliott, 1988b; Lamb *et al.*, 2000b; Wright & Doane, 1987). Wheat is most susceptible to wheat midge damage throughout heading (Zadoks stages 50-59), when the wheat head emerges from the boot (Ding & Lamb, 1999; Elliott & Mann, 1996; Zadoks *et al.*, 1974). Susceptibility drops off significantly after the onset of anthesis (flowering, Zadoks stage 60) (Ding & Lamb, 1999; Elliott & Mann, 1996; Zadoks *et al.*, 1974). After feeding for two to three weeks, larvae drop from the wheat heads and move into the soil, where they form overwintering cocoons (Doane & Olfert, 2008; Lamb *et al.*, 1999). The following spring, larvae break their diapause, emerge from their cocoons and return to the soil surface to pupate (Doane *et al.*, 1987; Elliott & Mann, 1996). Larvae are vulnerable to predation during life stages when they are found on the soil (Floate *et al.*, 1990).

Wheat midge is globally distributed in areas of wheat production between the 42nd and 62nd parallels (Affolter, 1990). Wheat midge was first introduced in North America in the early 1800s (Felt, 1912). The earliest report of this insect in Canada comes from Quebec in 1819 (Sanderson, 1915). In the Canadian Prairies, the insect was first reported in 1902 in Manitoba, but was not considered a significant pest until the 1950s (Allen, 1955; Fletcher, 1902). Wheat midge is now common across most of the wheat-growing areas in western Canada (Olfert *et al.*, 2009) including the Peace River region of northern Alberta, where it was first reported in 2011 (WCCP, 2011, 2013). In 2014, wheat midge populations were particularly high, reaching densities greater than 1800 midge per square metre in the northeastern portions of this region (Alberta Agriculture and Forestry, 2014).

Predation may be an important factor to consider in the management of the wheat midge. Reeher (1945) reported that small red mites, *Atomus pilosus* (Banks) (Trombidiformes: Trombidiidae), were found feeding on exposed wheat midge eggs. Spiders (e.g., Lycosidae, Micryphantidae, Linyphiidae) have been observed to feed on the egg, larval, and adult stages of wheat midge (Barnes, 1956; Basedow, 1973). Progress has been made in assessing carabids as potential predators of the wheat midge. Larval stages are particularly vulnerable to predation by Carabidae and Staphylinidae (Coleoptera) (Basedow, 1973; Floate *et al.*, 1990; Speyer & Waede, 1956). Basedow (1973) and Floate *et al.* (1990) indicated that wheat midge larvae are vulnerable to predation when they drop down to the soil surface and when they return to the surface in the spring. Basedow

(1973) found that, under laboratory conditions, the carabid species *Pterostichus vulgaris* L., *Agonum dorsale* Pontoppidan, and *Lorocera pilicornis* Fabricius (Coleoptera: Carabidae) killed an average of 3.3, 3.3, and 3.0 wheat midge larvae per beetle per day, respectively. *Bembidion quadrimaculatum* L., *Bembidion obscurellum* Motschulsky, *Agonum placidum* Say, and *Poecilus corvus* LeConte (Coleoptera: Carabidae) were observed to consume 9, 15, 43, and 48 larvae per beetle per day when no other prey were provided, respectively (Floate *et al.*, 1990). Daily predation in the field was estimated to range from <1 to 86 wheat midge larvae per square metre (Floate *et al.*, 1990). Holland and Thomas (2000) found that there was little impact from carabids on larval wheat midge populations before oviposition in an exclusion study. However, they indicated that carabids may still help reduce larval populations as they dropped to the soil (Holland & Thomas, 2000).

Understanding field crop rotation and seeding practices and their impact on wheat midge and its natural enemies will be very important for future management of these organisms. Crop rotation involves growing plants species sequentially on the same land over successive growing seasons (Yates, 1954). Crop rotation is an important component of integrated pest management, helping to control pest insect populations, weeds, and diseases (Bullock, 1992; Francis & Clegg, 1990). For instance, Dosdall *et al.* (2012) found that damage to canola taproots by root maggot larvae, *Delia* spp. (Diptera: Athomyiidae), increased after canola had been grown continuously for three years, especially when compared to more diverse cropping sequences. Dosdall *et al.* (2012) suggested that such effects could outweigh short-term incentives for continuous cropping in the long run. Crop canopy can affect many different abiotic factors including light, relative humidity, and soil moisture (Cárcamo & Spence, 1994; Kromp, 1999). Carabid beetles are particularly sensitive to changes in soil moisture and light (Kromp, 1999). In the Peace River region, the main field crop rotations used by growers are that of wheat following canola, and canola following canola (AAFC, 2011-2017). Though there have been many studies characterizing carabid assemblages in agro-ecosystems on the Canadian Prairies, none have characterized carabid species in the Peace River region (Holliday *et al.*, 2014). Bergeron *et al.* (2011) made strides in characterizing the carabid biodiversity found in the boreal forests of the region. However, no seasonal data is available to characterize carabid assemblages in the grasslands of the Peace River region (Holliday *et al.*, 2014).

In the current study, we examined effects of crop type and canopy structure on resident midge and carabid assemblages. The potential roles of the carabid beetles in regulating populations wheat midge were also assessed. Our four specific objectives were as follows: (1) the effects of crop type and canopy structure on carabid assemblages, (2) the effect of plant density on wheat midge populations, (3) the diversity of carabid assemblages found in the agro-ecosystems in the Peace River region, and (4) whether carabid assemblages have a significant effect on wheat midge populations.

2.3. Materials and Methods

2.3.1. Natural Enemies Plot Trial

2.3.1.1. Natural Enemies Plot Trial: Site Description and Experiment Design

To assess the effect of crop type and canopy structure on populations of midge and carabids, experimental plots were established for two years at the Agriculture and Agri-Food Canada research farm near Beaverlodge, Alberta (AAFC-Beaverlodge) (Lat: 55.199, Long: -119.396). The soil type at the site was clay loam (29.6% sand, 40.5% silt, and 29.9% clay) with a pH of 5.8. In the year prior to the study (2015), the land was seeded to barley (*Hordeum vulgare* L., cv. Metcalfe). Plots were organized into 16 treatments and four replicates. Plots were spatially paired to facilitate movement of carabid beetles within and between the two years of the study (Fig. 2.1). The 64 plots were arranged in replicates separated by 15 m of borders seeded to fall rye (*Secale cereal* L., cv. Common) (Fig. 2.1). The eight spatial pairings within each replicate were separated by 2 m borders seeded to fall rye. Individual plots measured 4 by 15 m with 23-cm row spacing (Fig. 2.1).

Plot treatments included different combinations of four plant species and three seeding rates to represent field crop rotations and canopies typical of the Peace River Region (Table 2.1). These included a wheat midge susceptible variety of wheat (*Triticum aestivum* L., cv. AC Stettler), a wheat midge tolerant variety of wheat (*Triticum aestivum* L., cv. CDC Utmost), canola (*Brassica napus* L., cv. Invigor L120), peas (*Pisum sativum* L., cv. CDC Meadow), and lambsquarters (*Chenopodium album* L.) (Table 2.1). Plant species were originally chosen to allow for comparison between more open (cereal species) and dense (broadleaf species) canopy structure and its effect on carabid densities

(Table 2.1). Seeding rates were selected to reflect low, standard, and high seeding rates in the region (Table 2.1). Susceptible and tolerant varieties of wheat were compared to assess differences in carabid assemblages in plots with high versus low densities of wheat midge (Table 2.1). Lambsquarters, a weed species ubiquitous to the region, was selected to assess how the canopy of a weed species and the presence of high numbers of weed seeds might encourage the presence of certain carabid species (Table 2.1). Crop rotations occurred in 2017 within the spatial pairings for all treatments except for paired wheat and lambsquarters treatments (Fig. 2.1a and 2.1b). Paired crops were seeded sequentially over one another within the pairing to simulate crop rotation within the region (Fig. 2.1a and 2.1b).

The experiment was laid out as a randomized complete-block design to account for variation in the field. Space constraints at the Beaverlodge Research Farm did not allow for full replication of seeding rates of all plant species for the trial. Both the tolerant and susceptible wheat were seeded at three rates: 200 seeds per m^2, 300 seeds per m^2, and 400 seeds per m^2 (Table 2.1). Canola was also seeded at three rates: 50 seeds per m^2, 100 seeds per m^2, and 125 seeds per m^2 (Table 2.1). Peas were seeded at a rate of 100 seeds per m^2 (Table 2.1). Lambsquarters was seeded at a high rate of 400 seeds per m^2 to ensure that the crop would germinate (Table 2.1). Plots were fertilized according to soil test recommendations for wheat, canola, or pea production depending on the crop species that was seeded that year.

Plots were seeded on May 10, 2016 and May 21, 2017 using a John Deere seeder (Conserva Pak CP129A, Manufacturer: Valcon Equipment, [Indian Head, Saskatchewan]). Plots were maintained using best management practices for the respective production systems. Once during each growing season, plots received herbicide applications. On June 13, 2016, wheat was treated pinoxaden (Axial® BIA applied at a rate of 61.7 g a.i. ha^{-1}). Peas and canola were treated with sethoxydim (Poast® Ultra applied at a rate of 50 g a.i. ha^{-1}). In 2017, wheat was treated with a spray mix of florasum + MCPA ester (Frontline^{TM} XL applied at a rate of 355 g a.i. ha^{-1}), pinoxaden (Axial® 100 EC applied at a rate of 59.3 g a.i. ha^{-1}), and methyl esters (Adigor® applied at a rate of 540 g a.i. ha^{-1}) on June 13. Treatments of canola were treated with glufosinate-ammonium (Liberty® applied at a rate of 500 g a.i. ha^{-1}) and clethodim + surfactant (Select® + 30% Amigo® applied at a rate of 30 g a.i. ha^{-1}) on June 15. Peas were sprayed once with imazamox + bentazon (Viper® + 28% UAN applied at a rate of 448.2 g a.i. ha^{-1})

Table 2.1. Crop, seeding rate, and target seeding densities seeded at Beaverlodge Research Farm in 2016 and 2017.

Treatment	**Crop**	**Seeding Rate (seeds/m^2)**
1	Susceptible Wheat	200
2	Tolerant Wheat	200
3	Susceptible Wheat	300
4	Tolerant Wheat	300
5	Susceptible Wheat	400
6	Tolerant Wheat	400
7	Susceptible Wheat	200
8	Canola	50
9	Susceptible Wheat	300
10	Canola	100
11	Susceptible Wheat	400
12	Canola	125
13	Susceptible Wheat	300
14	Peas	100
15	Susceptible Wheat	300
16	Lambsquarters	400

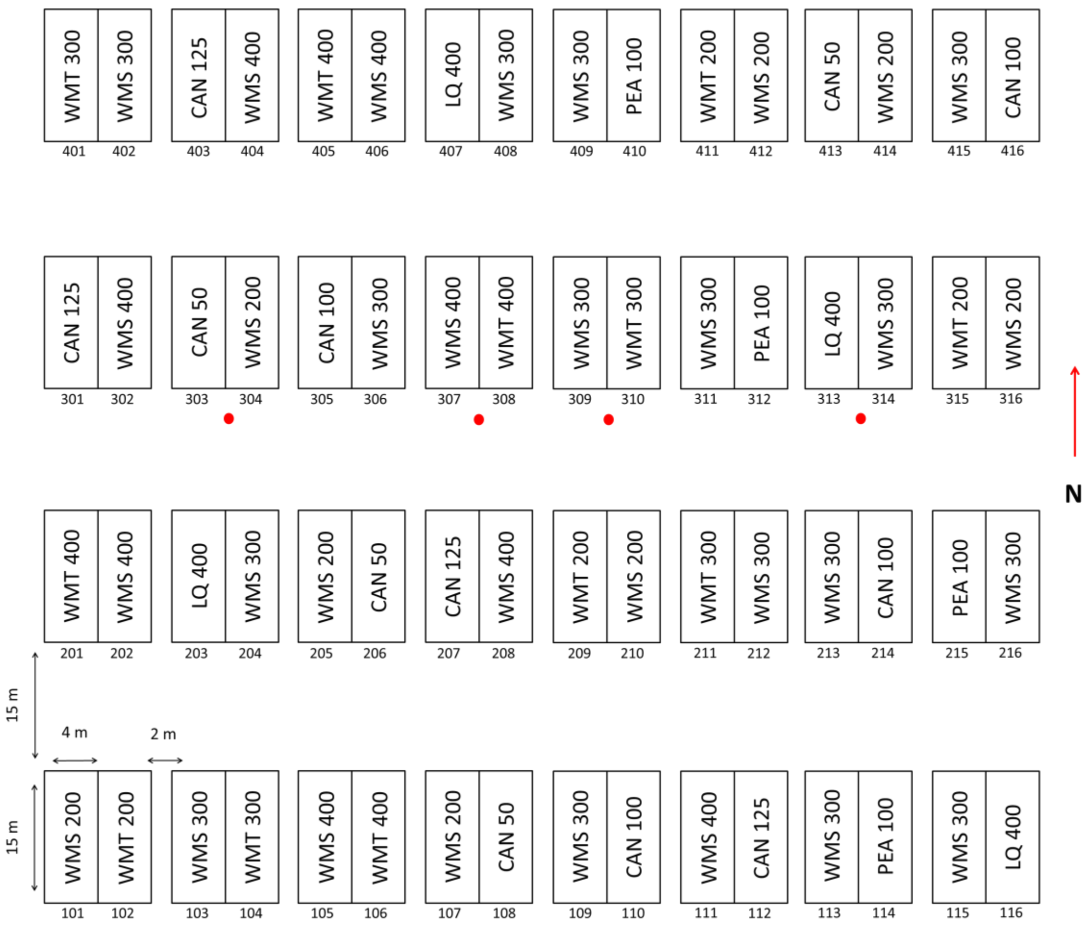

Figure 2.1a. Layout of field plot experiment seeded at the Beaverlodge Research Farm in 2016. Spatial arrangement was determined by random placement. Crops were seeded sequentially within their spatial pairs between 2016 and 2017 (indicated by blue arrow in 2.1b). Crop seeded is indicated using the following acronyms: WMS = wheat midge susceptible wheat, WMT = wheat midge tolerant wheat, CAN = canola, PEA = peas, LQ = lambsquarters. Seeding rates (seeds/m^2) are indicated by numbers listed on each corresponding plot. Red dots indicate the placement of a sticky card in the field. Plot number is indicated by the small number below each plot.

Figure 2.1b. Layout of field plot experiment seeded at the Beaverlodge Research Farm in 2017. Spatial arrangement was determined by random placement. Crops were seeded sequentially within their spatial pairs between 2016 and 2017 (indicated by blue arrow in 2.1b). Crop seeded is indicated using the following acronyms: WMS = wheat midge susceptible wheat, WMT = wheat midge tolerant wheat, CAN = canola, PEA = peas, LQ = lambsquarters. Seeding rates (seeds/m^2) are indicated by numbers listed on each corresponding plot. Red dots indicate the placement of a sticky card in the field. Plot number is indicated by the small number below each plot.

2.3.1.2. Natural Enemies Plot Trial: Carabid Collections

Pitfall traps were used to determine the activity density and species richness of arthropod predators within the plots. Pitfall trap catches represent activity densities rather than true densities as they are dependent on population density and the activity of different organisms collected. Each pitfall trap was constructed using two transparent plastic containers, one nested within the other (Diameter: 11.5 cm, Height: 13 cm) and placed into the row with the rim of the trap flush with the soil surface (Fig. 2.2). A plastic funnel constructed from a 2-litre soft-drink bottle (Diameter: 11.5 cm, Height: 8 cm) was placed into the inner container to reduce invertebrate by-catch (Fig. 2.2). To prevent excess water from entering the trap, rain shields constructed using corrugated plastic (16 cm x 16 cm) and nails were placed 2.54 cm above each trap (Fig. 2.2). One pitfall trap was placed in the centre row of each plot, 2 metres in from the eastern and western plot edges and 7.5 metres in from the northern and southern plot edges (Fig. 2.3). Each inner container was filled with a 1:1 solution of preservative propylene glycol (formulated in a commercial product, sold as nontoxic antifreeze) and water. Weekly, the inner container of each trap was removed and poured through two layers of cheese cloth, which was then labeled and bound shut. Pitfall collections occurred between May 24 and August 15 in 2016 and May 30 to September 4 in 2017. Traps were placed into the plots approximately one week following seeding and were removed prior to harvest. Trap catches were stored in 95% EtOH until they were sorted, counted, and identified. Arthropod predators were identified to species (Coleoptera: Carabidae) or family (Coleoptera).

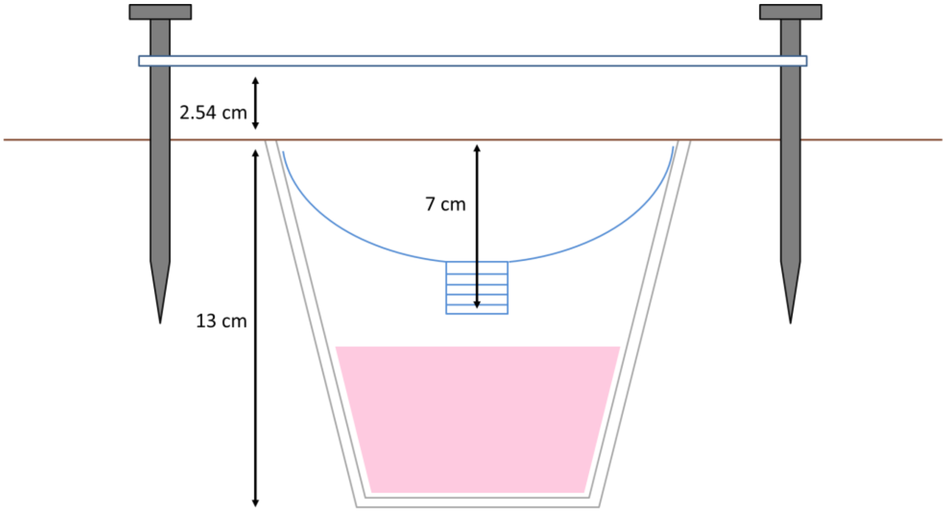

Figure 2.2. In-field pitfall trap design. Pitfall traps were placed in the centre row of each plot with the top of the trap flush with the soil surface.

2.3.1.3. Natural Enemies Plot Trial: Wheat Midge Collections

Yellow sticky cards (14 x 18 cm) (ACP Trap, Manufacturer: Alpha Scents, Inc. [Oregon, USA]) were placed at canopy height to determine presence and estimate peak populations of wheat midge during the growing season. Four sticky cards were placed at the southern edge of the third block to document seasonal activity (Fig. 2.1). Sticky cards were collected weekly from July 2 to August 9, 2016, and July 5 to August 1 Canon, 2017. In 2016, the first collection of the cards was delayed due to weather and represents a period of two weeks. Using a dissecting scope (Zeiss Stemi 2000-C, Manufacturer: Carl Zeiss Microscopy, LLC [New York, USA]), wheat midge adults were identified, counted and recorded for each card.

In each wheat plot, plants were collected from a two-metre row located four rows west of the centre of the plot. Entire plants (including the roots) were dug up and stored in paper bags held at 22° C until they could be processed. To obtain larval density per tiller, the number of wheat midge larvae was recorded for each tiller of 20 plants for each plot. Larval density per plot was determined by hand threshing 100 wheat heads per plot.

2.3.1.4. Natural Enemies Plot Trial: Canopy structure

In each plot, canopy structure was measured as plant density, canopy coverage, and growth ratings. Plant density was measured twice at the beginning of the growing season in both 2016 and 2017. The number of plants in a two-metre row was measured in the third row east of the plot edge, one metre north of the southern edge in 2016 (May 26 and June 3) (Fig. 2.3). In 2017, methods were altered so that measurements were taken in the row east of the pitfall, with the two-metre row beginning at the level of the pitfall and moving north (June 5 and June 16) (Fig. 2.3). All alterations to sampling locations between 2016 and 2017 were to ensure that the sampling location would more accurately represent canopy structure near the pitfall.

Canopy coverage was measured using percentage green cover. In 2016, weekly photographs were taken using a digital single-lens reflex camera (Canon EOS Rebel T5i, Manufacturer: Canon, [Japan]) two metres north of the centre of the plot (Fig. 2.3). Methods were altered in 2017 so that photographs were taken one metre north of the pitfall (Fig. 2.3). Canopy photographs were taken top-down from a height of 1.5 metres and encompassed sectioned defined by a t-square measure (measuring 0.5 m x 0.5 m). Photographs were taken from June 16 to August 9, 2016, and from May 30 to August 30, 2017. Photographs were analyzed using Leaf.exe version 1.21 (Liu, 2012), a program that assesses the percentage of green pixels within a defined area. During the final few weeks of the growing season, when the crops were ripening (and were no longer green), the sensitivity of the program was decreased to encapsulate the remaining canopy coverage.

Growth ratings that recorded the phenological stage of development were measured weekly in each plot throughout the growing season using Zadoks *et al.* (1974) (wheat), Harper and Berkenkamp (1975) (canola), and the BBCH Working Group (2001) (peas). Ten plants were rated in a two-metre row measured in the third row east of the plot edge, one metre north of the southern edge in 2016 (May 26 to August 16) (Fig. 2.3). In 2017, methods were altered so that measurements were taken in the row east of the pitfall, with the one-metre row beginning at the level of the pitfall and moving north (June 6 to September 5) (Fig. 2.3). From these ten growth ratings, an average growth rating per plot was calculated.

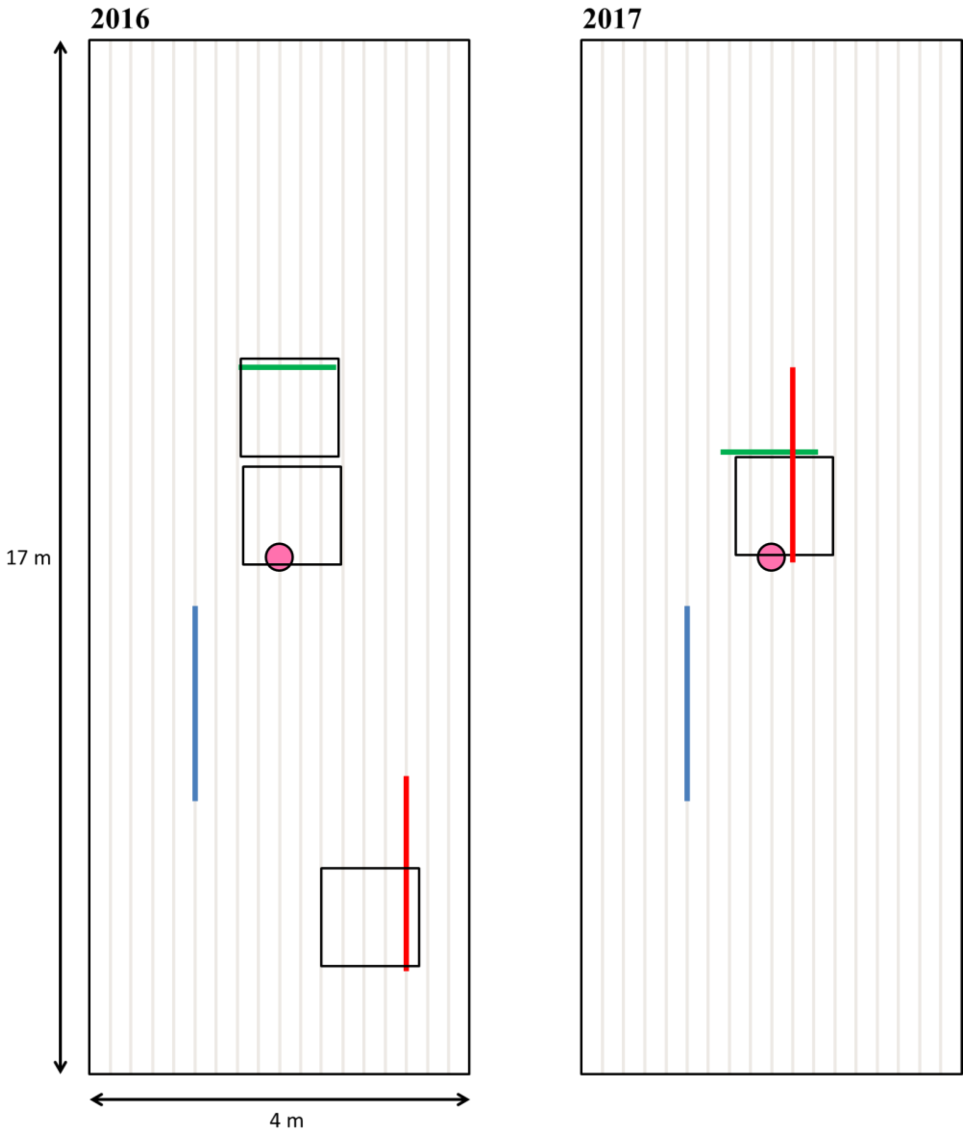

Figure 2.3. Layout of field plots seeded at the Beaverlodge Research Farm in 2016 and 2017. The pink circle indicates the position of the pitfall trap in the plot, green lines indicate where the t-square for the canopy coverage photographs was placed, red lines indicate where growth ratings and plant density counts were taken, blue lines indicate where wheat plants and heads were collected, and black squares indicate where quadrat samples (1 m^2) were harvested.

2.3.1.5. Natural Enemies Plot Trial: Tiller Susceptibility

In 2017, tiller susceptibility ratings were recorded for each wheat plot to determine the interval when wheat was susceptible to damage by wheat midge. Growth ratings were recorded for each tiller of ten plants in each plot seeded with susceptible wheat using the Zadoks *et al.* (1974) growth rating scale. Ratings were taken in the row east of the pitfall with the one-metre row beginning at the level of the pitfall and moving north in the same location as weekly growth ratings (Fig. 2.3). Ratings were recorded from the time the wheat plants were beginning to boot (Growth Stage 40, July 10) until all tillers had reached the anthesis (flowering) stage (Growth Stage 60, July 31) (Zadoks *et al.*, 1974). This was to ensure that the entire susceptibility period (Growth Stages 51-59) was captured for all plants (Zadoks *et al.*, 1974).

2.3.1.6. Natural Enemies Plot Trial: Harvest Parameters

Shortly before harvest, aboveground biomass samples were collected from all plots. Three quadrat samples (measuring 1 m x 1 m) were hand-harvested from each plot in 2016 to assess if biomass was consistent throughout the plot. These were collected at the location where growth ratings (Methods 2.3.1.4), canopy coverage ratings (Methods 2.3.1.4), and pitfalls samples were collected (Fig. 2.3). Because no significant differences in biomass were detected among these three quadrats, only results from the quadrat collected in the middle of the plot were used for analyses. Plants were cut just above ground level (2.54 cm above soil surface), counted, and then placed inside cotton harvest bags. In 2017, a single quadrat sample (measuring 1 m x 1 m) was hand-harvested from each plot. This sample was collected from the centre of the plot at the pitfall. Hand harvest methods were replicated between years. In both years, harvest samples were hung to dry outdoors for a period of approximately 3 weeks. Samples were then moved indoors and dried in a walk-in drier at 25 °C for approximately 2 weeks. Dried biomass samples were weighed and recorded.

Once plants reached maturity, all plots seeded to traditional crops (wheat, canola, and peas) were harvested and yield and moisture data were collected using a combine (Kincaid 8 XP, Manufacturer: Kincaid Equipment Manufacturing, [Kansas, USA]). Peas were harvested on August 19, 2016. Wheat and canola were harvested September 15, 2016. In 2017, peas were harvested on August 18. In 2017, wheat and canola were harvested September 27-28 and September 26, respectively.

Subsamples of seed from plots were assessed for percent protein (wheat, canola) and percent oil (canola). To determine the percent protein in wheat samples, the Dumas method of combustion was used on a Vario max cube (Manufacturer: Elementar, [Langenselbold Germany]) to obtain percent nitrogen of 120 g samples of seed. The combustion chamber was set at 900 °C and the oxygen flow rate was 125 mL per min. A conversion factor of 5.7 was used to convert percent nitrogen into percent protein (Jones, 1941).

2.3.2. Insect Identifications

Identification of wheat midge larvae and adults were determined using Barnes (1928) and Harris (1966). Family-level identifications of all Coleoptera were determined using Arnett *et al.* (1980). Carabid beetles were identified to species using Lindroth

(1961-1969) and by referring to voucher specimens provided by Owen Olfert (AAFC-Saskatoon) and by Héctor Cárcamo and Kevin Floate (AAFC-Lethbridge). Voucher specimens from this study have been stored at the Beaverlodge Research Farm.

2.3.3. Data Analyses

2.3.3.1. Data Analyses – Agronomic Parameters and Carabid Communities

Carabid communities and populations were studied in relation to crop type and canopy structure. The population parameter considered in this study was the activity density of species, as indicated by their catches. Seasonal carabid community structure was characterized by species richness. Abundance and species diversity data were log-transformed to help homogenize variances. For comparisons between carabid assemblages and canopy structure, treatments were split into the following three groups: paired susceptible and tolerant wheat treatments, paired susceptible wheat and canola treatments, paired susceptible wheat and pea treatments, and paired wheat and lambsquarters treatments. The first two groups were seeded to target low, standard, and high plant densities in the Peace River region.

Univariate split plot repeated measures ANCOVAs with standard least squares personalities were used for comparisons between canopy structure and carabid populations. Response variables for these models included carabid activity density and carabid species richness. For paired wheat treatments, models included plot nested within variety and block as a random effect. Fixed effects included collection week, variety, plant density or canopy coverage, and block.

For paired wheat and canola treatments, models included plot nested within crop type and block as a random effect. Fixed effects included collection week, crop type, plant density or canopy coverage, and block.

For paired wheat and broadleaf (peas or lambsquarters), models included plot nested within crop type and block as a random effect. Fixed effects for this model included collection week, crop type, canopy coverage, and block.

The overall abundance for the three dominant carabid species collected during each year of the plot trial was compared between the agronomic treatments seeded. A univariate split plot ANOVA was used to compare all treatments for each year. This model included plot nested wihtin treatment and block as a random effect. Fixed effects included treatment and block. All analyses were completed in JMP version 14.0.0.

2.3.3.2. Data Analyses – Agronomic Parameters, Carabid Communities, and Wheat Midge Larval Populations

Wheat midge larval populations were studied in relation to plant density, tiller density, and the period of susceptibility. Larval density was measured as the number of larvae per 100 heads. Univariate split plot ANCOVAs with a standard least squares personalities were used to compare the paired wheat treatments seeded. For models where wheat midge larval density was the response variable, plot was nested within variety and block as a random effect. Fixed effects included variety, plant density or tiller density or carabid activity density or period of susceptibility, and block. All analyses were completed in JMP version 14.0.0.

2.3.3.3. Data Analyses – Harvest Parameters

Harvest parameters (biomass, yield, and percent protein) were studied in relation to plant density, carabid abundance, and wheat midge larval density. Carabid abundance was measured as the mean activity density for the entire season per treatment. For comparisons, treatments were split into the following three groups: paired wheat treatments, paired wheat and canola treatments, and paired wheat and broadleaf treatments seeded at rates considered standard for the Peace River region. Univariate split plot repeated measures ANCOVAs with standard least squares personalities were used for comparisons harvest parameters and insect populations. For paired wheat treatments, plot was nested within variety and block as a random effect. Fixed effects for this model included variety, plant density, wheat midge larval density or carabid abundance, and block.

For paired wheat and canola treatments, models included plot nested within crop type and block nested as a random effect. Fixed effects for this model included variety, plant density, carabid abundance, and block.

For paired wheat and broadleaf (peas and lambsquarters) models included plot nested within crop type and block as a random effect. Fixed effects for this model included crop type, carabid abundance, and block. All analyses were completed in JMP version 14.0.0.

2.4. Results

2.4.1. Canopy structure

2.4.1.1. Canopy structure – Paired Wheat (Treatments 1-6)

Plant Density & Variety

In 2016, for paired wheat treatments during the season, no effect of plant density was detected on the total number of carabids recovered ($F_{1,\ 17} = 1.2611$, $p = 0.2771$; Fig. 2.4) or on species richness ($F_{1,\ 17} = 3.4025$, $p = 0.0826$; Fig. 2.5). Neither was there an effect of variety (susceptible vs. tolerant) on carabid activity density ($F_{1,\ 17} = 0.7814$, $p = 0.7814$; Fig. 2.4) or species richness ($F_{1,\ 17} = 0.0634$, $p = 0.8402$; Fig. 2.5) in paired wheat treatments in 2016.

This differed in 2017, when there was a significant negative effect of plant density on activity density of carabid beetles captured throughout the season ($F_{1,\ 17.06} = 10.8667$, $p = 0.0042$; Fig. 2.4). There was a significant interaction between plant density and wheat variety on activity density of carabid beetles ($F_{1,\ 17.09} = 4.5416$, $p = 0.0479$; Fig. 2.4). Plant density negatively affected the mean activity density of carabids collected in paired wheat treatments. Plant density also had a significant effect on the species richness of carabid beetles captured ($F_{1,\ 16.91} = 11.0354$, $p = 0.0041$; Fig. 2.5). Mean species richness of carabids decreased as plant density increased. There was no significant effect of variety on species richness ($F_{1,\ 16.94} = 0.2005$, $p = 0.6600$; Fig. 2.5). All other interaction terms were non-significant ($p > 0.05$).

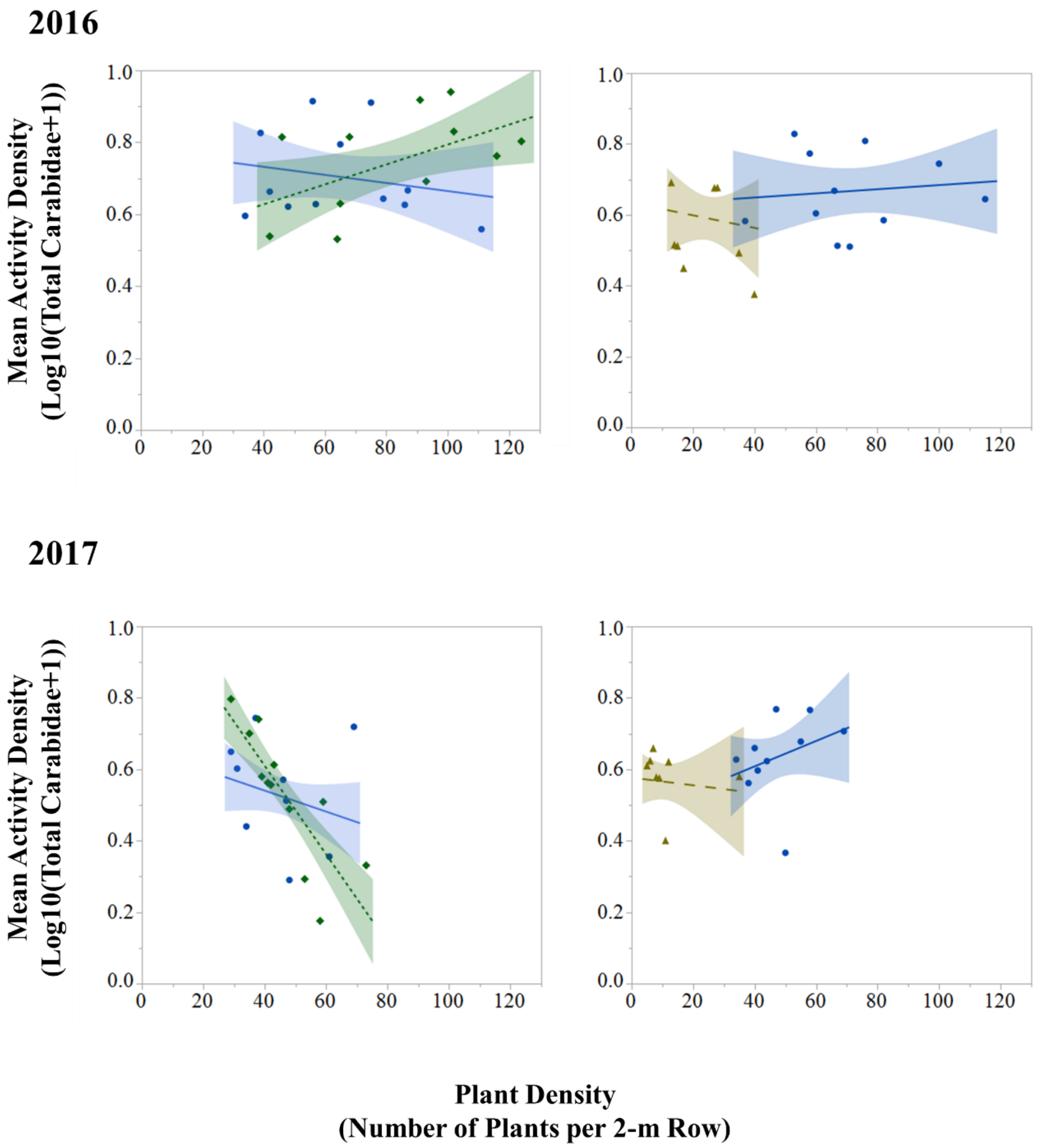

Figure 2.4. Effect of plant density on the mean activity density of carabid beetles collected weekly in paired wheat treatments (left) and paired wheat and canola treatments (right). Carabid activity density data were log-transformed to help homogenize the variances. Solid lines represent lines of best fit, while dark shading represents 95% confidence bands. Blue, solid line with circular marker = wheat midge susceptible wheat, green, dotted line with diamond marker = tolerant wheat, yellow, dashed line with triangle marker = canola.

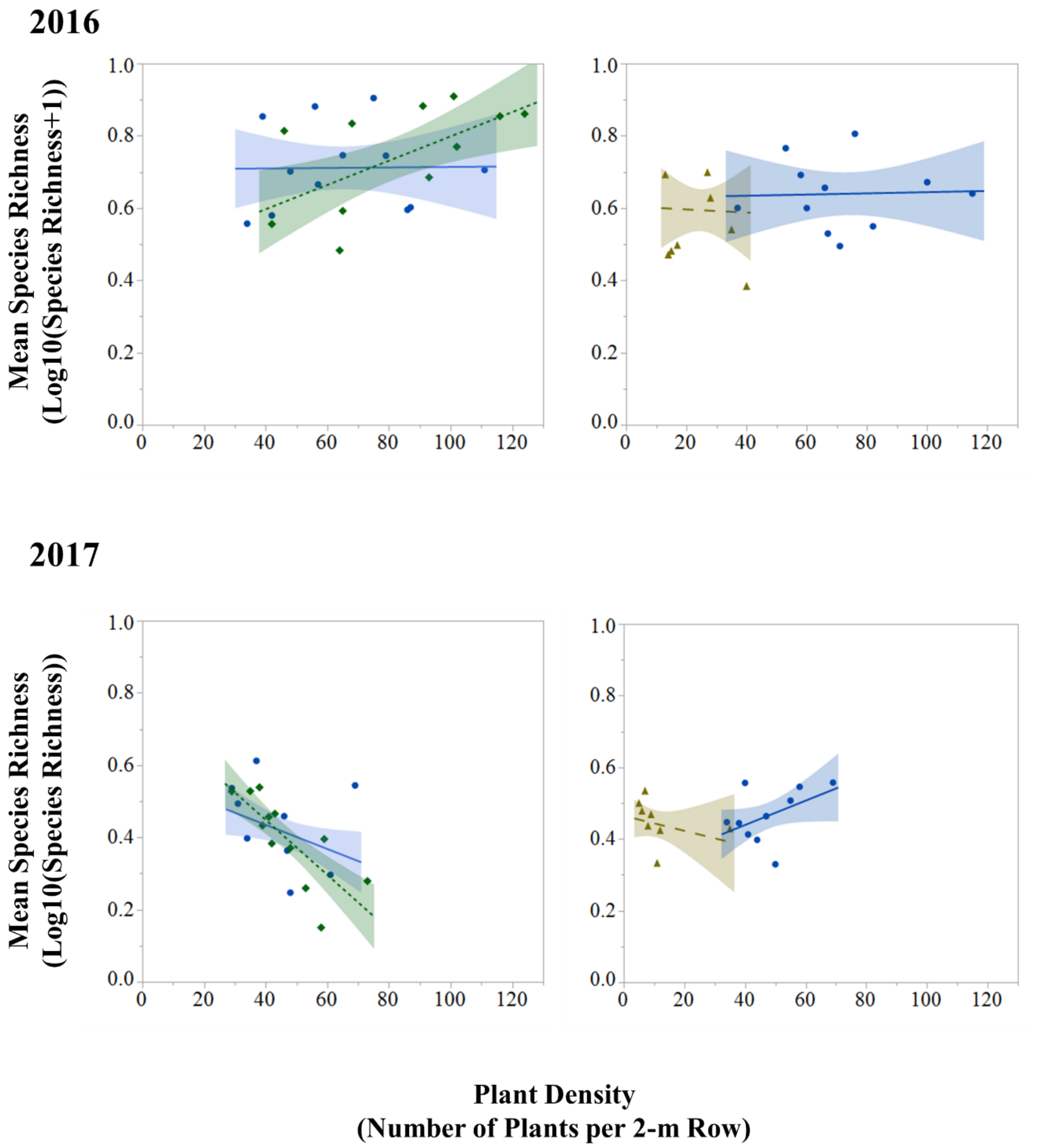

Figure 2.5. Effect of plant density on the mean species richness of carabid beetles collected weekly in paired wheat treatments (left) and paired wheat and canola treatments (right). Carabid activity density data were log-transformed to help homogenize the variances. Solid lines represent lines of best fit, while dark shading represents 95% confidence bands. Blue, solid line with circular marker = wheat midge susceptible wheat, green, dotted line with diamond marker = tolerant wheat, yellow, dashed line with triangle marker = canola.

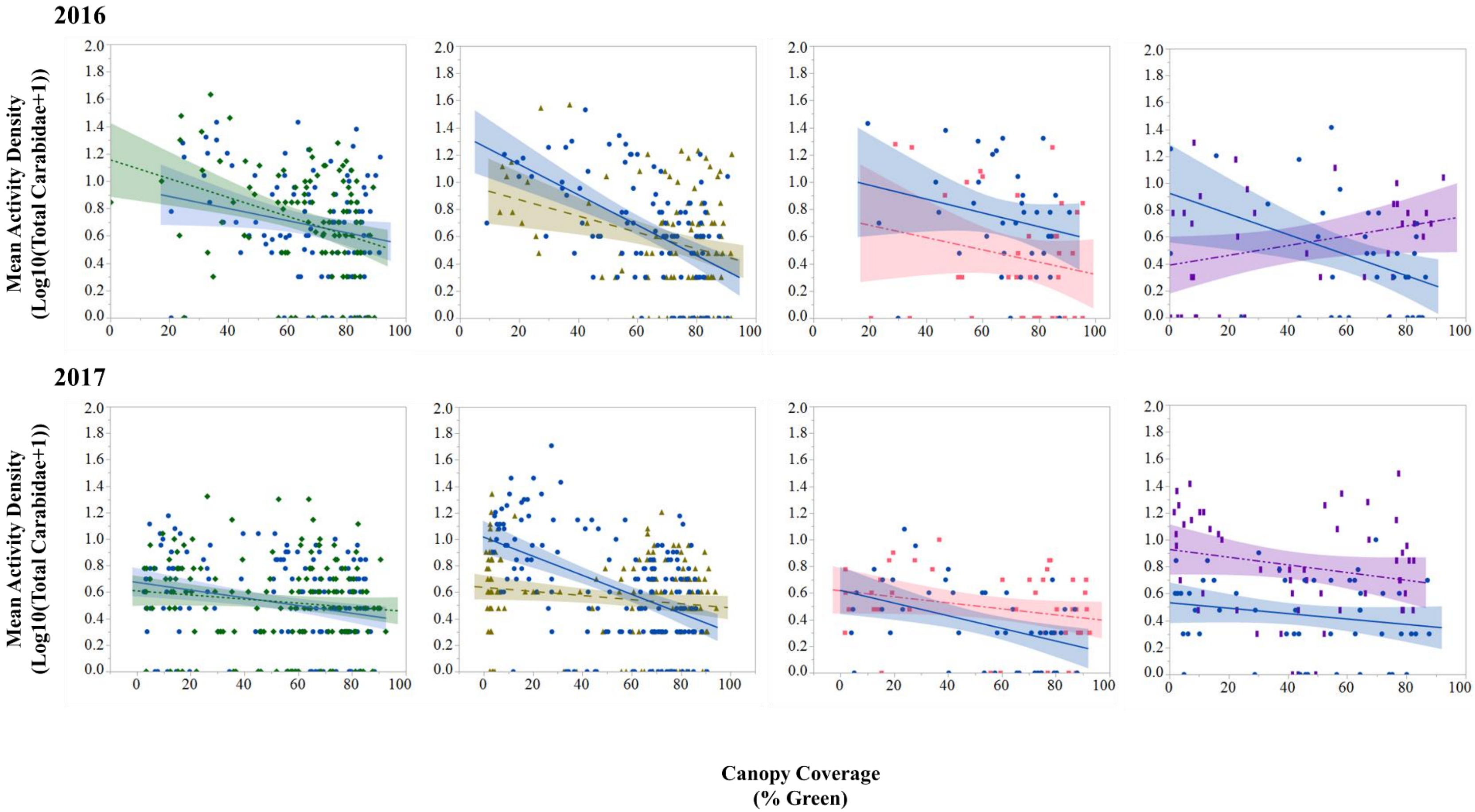

Figure 2.6. Effect of canopy coverage on the mean activity density of carabid beetles collected weekly in paired wheat treatments and paired wheat and canola treatments. Carabid activity density data were log-transformed to help homogenize the variances. Solid lines represent lines of best fit, while dark shading represents 95% confidence bands. Blue, solid line with circular marker = susceptible wheat; green, dotted line with diamond marker = tolerant wheat; yellow, dashed line with triangle marker = canola; pink, dash-dotted line with square marker = peas; purple, dash-double dotted line with rectangular marker = lambsquarters

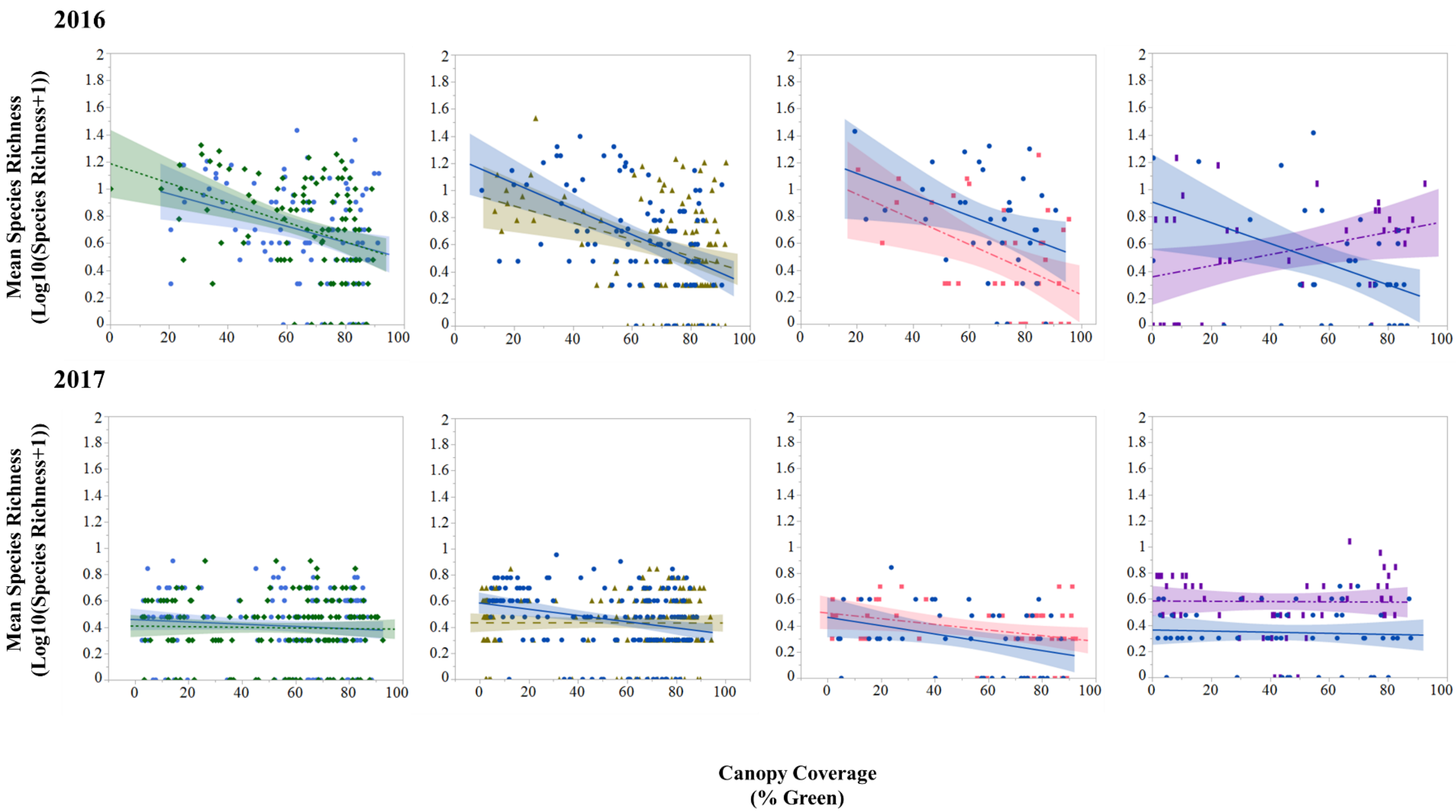

Figure 2.7. Effect of canopy coverage on the mean species richness of carabid beetles collected weekly in paired wheat treatments and paired wheat and canola treatments. Carabid activity density data were log-transformed to help homogenize the variances. Solid lines represent linear lines of best fit with dark shading identifying 95% confidence bands. Blue, solid line with circular marker = susceptible wheat; green, dotted line with diamond marker = tolerant wheat; yellow, dashed line with triangle marker = canola; pink, dash-dotted line with square marker = peas; purple, dash-double dotted line with rectangular marker = lambsquarters

Canopy Coverage

There was no significant effect of canopy coverage on carabid activity density of in paired wheat treatments in 2016 ($F_{1,\ 170.7}$ = 2.8441, p = 0.0935; Fig. 2.6). However, there was a significant interaction between canopy coverage and variety of wheat (susceptible vs. tolerant) on activity density ($F_{1,\ 171.7}$ = 4.5015, p = 0.0457) (Fig. 2.6). As canopy coverage increased, activity density decreased more strongly in tolerant wheat than in susceptible wheat. There was no significant effect of canopy coverage on species richness of carabid beetles collected from paired wheat treatments in 2016 ($F_{1,\ 168}$ = 1.6998, p = 0.1941) (Fig. 2.7). All other interaction terms were non-significant ($p > 0.05$).

In 2017, there was no significant effect of canopy coverage on the activity density of carabid beetles collected in paired wheat treatments ($F_{1,\ 252.7}$ = 1.2237, p = 0.2697) (Fig. 2.6). There was no significant effect of canopy coverage on species richness of carabid beetles collected in paired wheat treatments ($F_{1,\ 253.9}$ = 2.8191, p = 0.0944) (Fig. 2.7). All interaction terms were non-significant ($p > 0.05$).

2.4.1.2. Canopy structure – Paired Wheat and Canola (Treatments 7-12)

Plant Density & Crop Type

In 2016, there was no significant effect of plant density on the activity density ($F_{1,\ 17.3}$ = 0.0149, p = 0.9403) or species richness ($F_{1,\ 16.96}$ = <0.001, p = 0.9984) of carabids in paired canola and wheat (Fig. 2.4 and 2.5). There was also no effect of crop type on activity density ($F_{1, 17.17}$ = 0.5467, p = 0.4697) and species richness ($F_{1, 17.11}$ = 0.3687, p = 0.5517) (Figures 4 and 5). All interaction terms were non-significant ($p > 0.05$).

There was no significant effect of plant density ($F_{1, 29.65}$ = 0.001, p = 0.9961) or crop type ($F_{1, 30.91}$ = 0.3656, p = 0.5498) on the activity density of carabid beetles collected in 2017 from paired canola and wheat treatments (Fig. 2.4). There was also no significant effect of plant density ($F_{1, 27.43}$ = 0.0124, p = 0.9121) or crop type ($F_{1, 28.39}$ = 0.0381, p = 0.8465) on the species richness of carabid beetles collected in paired wheat and canola treatments in 2017 (Fig. 2.5). All interaction terms were non-significant ($p > 0.05$).

Canopy Coverage

In 2016, there was no effect of canopy coverage on the activity density of carabid beetles collected in paired wheat and canola ($F_{1, 172.6}$ = 0.9252, p = 0.3375) (Fig. 2.6). There was no effect of canopy coverage on the species richness of carabid beetles collected in paired wheat and canola treatments in 2016 ($F_{1, 173.3}$ = 0.5960, p = 0.4412) (Fig. 2.7). There was a significant interaction between collection week, crop type, and canopy coverage on species richness ($F_{8, 170.1}$ = 2.8586, p = 0.0052). All interaction terms were non-significant ($p > 0.05$).

There was no effect of canopy coverage on the activity density of carabid beetles collected in paired wheat and canola treatments in 2017 ($F_{1, 236.2}$ = 0.2472, p = 0.6195) (Fig. 2.6). There was also no effect of canopy coverage on species richness in paired wheat and canola treatments ($F_{1, 246.1} = 0.0612$, $p = 0.8048$) (Fig. 2.7). All interaction terms were non-significant ($p > 0.05$).

2.4.1.3. Canopy structure – Paired Wheat and Peas (Treatments 13-14)

In 2016, there was no significant effect of canopy coverage ($F_{1, 32.34} = 0.8519$, $p = 0.3629$) or crop type ($F_{1, 29.82} = 0.1382$, $p = 0.7127$) on carabid activity density (Fig. 2.6). There was also no significant effect of canopy coverage ($F_{1, 19.45} = 0.2.5275$, $p = 0.1280$) or crop type ($F_{1, 23.02} = 0.4941$, $p = 0.4892$) on species richness in paired wheat and pea treatments (Fig. 2.7). All interaction terms were non-significant ($p > 0.05$).

In 2017, there was no significant effect of canopy coverage ($F_{1, 40.75} = 0.0610$, $p = 0.8062$) and crop type ($F_{1, 39.64} = 0.1056$, $p = 0.7469$) on the activity density of carabid beetles collected in paired wheat and pea treatments (Fig. 2.6). There was a significant interaction between collection week and canopy coverage on the activity density of carabid beetles collected in these treatments ($F_{10, 38.94} = 2.1299$, $p = 0.0452$). There was no significant effect of canopy coverage ($F_{1, 40.17} = 0.0135$, $p = 0.9080$) or crop type ($F_{1, 38.55} = 0.1915$, $p = 0.6641$) on species richness in paired wheat and pea treatments (Fig. 2.7). All other interaction terms were non-significant ($p > 0.05$).

2.4.1.4. Canopy structure – Paired Wheat and Lambsquarters (Treatments 15-16)

Canopy Coverage & Plant Species

In 2016, there was no significant effect of canopy coverage ($F_{1,\,31.85} = 0.4477$, $p = 0.5082$) or plant species ($F_{1,\,32.79} = 0.7347$, $p = 0.3976$) on the activity density of carabid beetles collected in paired wheat and lambsquarters treatments (Fig. 2.6). There was no significant effect of canopy coverage ($F_{1,\,32.26} = 2.7506$, $p = 0.1069$) or plant species ($F_{1,\,32.95} = 2.0004$, $p = 0.1666$) on species richness (Fig. 2.7). All interaction terms were non-significant ($p > 0.05$).

In 2017, there was no significant effect of canopy coverage on the activity density of carabid beetles collected in paired wheat and lambsquarters treatments ($F_{1,\,13.71} = 0.6577$, $p = 0.4362$) (Fig. 2.6). There was also no effect of crop type on activity density in these treatments ($F_{1,\,44.5} = 0.1636$, $p = 0.6882$) (Fig. 2.6). There were no significant effects of canopy coverage ($F_{1,\,13.4} = 0.0553$, $p = 0.8177$) or crop type ($F_{1,\,44.81} = 0.2371$, $p = 0.6287$) on species richness of carabid beetles collected in paired wheat and lambsquarters treatments in 2017 (Fig. 2.7). All other interaction terms were non-significant ($p > 0.05$).

2.4.2. Carabid Community Structure

A total of 8,025 Coleoptera were collected in 2016. Of these, 56.2% were Carabidae (n = 4,511) and 19.6% were Staphylinidae (n = 1,569). Of the 4,511 carabid beetles collected, 45 species were identified in the plot trial (Table 2.2). The dominant three species were *Poecilus lucublandus* Say (n = 2,424; Fig. 2.8), *Amara thoracica*

Hayward (n = 368), and *Carabus taedatus* (n = 358) (Fabricius) (Coleoptera: Carabidae) (Table 2.2). *Poecilus lucublandus* populations peaked in early June (June 6, 2016) (Fig. 2.10). Both *A. thoracica* and *C. taedatus* populations peaked later on in the season in early August (August 1, 2016) and late July (July 25, 2018), respectively (Fig. 2.10).

Figure 2.8. Dorsal view of *Poecilus lucublandus* collected from Beaverlodge, AB.

Table 2.2. Carabid species collected from the plot trial in Beaverlodge, AB in 2016.

Species	Number Collected	Proportion of Total Catch	Proportion Female
Poecilus lucublandus Say	2424	0.54	0.54
Amara thoracica Hayward	368	0.08	0.40
Carabus taedatus Fabricius	358	0.08	0.68
Amara obesa Say	302	0.07	0.49
Harpalus carbonatus LeConte	165	0.04	0.46
Agonum cupreum Dejean	121	0.03	0.67
Harpalus amputatus Say	117	0.03	0.65
Amara sinuosa Casey	104	0.02	0.51
Pterostichus adstrictus Eschscholtz	100	0.02	0.44
Amara torrida Panzer	61	0.01	0.66
Pterostichus melanarius Illiger	51	0.01	0.31
Harpalus cordifer Notman	51	0.01	0.61
Cymindis cribricollis Dejean	43	0.01	0.47
Metabletus americanus Dejean	30	0.01	0.80
Amara littoralis Dejean	30	0.01	0.36
Amara sp. 1	28	0.01	0.44
Cicindela longilabris Say	26	0.01	0.88
Bembidion rupicola Kirby	22	<0.01	0.45
Calathus advena LeConte	19	<0.01	0.58
Calathus ingratus Dejean	13	<0.01	0.54
Bembidion mutatum Gemminger & Harold	12	<0.01	0.58
Bembidion versicolor LeConte	11	<0.01	0.18
Amara bifrons Gyllenhal	10	<0.01	0.30
Chlaenius purpuricollis Randall	6	<0.01	0.83
Amara quenseli Schonherr	5	<0.01	0.60
Harpalus opacipennis Haldemann	5	<0.01	0.40
Harpalus hoppingi Lindroth	5	<0.01	0.40
Cymindis pilosa Say	5	<0.01	0.60
Notiophilus semistriatus Say	3	<0.01	1.00
Amara cupreolata Putzeys	2	<0.01	1.00
Notiophilus borealis Harris	1	<0.01	0.00
Amara aenea De Geer	1	<0.01	1.00
Harpalus seclusus Casey	1	<0.01	1.00
Harpalus funerarius Csiki	1	<0.01	1.00
Agonum affine Kirby	1	<0.01	0.00
Bembidion timidum LeConte	1	<0.01	1.00
Agonum placidum Say	1	<0.01	1.00
Clivina oregona Fall	1	<0.01	0.00
Calathus ruficollis Dejean	1	<0.01	1.00
Diplocheila obtusa LeConte	1	<0.01	0.00
Platynus decentis Say	1	<0.01	1.00
Harpalus pleuriticus Kirby	1	<0.01	1.00
Harpalus herbivagus Say	1	<0.01	0.00
Harpalus erraticus Say	1	<0.01	1.00

Table 2.3. Carabid species collected from the plot trial in Beaverlodge, AB in 2017.

Species	Number Collected	Proportion of Total Catch	Proportion Female
Poecilus lucublandus Say	880	0.27	0.38
Amara obesa Say	443	0.14	0.47
Amara littoralis Dejean	424	0.13	0.62
Amara thoracica Hayward	260	0.08	0.55
Carabus taedatus Fabricius	259	0.08	0.46
Pterostichus adstrictus Eschscholtz	218	0.07	0.49
Harpalus carbonatus LeConte	206	0.06	0.31
Harpalus amputatus Say	126	0.04	0.37
Pterostichus melanarius Illiger	92	0.03	0.32
Amara apricaria Paykull	56	0.02	0.64
Amara anthobia Villa & Villa	45	0.01	0.59
Agonum placidum Say	38	0.01	0.39
Agonum cupreum Dejean	37	0.01	0.70
Bembidion mutatum Gemminger & Harold	28	0.01	0.68
Cymindis cribricollis Dejean	26	0.01	0.31
Bembidion versicolor LeConte	24	<0.01	0.42
Amara sinuosa Casey	15	<0.01	0.40
Notiophilus borealis Harris	14	<0.01	0.43
Calathus ruficollis Dejean	13	<0.01	0.54
Notiophilus semistriatus Say	13	<0.01	0.69
Harpalus somnulentus Dejean	9	<0.01	0.33
Calathus advena LeConte	8	<0.01	0.63
Harpalus opacipennis Haldemann	8	<0.01	0.25
Bembidion rupicola Kirby	5	<0.01	0.80
Metabletus americanus Dejean	5	<0.01	0.40
Cymindis pilosa Say	5	<0.01	0.40
Amara quenseli Schonherr	3	<0.01	0.67
Pterostichus pensylvanicus LeConte	3	<0.01	0.67
Dyschirius globulosus Herbst	3	<0.01	1.00
Chlaenius purpuricollis Randall	3	<0.01	0.67
Pterostichus femoralis Kirby	2	<0.01	1.00
Clivina oregona Fall	2	<0.01	1.00
Agonum retractum LeConte	2	<0.01	1.00
Synuchus impunctatus Say	1	<0.01	0.00
Bembidion timidum LeConte	1	<0.01	0.00
Patrobus lecontei Chaudoir	1	<0.01	0.00
Patrobus longicornis Say	1	<0.01	0.00
Bembidion quadrimaculatum Linneaus	1	<0.01	0.00
Agonum errans Say	1	<0.01	1.00
Trichocellus cognatus Gyllenhal	1	<0.01	0.00
Harpalus herbivagus Say	1	<0.01	1.00
Calosoma calidum Fabricius	1	<0.01	1.00

In 2017, a total of 11,189 Coleoptera were collected. Of these, 29.4% collected were Carabidae (n = 3,284) and 25.1% were Staphylinidae (n = 2,813). Of the 3,284 carabid beetles collected from the plot trial in 2017, 42 species were identified (Table 2.3). Of these, *P. lucublandus* (n = 880), *Amara obesa* Say (n = 443), and *Amara littoralis* Dejean (n = 424) were the three dominant species collected that year (Table 2.3). Populations of *P. lucublandus* peaked in the middle of June (June 19, 2018; Fig. 2.10). *Amara obesa* populations peaked in the middle of the season near the end of July (July 24, 2018; Fig. 2.10). Populations of *A. littoralis* peaked near the beginning of the season in early June (June 12, 2018; Fig. 2.10).

The abundance of the three dominant species was compared between treatments for each of the two years. In 2016, there was no difference in abundance of *P. lucublandus* between treatments ($F_{15, 45}$ = 0.9377, p = 0.5317; Fig. 2.9). Abundance of *A. thoracica* was significantly higher in susceptible wheat at 400 seeds per m^2 paired with canola than in tolerant wheat at 200 seeds per m^2 paired with susceptible wheat), susceptible wheat at 400 seeds per m^2 paired with tolerant wheat, susceptible wheat at 300 seeds per m^2 paired with canola, and 14 peas at 100 seeds per m^2 paired with wheat ($F_{15, 45}$ = 2.4017, p = 0.0119; Fig. 2.9). There was no difference in abundance of *C. taedatus* between treatments ($F_{15, 45}$ = 1.4330, p = 0.1736; Fig. 2.9).

In 2017, there was no difference in the abundance of *P. lucublandus* between treatments ($F_{15,45}$ = 1.0103, p = 0.4622; Fig. 2.9). There was a significant difference in the abundance of *Amara obesa* but no treatments were discernably more or less abundant when a post-hoc Tukey test was performed ($F_{15,45}$ = 1.8958, p = 0.0499; Fig. 2.9). There was no significant difference in the abundance of *Amara littoralis* between treatments ($F_{15,45}$ = 1.8597, p = 0.0551; Fig. 2.9).

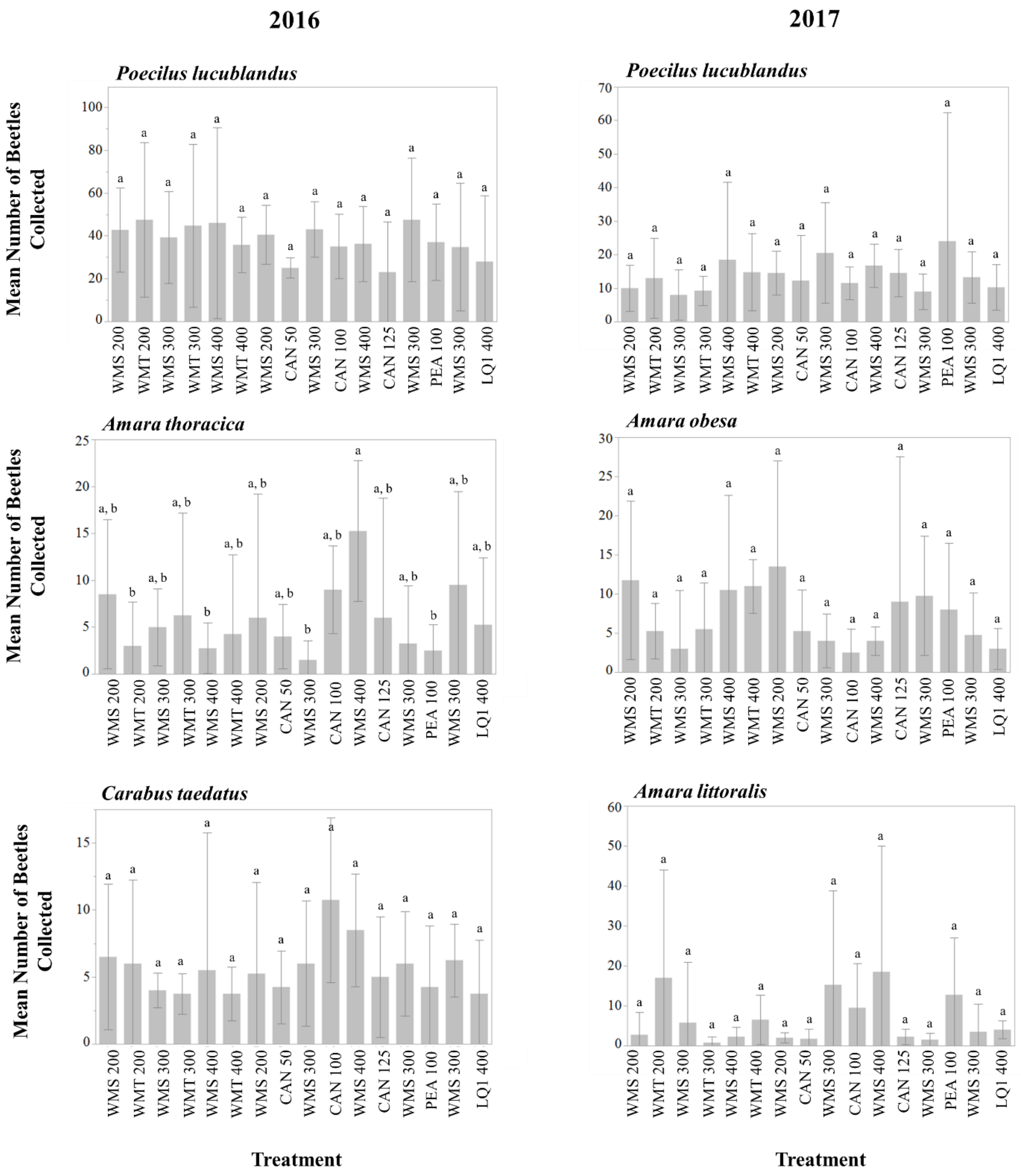

Figure 2.9. Effect of treatment on mean abundance (± 95% CI) of dominant species collected in 2016 and 2017. Letters above the bars indicate significant differences among groups (post-hoc Tukey test, α = 0.05).

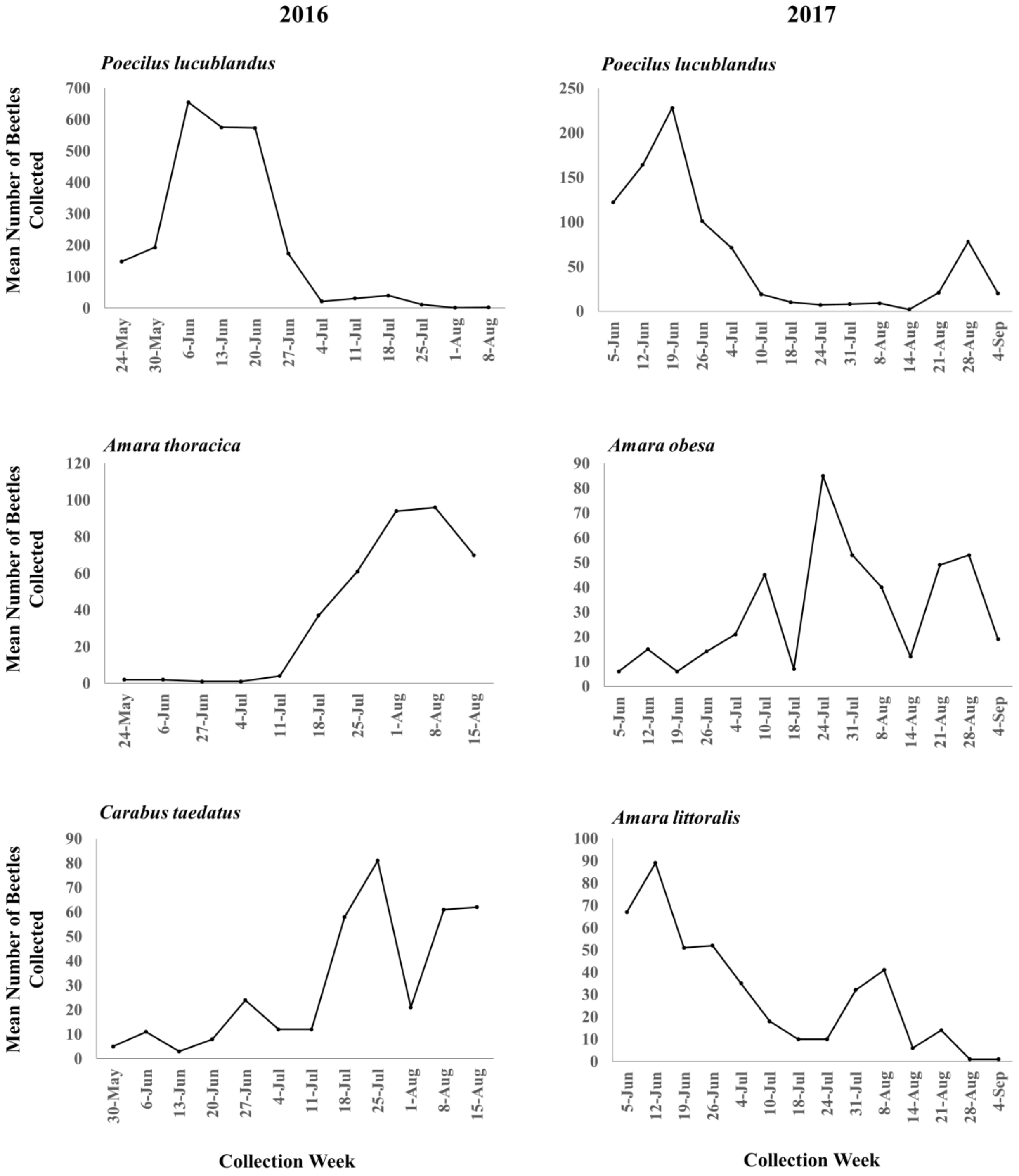

Figure 2.10. Seasonal activity of the three dominant carabid species collected in Beaverlodge, AB in 2016 and 2017. Mean activity density for each species was recorded weekly throughout each season.

2.4.3 Wheat Midge Populations

Only seven wheat midge adults were collected on sticky cards during the study (n = 3 in 2016, n = 4 in 2017; Fig. 2.11). Based on this, it is difficult to draw any conclusions on the seasonal activity of adult wheat midge in the plot trial.

In 2016, there was no significant effect of plant density on larval density (number of larvae per 100 heads) ($F_{1, 17}$ = 1.7120, p = 0.2081; Fig. 2.11). There was a significant effect of variety on wheat midge larval density ($F_{1, 17}$ = 45.5273, p = <0.0001) with susceptible varieties showing far higher densities. There was a significant effect of average tiller density on larval density ($F_{1, 7}$ = 9.1878, p = 0.0075; Fig. 2.11). This indicates that plants with lower numbers of tillers had lower larval densities. No significant effect of carabid abundance on larval density was found in paired wheat treatments ($F_{1, 17}$ = 0.6307, p = 0.4381). All interaction terms were non-significant ($p > 0.05$). No susceptibility ratings occurred in 2016. Growth ratings indicated that wheat was susceptible to wheat midge from approximately July 5-15, 2016.

There was no significant effect of plant density on larval density in 2017 ($F_{1, 17}$ = 1.0455, p = 0.3209). There was a significant effect of variety on wheat midge larval density ($F_{1, 17}$ = 39.9365, p = <0.0001; Fig. 2.11). Overall, there were significantly lower larval densities in 2017, with no larvae found in the tolerant wheat variety. There was no significant effect of average tiller density on larval density ($F_{1, 17}$ = 0.5793, p = 0.4570; Fig. 2.11). There was no significant effect of carabid activity density on larval density in paired wheat treatments ($F_{1, 17}$ = 0.0469, p = 0.8312). All interaction terms were non-significant ($p > 0.05$).

In 2017, There was no significant effect of average tiller density ($F_{1, 17} = 0.3777$, $p = 0.5470$) or plant density ($F_{1, 17} = 0.5045$, $p = 0.4872$) on the duration of susceptibility in susceptible wheat treatments. In turn, there was no significant effect of the period of susceptibility on the wheat midge larval density ($F_{1, 17} = 0.0668$, $p = 0.7992$). In 2017, wheat plants were susceptible between July 10 and July 31. All interaction terms were non-significant ($p > 0.05$).

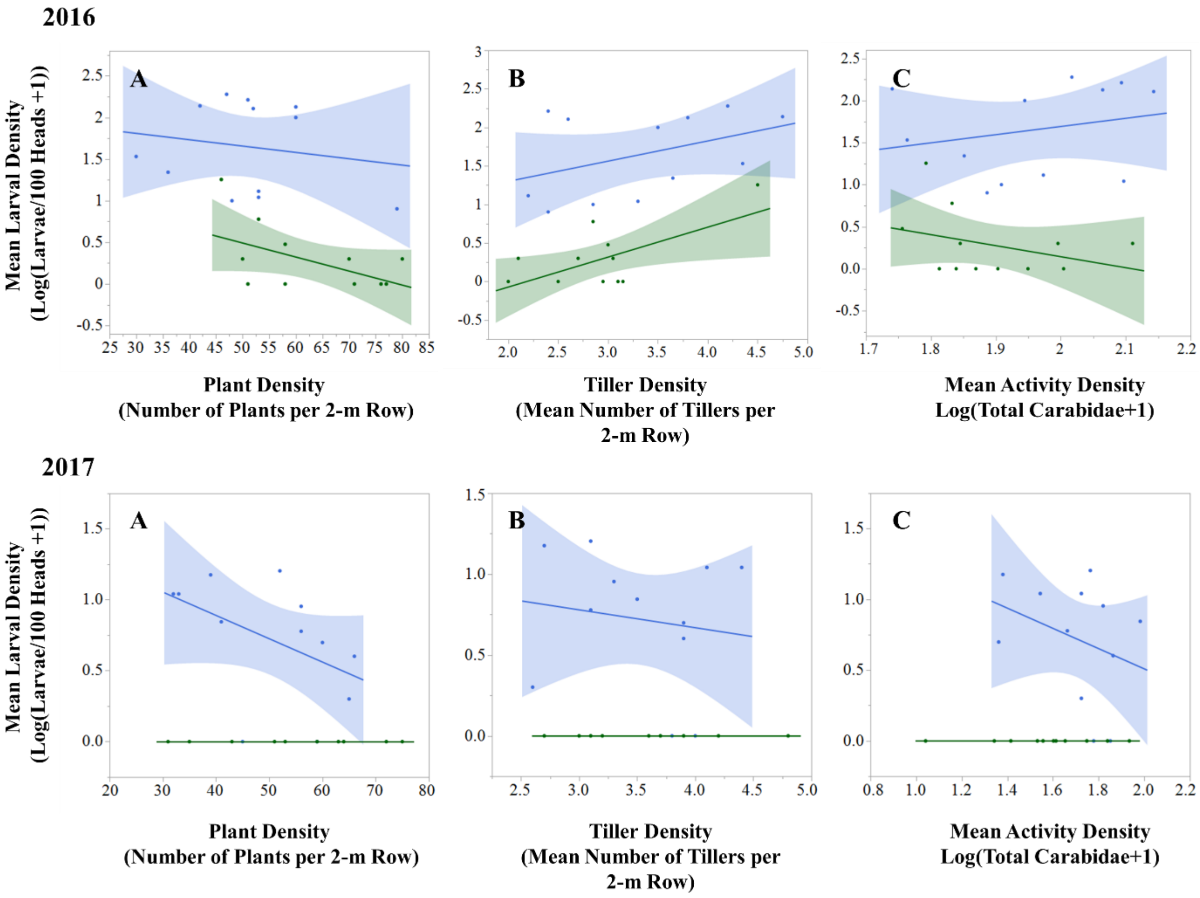

Figure 2.11. Effects of canopy structure and carabid assemblages on mean wheat midge larval populations in paired wheat treatments in 2016 and 2017. Carabid activity density and wheat midge larval density data were log-transformed to help homogenize the variances. Solid lines represent lines of best fit, while dark shading represents 95% confidence bands. Blue = midge susceptible wheat and green = midge tolerant wheat. (A) Effect of plant density on mean wheat midge larval density. (B) Effect of tiller density on mean wheat midge larval density (C) Effect of mean carabid activity density on wheat midge larval density.

2.4.4. Harvest Parameters

2.4.4.1. Harvest Parameters - Paired Wheat (Treatments 1-6)

All results in this section are summarized in Table 2.4. In paired wheat treatments, there were no significant effects of wheat midge larval populations on plant biomass in either year of the study (2016: $F_{1,13} = 0.0226$, $p = 0.8828$; 2017: $F_{1,5} = 6.0739$, $p = 0.0569$). There was no significant effect of mean carabid abundance on biomass in either year (2016: $F_{1,13} = 0.7049$, $p = 0.4135$; 2017: $F_{1,13} = 0.5111$, $p = 0.4873$). For both analyses, plant density did not have an effect on biomass in either year of the study. (Larval Population Analysis: 2016: $F_{1,13} = 0.0283$, $p = 0.8690$; 2017: $F_{1,5} = 3.7218$, $p = 0.1116$; Carabid Population Analysis: 2016: $F_{1,13} = 0.9397$, $p = 0.3468$; 2017: $F_{1,13} = 0.7987$, $p = 0.3877$). There was similarly no effect of wheat variety on biomass in either year of the study for both analyses (Larval Population Analysis: 2016: $F_{1,13} = 0.0303$, $p = 0.8646$; 2017: not compared [no larvae obtained from tolerant wheat]; Carabid Population Analysis: 2016: $F_{1,13} = 0.9036$, $p = 0.3506$; 2017: $F_{1,13} = 0.1210$, $p = 0.7335$). All interaction terms were non-significant ($p > 0.05$).

Yield was similarly unaffected both years by wheat midge larval populations (2016: $F_{1,13} = 0.4671$, $p = 0.5063$; 2017: $F_{1,5} = 1.4979$, $p = 0.2755$). There was no significant effect of carabid abundance on yield in either year of the study (2016: $F_{1,13} = 0.3110$, $p = 0.5866$; 2017: $F_{1,13} = 2.5422$, $p = 0.1349$). For both analyses, plant density did not have an effect on yield in either year of the study (Larval Population Analysis: 2016: $F_{1,13} = 1.3349$, $p = 0.2687$; 2017: $F_{1,5} = 1.7392$, $p = 0.2444$; Carabid Population Analysis: 2016: $F_{1,13} = 0.7363$, $p = 0.4064$; 2017: $F_{1,13} = 0.2286$, $p = 0.6405$). There was similarly no effect of wheat variety on yield in either year of the study for both analyses

(Larval Population Analysis: 2016: $F_{1, 13} = 1.1323$, $p = 0.3066$; 2017: not compared [no larvae obtained from tolerant wheat]; Carabid Population Analysis: 2016: $F_{1, 13} = 0.3444$, $p = 0.5673$; $F_{1, 13} = 0.1823$, $p = 0.6764$). All interaction terms were non-significant ($p > 0.05$).

Percent protein was significantly greater when larval populations were higher in 2016 ($F_{1, 13} = 5.5638$, $p = 0.0347$) (Fig. 2.12). There was no effect of larval populations on percent protein in 2017 ($F_{1, 5} = 0.1409$, $p = 0.7228$) in susceptible wheat. Plant density did not have an effect on percent protein in either year of the study (2016: $F_{1, 13} = 0.9529$, $p = 0.3535$; 2017: $F_{1, 5} = 1.3767$, $p = 0.2935$). There was similarly no effect of wheat variety on percent protein in either year of the study (2016: $F_{1, 13} = 2.3199$, $p = 0.1517$; 2017: not compared [no larvae obtained from tolerant wheat]). All interaction terms were non-significant ($p > 0.05$).

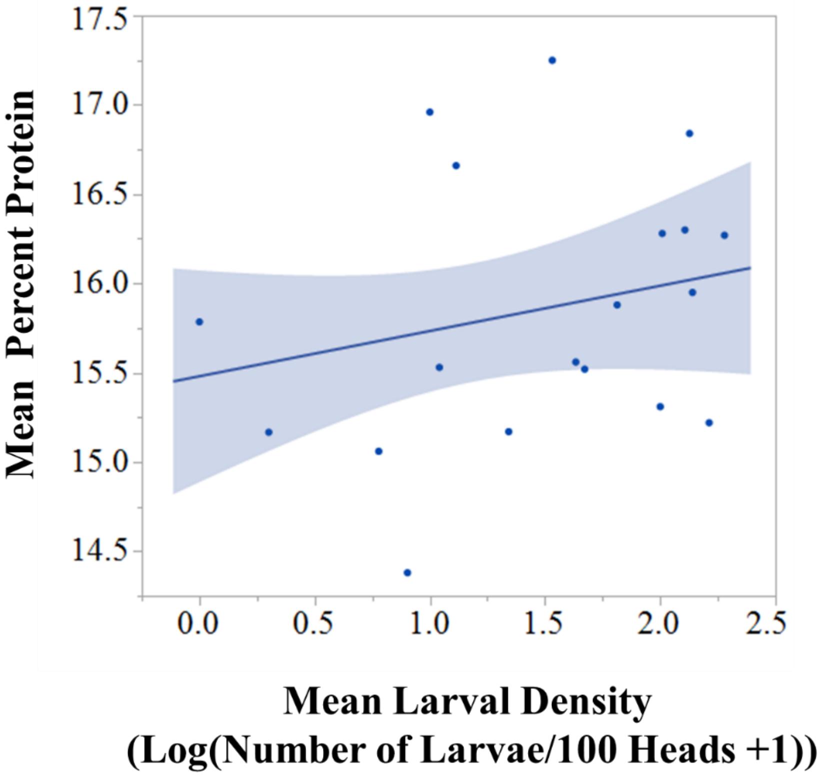

Figure 2.12. Effect of mean larval density of wheat midge collected from paired wheat treatments (treatments 1-6) on mean percent protein content in wheat in 2016. Wheat midge larval density data were log-transformed to help homogenize the variances. Solid lines represent lines of best fit, while dark shading represents 95% confidence bands.

Table 2.4. Effects of variety, plant density, larval density, and carabid abundance on biomass, yield, and protein in paired wheat treatments in 2016 and 2017. All interaction terms were non-significant ($p > 0.05$).

			2016		
Treatment	**Crop**	**Seeding Rate (seeds/m²)**	**Biomass (kg/plot)**	**Yield (kg/plot)**	**Protein (%)**
1	Susceptible Wheat	200	0.92 ± 0.09	19.45 ± 0.83	16.31 ± 0.71
2	Tolerant Wheat	200	0.87 ± 0.18	19.40 ± 0.76	15.81 ± 0.31
3	Susceptible Wheat	300	0.84 ± 0.04	18.85 ± 1.17	16.01 ± 0.75
4	Tolerant Wheat	300	0.84 ± 0.13	18.21 ± 1.54	15.43 ± 0.77
5	Susceptible Wheat	400	0.87 ± 0.10	17.61 ± 2.29	15.64 ± 0.90
6	Tolerant Wheat	400	0.96 ± 0.05	18.04 ± 1.82	15.49 ± 0.70
	Statistics				
	Variety		$F_{1,13} = 0.0303, p = 0.8646$	$F_{1,13} = 1.1323, p = 0.3066$	$F_{1,13} = 2.3199, p = 0.1517$
	Plant Density		$F_{1,13} = 0.0283, p = 0.8690$	$F_{1,13} = 1.3349, p = 0.2687$	$F_{1,13} = 0.9529, p = 0.3535$
	Larval Density		$F_{1,13} = 0.0226, p = 0.8828$	$F_{1,13} = 0.4671, p = 0.5063$	**$F_{1,13} = 5.5638, p = 0.0347$***
	Variety		$F_{1,13} = 0.9036, p = 0.3506$	$F_{1,13} = 0.3444, p = 0.5673$	.
	Plant Density		$F_{1,13} = 0.9397, p = 0.3468$	$F_{1,13} = 0.7363, p = 0.4064$	.
	Carabid Abundance		$F_{1,13} = 0.7049, p = 0.4135$	$F_{1,13} = 0.3110, p = 0.5866$	.

			2017		
Treatment	**Crop**	**Seeding Rate (seeds/m²)**	**Biomass (kg/plot)**	**Yield (kg/plot)**	**Protein (%)**
1	Susceptible Wheat	200	0.90 ± 0.08	16.73 ± 1.61	15.63 ± 0.56
2	Tolerant Wheat	200	0.87 ± 0.09	16.80 ± 1.01	16.29 ± 0.20
3	Susceptible Wheat	300	0.74 ± 0.09	15.99 ± 2.54	15.72 ± 0.43
4	Tolerant Wheat	300	0.83 ± 0.09	16.93 ± 1.48	16.16 ± 0.49
5	Susceptible Wheat	400	0.92 ± 0.10	17.24 ± 1.87	15.62 ± 0.22
6	Tolerant Wheat	400	0.88 ± 0.05	16.19 ± 0.91	16.29 ± 0.12
	Statistics				
	Variety		.	.	.
	Plant Density		$F_{1,5} = 3.7218, p = 0.1116$	$F_{1,5} = 1.7392, p = 0.2444$	$F_{1,5} = 1.3767, p = 0.2935$
	Larval Density		$F_{1,5} = 6.0739, p = 0.0569$	$F_{1,5} = 1.4979, p = 0.2755$	$F_{1,5} = 0.1409, p = 0.7228$
	Variety		$F_{1,13} = 0.1210, p = 0.7335$	$F_{1,13} = 0.1823, p = 0.6764$	.
	Plant Density		$F_{1,13} = 0.7987, p = 0.3877$	$F_{1,13} = 0.2286, p = 0.6405$	.
	Carabid Abundance		$F_{1,13} = 0.5111, p = 0.4873$	$F_{1,13} = 2.5422, p = 0.1349$	.

2.4.4.2. Harvest Parameters - Paired Wheat and Canola (Treatments 7-12)

All results for this section are summarized in Table 2.5. In 2016, there were no significant effects of crop type ($F_{1, 13}$ = 0.8797, p = 0.3654), plant density ($F_{1, 13}$ = 1.3140, p = 0.2723), or overall carabid activity density ($F_{1, 13}$ = 0.8755, p = 0.3655) on biomass in paired wheat and canola treatments. This differed in 2017, when all three factors significantly affected biomass: crop ($F_{1, 13}$ = 7.4045, p = 0.0175), plant density ($F_{1, 13}$ = 7.9279, p = 0.0146), and carabid activity density ($F_{1, 13}$ = 10.5449, p = 0.0064) (Figure 2.12). There were significant interactions between crop type and plant density ($F_{1, 13}$ = 13.4032, p = 0.0029) and between crop type and mean carabid activity density ($F_{1, 13}$ = 14.0489, p = 0.0024) on biomass (Fig. 2.13). For both wheat and canola treatments, as the number plants increased, biomass decreased. In canola treatments, there was a negative relationship between mean carabid activity and mean biomass. In contrast, there was a positive relationship between mean carabid activity density and biomass in wheat treatments. There was also a significant interaction between crop type, plant density, and mean carabid activity density on biomass ($F_{1, 13}$ = 10.8256, p = 0.0059).

Table 2.5. Effects of crop type, plant density, and carabid abundance on biomass and yield in paired wheat and canola treatments in 2016 and 2017. All interaction terms were non-significant ($p > 0.05$).

			2016		
Treatment	**Crop**	**Seeding Rate (seeds/m²)**	**Biomass (kg/plot)**	**Yield (kg/plot)**	**Protein (%)**
7	Susceptible Wheat	200	0.79 ± 0.08	19.95 ± 0.60	15.68 ± 0.81
8	Canola	50	0.90 ± 0.14	18.97 ± 1.85	22.49 ± 1.48
9	Susceptible Wheat	300	0.87 ± 0.05	19.57 ± 0.33	16.32 ± 1.23
10	Canola	100	0.89 ± 0.04	18.46 ± 0.96	22.47 ± 0.74
11	Susceptible Wheat	400	1.01 ± 0.09	19.17 ± 0.69	14.93 ± 0.79
12	Canola	125	0.80 ± 0.06	17.53 ± 1.53	21.39 ± 2.23
	Statistics				
	Crop		$F_{1,13} = 0.8797, p = 0.3654$	$F_{1,13} = 4.0900, p = 0.1995$	.
	Plant Density		$F_{1,13} = 1.3140, p = 0.2723$	$F_{1,13} = 1.8272, p = 0.1995$	.
	Carabid Abundance		$F_{1,13} = 0.8755, p = 0.3655$	$F_{1,13} = 0.6838, p = 0.4232$	.

			2017		
Treatment	**Crop**	**Seeding Rate (seeds/m²)**	**Biomass (kg/plot)**	**Yield (kg/plot)**	**Protein (%)**
7	Susceptible Wheat	200	0.89 ± 0.13	16.67 ± 1.69	16.01 ± 0.45
8	Canola	50	0.71 ± 0.20	13.14 ± 2.78	23.17 ± 0.47
9	Susceptible Wheat	300	0.82 ± 0.10	17.26 ± 1.43	15.93 ± 0.49
10	Canola	100	0.86 ± 0.21	15.21 ± 2.49	23.41 ± 0.13
11	Susceptible Wheat	400	0.82 ± 0.07	17.92 ± 1.04	15.80 ± 0.36
12	Canola	125	0.68 ± 0.10	16.20 ± 1.23	22.80 ± 0.27
	Statistics				
	Crop		**$F_{1,13} = 7.4045, p = 0.0175$***	**$F_{1,13} = 4.7481, p = 0.0483$***	.
	Plant Density		**$F_{1,13} = 7.9279, p = 0.0146$***	**$F_{1,13} = 2.8500, p = 0.1152$***	.
	Carabid Abundance		**$F_{1,13} = 10.5449, p = 0.0064$***	**$F_{1,13} = 7.3899, p = 0.0176$***	.

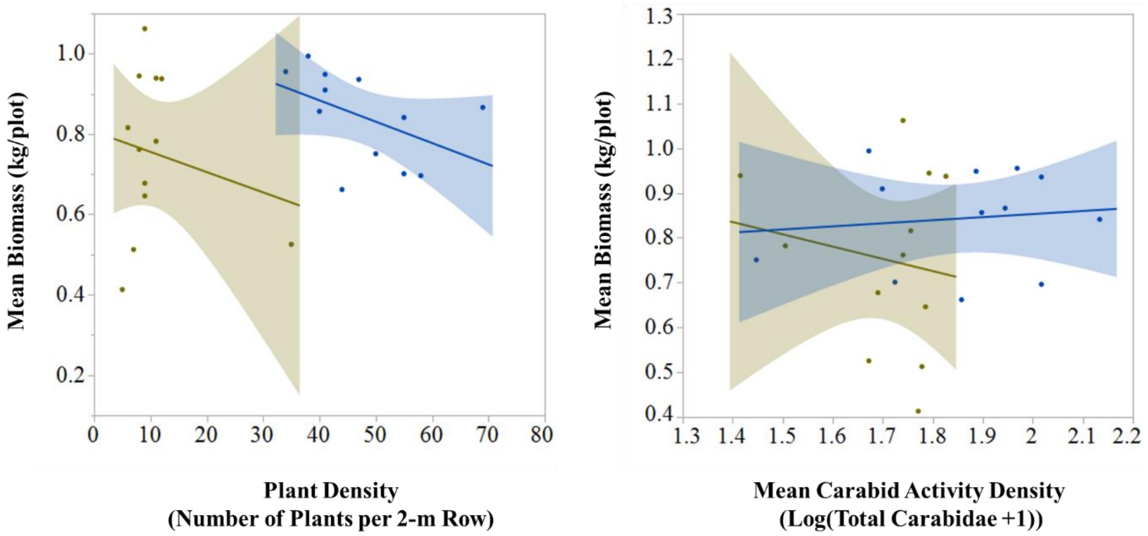

Figure 2.13. Effects of plant density and mean carabid activity density on mean biomass in paired wheat and canola treatments in 2017. Carabid activity density data were log-transformed to help homogenize the variances. Solid lines represent lines of best fit, while dark shading represents 95% confidence bands. Blue = wheat midge susceptible wheat, yellow = canola.

In 2016, there were no significant effects of crop type ($F_{1, 13} = 4.0900$, $p = 0.1995$), plant density ($F_{1, 13} = 1.8272$, $p = 0.1995$), and mean carabid activity density ($F_{1, 13} = 0.6838$, $p = 0.4232$) on yield in paired wheat and canola treatments. However, in 2017, there were significant effects of crop type ($F_{1, 13} = 4.7481$, $p = 0.0483$) and plant density ($F_{1, 13} = 7.3899$, $p = 0.0176$) on yield (Table 2.5; Fig. 2.14). For both wheat and canola treatments, there was a positive relationship plant density and yield. There was no significant effect of mean carabid activity density on yield in paired wheat and canola treatments ($F_{1, 13} = 2.8500$, $p = 0.1152$). All interaction terms were non-significant ($p > 0.05$).

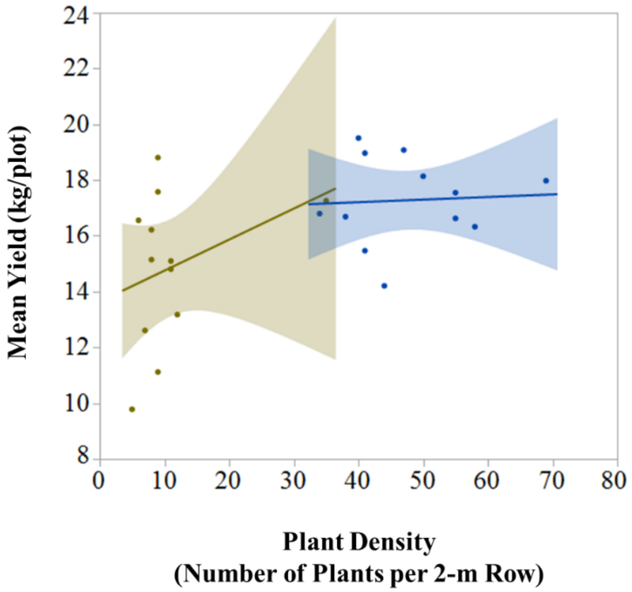

Figure 2.14. Effect of plant density on yield in paired wheat and canola treatments in 2017. Solid lines represent lines of best fit, while dark shading represents 95% confidence bands. Blue = wheat midge susceptible wheat, yellow = canola.

2.4.4.3. Harvest Parameters - Paired Wheat and Pea (Treatments 13-14)

All results for this section are summarized in Table 2.6. In both years of the study, there were no significant effects of crop type (2016: $F_{1,1} = 3.1062$, $p = 0.3286$; 2017: $F_{1,1} = 0.0807$, $p = 0.8238$) or mean carabid activity density (2016: $F_{1,1} = 1.2070$, $p = 0.4701$; 2017: $F_{1,1} = 3.9181$, $p = 0.2978$) on biomass in paired wheat and pea treatments. All interaction terms were non-significant ($p > 0.05$).

There was a significant effect of crop type on yield in paired wheat and pea treatments ($F_{1,1} = 309.7155$, $p = 0.0361$) in 2016 but no effect was seen in 2017 ($F_{1,1} = 3.9181$, $p = 0.2978$). There was no significant effect of mean carabid activity density on yield in either year of the study for paired wheat and pea treatments (2016: $F_{1,1} =$

22.8651, p = 0.1321; 2017: $F_{1,1}$ = 24.1995, p = 0.1277). All interaction terms were non-significant ($p > 0.05$).

2.4.4.4. Harvest Parameters - Paired Wheat and Lambsquarters (Treatments 15-16)

All results for this section are summarized in Table 2.6. In both years of the study, there were no significant effects of crop type (2016: $F_{1,1}$ = 49.9507, p = 0.0895; 2017: $F_{1,1}$ = 4.5231, p = 0.2798) or mean carabid activity density (2016: $F_{1,1}$ = 3.5336, p = 0.3112; 2017: $F_{1,1}$ = 0.3237, p = 0.6707) on biomass in paired wheat and lambsquarters treatments. All interaction terms were non-significant ($p > 0.05$).

Table 2.6. Effects of crop type and carabid abundance on biomass and yield in paired wheat and peas and paired wheat and lambsquarters treatments in 2016 and 2017. All interaction terms were non-significant (p > 0.05).

			2016		
Treatment	**Crop**	**Seeding Rate (seeds/m²)**	**Biomass (kg/plot)**	**Yield (kg/plot)**	**Protein (%)**
13	Susceptible Wheat	300	0.87 ± 0.05	20.21 ± 0.74	15.89 ± 0.69
14	Peas	100	0.82 ± 0.02	28.61 ± 1.89	.
	Statistics				
	Crop		$F_{1,1}$ = 3.1062, p = 0.3286	**$F_{1,1}$ = 309.7155, p = 0.0361***	-
	Carabid Abundance		$F_{1,1}$ = 1.2070, p = 0.4701	$F_{1,1}$ = 22.8651, p = 0.1321	-
15	Susceptible Wheat	300	0.84 ± 0.11	18.24 ± 1.90	15.15 ± 0.77
16	Lambsquarters	400	0.45 ± 0.13	.	.
	Statistics				
	Crop		$F_{1,1}$ = 49.9507, p = 0.0895	.	.
	Carabid Abundance		$F_{1,1}$ = 3.5336, p = 0.3112	.	.

			2017		
Treatment	**Crop**	**Seeding Rate (seeds/m²)**	**Biomass (kg/plot)**	**Yield (kg/plot)**	**Protein (%)**
13	Susceptible Wheat	300	0.82 ± 0.05	18.09 ± 1.75	15.24 ± 2.01
14	Peas	100	0.74 ± 0.17	19.59 ± 1.96	.
	Statistics				
	Crop		$F_{1,1}$ = 0.0807, p = 0.8238	$F_{1,1}$ = 13.1377, p = 0.1714	.
	Carabid Abundance		$F_{1,1}$ = 3.9181 p = 0.2978	$F_{1,1}$ = 24.1995 p = 0.1277	.
15	Susceptible Wheat	300	0.74 ± 0.12	16.47 ± 2.02	15.80 ± 0.30
16	Lambsquarters	400	0.22 ± 0.12	.	.
	Statistics				
	Crop		$F_{1,1}$ = 4.5231, p = 0.2798	.	.
	Carabid Abundance		$F_{1,1}$ = 0.3237, p = 0.6707	.	.

2.5. Discussion

2.5.1. Crop Type and Canopy structure

Plant density affected both carabid activity density and species richness in paired susceptible and tolerant wheat treatments in 2017. The dominant species in the plot trial for both years was *P. lucublandus,* which is a species reported by Lindroth (1961-1969) to inhabit open, moderately dry grassland habitats. This may indicate why the activity density in wheat treatments with lower plant densities is higher, with *P. lucublandus* strongly influencing the activity densities in those treatments. This effect was not detected in 2016, which may have been due to the shorter trapping period. In 2017, a second, smaller peak of seasonal activity occurred for *P. lucublandus* later in the season. If this was missed in 2016, it may explain the lack of treatment effect. Further analyses focusing solely on this species could provide more insight. Vegetation can act as an impediment to carabid movement on the soil surface (Honek, 1988; Thomas *et al.*, 2006). A lower plant density might therefore allow for more movement of carabid beetles and higher activity densities (Thomas *et al.*, 2006). This might also explain why species richness was higher in paired wheat treatments with lower plant densities. Because pitfall traps capture more active species, dense vegetation may hinder the movement and capture of less active species. However, it is important to note that vegetation acting as a physical deterrent for carabid beetles is only one small aspect that affects activity density and species richness.

Other than the relationship found between plant density and carabid assemblages in paired wheat treatments in 2017, there were no significant effects of crop type, plant density, and canopy coverage on carabid assemblages. This was unexpected and differs from much of the established literature, where these factors strongly influence carabid assemblages. Perfecto *et al.* (1986) found that the emigration rate of *Harpalus pennsylvanicus* Degeer and *Evarthus sodalis* LeConte (Coleoptera: Carabidae) was much lower in cropping systems with high plant densities. Honek (1988) found that activity of arthropods was higher in open areas than in the dense stands of wheat adjacent to them.

Carabids are highly influenced by a variety of factors associated with crop type and canopy coverage. These include light, food availability, the presence of weeds, and moisture (Cárcamo & Spence, 1994; Honek, 1988; Lindroth, 1961-1969; Lovei & Sunderland, 1996; Rivard, 1966; Thomas *et al.*, 2006; Varis *et al.*, 1984). Speight and Lawton (1976) found that in fields of winter wheat, carabid abundance was positively related to the frequency and density of weeds. Varis *et al.* (1984) hypothesized that the influence of crop type on carabid activity density was due to the different microclimate and light at the soil surface. It is possible that certain factors may have a stronger influence on carabid assemblages than those that we tested or that a combination of factors is responsible.

Another factor that might be influencing the lack of effect of crop type, canopy coverage, and plant density on carabid assemblages could be plot size. The plot size used in this study (4 m by 15 m) is relatively small compared with similar studies (Cárcamo *et al.*, 1995). Ground beetles are able to cover large distances by flying and have walking dispersal rates of a few metres per day (Floate *et al.*, 2007; Mitchell, 1963; Thiele, 2012). As a result, the potential scale of movement of some carabid species could have masked any observable effects (Cárcamo *et al.*, 1995; Floate *et al.*, 2007). Butts *et al.* (2003) were able to detect treatment effects in smaller plots (measuring 7 m by 10 m) in their study assessing the effects of intercropping regimes on carabid beetles. To compensate for arthropod movement, Prasifka *et al.* (2005) recommended that plot widths below nine metres should be avoided.

Crop rotation could have been an influencing factor on carabid assemblages in this study. Many carabids typically live for 1-2 years, though development can take much longer under adverse conditions (Lovei & Sunderland, 1996). Additionally, carabids overwinter in different life stages depending on the species (Lovei & Sunderland, 1996). These factors could mean that previous growing conditions could mask effects of crop type and canopy structure on carabid activity density and species richness. O'Rourke *et al.* (2014) found that low input, four-year crop rotations resulted in increased carabid activity density and species richness when compared with high input, two-year rotations. They hypothesized that more diverse crop habitats might bolster populations of natural enemies (O'Rourke *et al.*, 2014). Plots were spatially paired in this study to facilitate carabid movement within and between the two growing seasons. However, the effect of crop rotation on carabid assemblages was not analyzed.

2.5.2. Carabid Assemblages in the Peace River Region

Twelve of the 58 species collected during the two-year study accounted for 90% of the catch; i.e., *Poecilus lucublandus*, *Amara obesa, Amara thoracica*, *Carabus taedatus, Amara littoralis, Harpalus carbonatus, Pterostichus adstrictus, Harpalus amputatus, Agonum cupreum*, *Pterostichus melanarius,* and *Amara sinuosa* (Tables 2.2 and 2.3). Ten of these species are typically associated with dry, open grasslands having sparse vegetation (Lindroth (1961-1969). Two species (*P. melanarius, P. adstrictus*) are more typical of open habitats and cultivated land (Lindroth (1961-1969).

The species assemblage found in the Peace River region is fairly unique to the Canadian Prairies, with *Poecilus lucublandus* dominating both years of the study and making up 42.1% of the total catch. This species has been reported in many other studies throughout the prairies but typically only makes up less than 5% of the total capture (Bell *et al.*, 2014; Bourassa *et al.*, 2008; Bourassa *et al.*, 2010; Broatch, 2008; Floate, 1987; Frank, 1971; Holliday *et al.*, 2014; Melnychuk *et al.*, 2003; Pepper, 1999; Stjernberg, 2011). Uddin (2005) found this species made up 6% of the total capture in alfalfa fields in Manitoba. In their experimental plot trial looking at the effects of organic versus conventional farming in barley, faba beans, and intercropping of barley and pea, Cárcamo *et al.* (1995) found that *P. lucublandus* made up around 14% of the relative abundance at the Ellerslie Research Farm near Edmonton, AB. The work done at the Ellerslie Research Farm was conducted approximately 500 km southeast of the Peace River region and is one of the most geographically proximal studies conducted in an agricultural landscape on carabids, to the study conducted here in Beaverlodge, AB. The species assemblage described by Cárcamo *et al.* (1995) contains many similar species to those found in the Peace River region. However, the breakdown in relative abundance for those species is quite different, with eight species making up 90% of the total catch: *Pterostichus melanarius, Agonum cupreum, Poecilus lucublandus, Bembidion quadrimaculatum, Pterostichus adstrictus, Agonum placidum, Amara torrida*, and *Bembidion mutatum* (Cárcamo *et al.*, 1995). A study in northern Saskatchewan examined carabid diversity in wheat fields in an ecoregion similar to both the Peace River region and Edmonton, AB

(Floate, 1987). Floate (1987) found that four species: *B. quadrimaculatum*, *Bembidion obscurellum* Motschulsky, *Poecilus corvus* LeConte, and *A. placidum* dominated the catch in both years of the study, making up 67% (n = 5,290) and 62% (n = 8,692) of each year's catch.

Of the species recovered, only three are not native to North America; i.e., *Amara anthobia*, *Amara bifrons*, and *Pterostichus melanarius*. *Amara bifrons* was first recorded in eastern North America in Nova Scotia in 1929 and was reported to be spreading westward by Lindroth (1961-1969) in the 1950s. It is has not been reported in Alberta before (Holliday *et al.*, 2014; Lindroth, 1961-1969). *Amara anthobia* is a European species first recorded in Washington and Oregon in 1945 (Lindroth, 1961-1969), and subsequently in Alberta by Cárcamo *et al.* (1995), indicating that it had expanded its range northward into Canada by the mid-1990s. *Pterostichus melanarius* is also European in origin and was introduced in North America on both the Pacific and the Atlantic coasts in the 1920s. It has since rapidly spread throughout North America and is a common species in agro-ecosystems in Canada and the northern United States (Lindroth, 1961-1969). This holds true for the Canadian Prairies, where much of the existing literature cites *P. melanarius* as common (making up 5-50% of the total catch) or abundant (making up greater than 50% of the total catch) (Bourassa *et al.*, 2008; Bourassa *et al.*, 2010; Broatch, 2008; Cárcamo *et al.*, 1995; Floate *et al.*, 2007; Uddin, 2005). In 2019, this species is very common in many of the agricultural landscapes, with *P. melanarius* making up approximately 73% of the total catch (n = approximately 34,000) of carabids caught in agricultural fields between May and August in 2017 near Lethbridge, AB (Haley Catton, 2019, unpublished data). In Lacombe, AB, this species made up 88% of the total catch (n = 46,179) in a 3-year field plot trial seeded to faba beans (Héctor Cárcamo, 2019, unpublished data).

The Peace River region differs greatly in this respect from most of rest of the Prairies, with only 143 *P. melanarius* collected between both years of the study (Tables 2 and 3). It is possible that *P. melanarius* is relatively new to the Peace River region and still becoming established. This species has wing dimorphism, with long winged individuals signaling a recent arrival of a population (Lindroth, 1961-1969; Niemelä & Spence, 1999). Niemelä and Spence (1999) studied the local expansion of *P. melanarius* into aspen-poplar forest and found that the percentage of long-winged individuals was higher within the forest (80%) than along the road and forest edge (54%). Of the 92 *P. melanarius* collected in this study in 2017, 76.4% were of the long-winged form. For sites ranging across southern Alberta and Saskatchewan, the percentage of long-winged individuals ranges from 16-46% (n = ca. 200 beetles/field) (Kevin Floate, 2019, unpublished data). More than 50% of the individuals collected in this study were of the long-winged form. The presence of low numbers of introduced species, especially that of *P. melanarius*, could indicate the possibility of a reduction in species diversity for the Peace River region in the future. However, it is important to note that these species do have some beneficial aspects including their propensity to feed on weed seeds, in the case of both *Amara* species, or their ability as predators, in the case of *P. melanarius*.

2.5.3. Agronomic Parameters and Wheat Midge Populations

There was no effect of plant density on wheat midge larval density (number of wheat midge larvae per 100 heads) during either year of the study. In 2016, however, there was an effect of the mean tiller density on larval density. As the number of tillers increased, mean larval density also increased. This is in keeping with the literature, with higher seeding densities tending to tiller out less and sustain lower larval populations (Elliott *et al.*, 2011b). This effect was not seen in 2017. However, larval densities were also much lower during this year, which may have masked any effects. This is because when there are higher numbers of tillers present on a plant, the plant remains susceptible to wheat midge for a much longer period of time with tillers heading out and becoming susceptible at different times. In 2016, the period of susceptibility was not monitored. Growth ratings collected for wheat plots in 2016 indicate that plots were susceptible roughly between July 5-15, 2016 for all seeding rates.

Sticky cards in the plot trial caught very few adult wheat midge in 2016 (n = 3), which precludes making any conclusions regarding peak adult activity. These low capture rates could indicate that the traps were poorly placed or that there were not enough traps placed out into the plot trial. Lamb *et al.* (2002a) found that ten traps placed within the crop at 10-m apart yielded accurate control decisions about 75% of the time. However, Bruce *et al.* (2007) found that the correlation between sticky traps and the subsequent midge infestation was much lower in sticky cards when compared with pheromone traps. In future studies, it might be more accurate to measure wheat midge populations using pheromone traps as opposed to sticky traps. Based upon the data collected for the plot trial, it is likely that the peak flight of adult wheat midge occurred during the period in which wheat plants were susceptible to attack.

There was no effect of plant density or mean tillering density on the period of susceptibility or larval densities in 2017. The recovery of only four adults on sticky cards in 2017 precludes making any conclusions regarding periods of peak adult activity. Susceptibility growth ratings conducted during this year indicate that wheat was susceptible from July 10-31, 2017.

2.5.4. Carabids as Predators of Wheat Midge

Mean carabid activity density did not have a significant effect on wheat midge larval densities during either year of the study. Larvae would have been vulnerable to predation in June when they moved out of their cocoons to the surface of the soil and in August when they would be dropping down from the wheat heads (Floate *et al.*, 1990). Floate *et al.* (1990) found that *Bembidion quadrimaculatum*, *Bembidion obscurellum*, *Agonum placidum*, and *Poecilus corvus* consumed wheat midge larvae under laboratory conditions. Both *B. quadrimaculatum* (n = 1) and *A. placidum* (n = 39) were found in the plot trial, though in relatively low abundance.

As part of their study, Floate *et al.* (1990) also identified fourteen species to have preyed on wheat midge in the field using serological assays to detect prey from extracts of carabids. Seven of these species were recovered in the current study; i.e., *A. placidum*, *B. quadrimaculatum*, *B. rupicola*, *B. timidum*, *P. adstrictus*, *P. femoralis*, and *P. lucublandus*. Most of these species comprised less than 5% of the total catch for both years. In contrast, *P. lucublandus* comprised 54% (in 2016) and 28% (in 2017) of the carabids recovered with peak populations occurring in early to mid June. This would coincide with the period during which wheat midge larvae return to the surface after overwintering. Comparing this species to *P. corvus*, a carabid of similar size and shape, it could be hypothesized that that *P. lucublandus* might have a similar predation rate (48 wheat midge larvae killed per beetle per day) (Floate, 1990).

2.5.5. Harvest Parameters

Wheat midge larval densities did not affect on biomass or yield in paired wheat treatments in either year of the study, likely because of relatively low larval densities. Lamb *et al.* (2000b) indicated that yield and quality are not affected below thresholds of 11% and 6% infested seeds, respectively. Infestation levels were below 5% infested seeds in for both wheat varieties during both years of the plot trial. Larval density did have a significantly positive effect on percent protein in 2016. Typically, wheat that is damaged by wheat midge larvae tends to have very high protein content (Dexter *et al.*, 1987). This effect was not observed in 2017 and may have been due to the very low larval densities in the plot trial. There were no effects of plant density or carabid abundance on biomass or yield in either year of the study.

In 2016, there were no effects of plant density or carabid abundance on biomass or yield in paired wheat and canola treatments. However, in 2017, plant density had a significant effect on both biomass and yield in canola and wheat. Higher plant densities resulted in lower biomass and higher yield. In canola, increased seeding rates often result in higher yields (Harker *et al.*, 2003; Harker *et al.*, 2012). In 2017, carabid abundance had a negative effect on the biomass in canola treatments and a positive effect on biomass in wheat treatments. Carabid beetles could be reducing pest populations in wheat treatments and in, turn, increasing the biomass. However, since carabid beetles did not affect wheat midge larval densities and there were no other pests examined in this study, further investigation would be required determine the validity of this hypothesis. In canola treatments, mean biomass decreased with mean carabid abundance. There were no effects of carabid abundance on paired wheat and pea treatments or paired wheat and lambsquarters treatments in either year of the study.

2.6. Conclusions

In this study, carabid populations did not affect wheat midge larval densities. Wheat midge populations were relatively low during both years of the study and further investigation into carabids as predators of wheat midge in the Peace River region could prove very beneficial. *Poecilus lucublandus*, the dominant species in this study, is a good candidate for wheat midge control. To bolster populations of this species, growers may consider seeding wheat at lower densities.

CHAPTER 3: EVALUATION OF THE DIVERSITY, DENSITY, AND DISTRIBUTION OF WHEAT MIDGE PARASITOIDS IN THE PEACE RIVER REGION OF ALBERTA, CANADA

3.1. Abstract

Orange wheat blossom midge, *Sitodiplosis mosellana* (Géhin), is an economic pest of wheat that has recently expanded its range into the Peace River region of Alberta, Canada. Parasitoids have been found to be important mortality factors of wheat midge throughout the rest of its range, but no information is currently available for the Peace River region. The wheat midge has four main ovo-larval endoparasitoids, three of which have been reported in North America. Following the first report of wheat midge in the Peace River region of Alberta in 2011, there have been limited surveys assessing the distribution its parasitoids. A survey was conducted in 2016 and 2017 where wheat midge larvae were collected from wheat heads obtained from commercial wheat fields throughout the region. Parasitoids and adult wheat midge were reared from the overwintered larvae. Parasitism rates ranging from 35.5 ± 13.4% to 71.3 ± 12.3% were observed across 22 commercial fields. Almost all parasitoids (n = 2,140) recovered were *Macroglenes penetrans* (Kirby). The sole exception was a single individual in the genus *Inostemma*. This is the first report of an *Inostemma* species from *S. mosellana* in Canada.

3.2. Introduction

Wheat midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae), is an invasive, economically important pest in wheat grown in North America. This species is believed to be palearctic in origin and is widespread, occurring globally in most areas where wheat production occurs (Affolter, 1990). Introduced into North America in the 1800s, it is now a major pest in spring wheat on the Canadian Prairies. In 1985, a major outbreak of wheat midge in Saskatchewan caused $65 million CAD (value adjusted to 2018 dollars) in yield loss (Olfert *et al.*, 1985).

In Canada, adult wheat midge emerge in late June or early July over a five- to six-week period (Olfert *et al.*, 1985). Adults lay their eggs on the florets of emerging wheat heads singly or in groups of up to four (Olfert *et al.*, 2009). The eggs hatch within four to seven days and the neonate larvae crawl into the florets, where they feed on the kernel for two to three weeks (Doane & Olfert, 2008; Elliott *et al.*, 2009). This feeding results in kernels that are shrivelled and cracked, reducing grade and causing decreased yield (Dexter *et al.*, 1987). At the end of the feeding period and when moist conditions occur, the larvae drop down to the soil from the wheat heads, bury themselves, and form an overwintering cocoon (Doane & Olfert, 2008; Lamb *et al.*, 1999). In the spring, providing temperature and soil moisture conditions are amenable, larvae break diapause and return to the soil surface to pupate (Doane *et al.*, 1987; Elliott & Mann, 1996).

Parasitoids are important beneficial insects in agriculture, acting as specialized predators of many pest insects. Parasitoids lay their eggs on, or in their hosts, where they develop until they eventually emerge and kill their host (Wheeler, 1914). There are 27 species recorded for the parasitoid complex of *S. mosellana* and the closely-related wheat pest, *Contarinia tritici* Kirby (Diptera: Cecidomyiidae) (Affolter, 1990). However, in Switzerland, Affolter (1990) observed that the parasitoid complex of *S. mosellana* comprised only eight species and that each midge species had a distinct parasitoid complex. Chavalle *et al.* (2018) reported that the parasitoid complex of the wheat midge was made up of eight species (Hymenoptera: Pteromalidae and Platygasteridae) in Belgium as well. These included: *Macroglenes penetrans* Kirby, *Amblypasis tritici* Walker, *Euxestonotus error* Fitch, *Euxestonotus* sp. Fouts, *Leptacis* sp. Foerster, *Platygaster gracilipes* Huggert, *Platygaster nisus* Walker, and *Platygaster tuberosula* Kieffer. The four main ovo-larval endoparasitoids specific to the wheat midge include *M. penetrans* Kirby plus *P. tuberosula*, *E. error* Fitch and *Inostemma mosellanae* Flight (Hymenoptera: Platygasteridae) (Affolter, 1990; Chavalle *et al.*, 2015a; Olfert *et al.*, 2003). These parasitoids oviposit into the eggs of their hosts where the parasitoid larvae will develop until they are ready to emerge (Doane *et al.*, 1989). Parasitoids of the wheat midge have been reported to substantially affect their densities (Affolter, 1990; Basedow & Schuette, 1982). Basedow and Schuette (1982) reported that the degree of parasitism changed inversely to the population density of wheat midge and that parasitism reached a maximum observed rate of 74.4% in the Brodersdorf area of Germany. Parasitism levels of 64% are commonly reported in Europe (Carl & Raps, 1992). Variable rates of parasitism were reported in the United Kingdom between 1929-1956, reaching 99% (Barnes, 1956). To date, only *M. penetrans*, *P. tuberosula*, and *E. error* have been reported in North America whereas *I. mosellanae* is only found in Europe. The following chapter will only cover species that have been reported in North America.

In North America, the most common parasitoid of the wheat midge is *Macroglenes penetrans* (Affolter, 1990). This parasitoid was originally reported in the 1950s in Manitoba, but it is believed to have been accidentally introduced to North America as early as the 1800s from Europe (Affolter, 1990; Mason *et al.*, 2017). This species is characterized by its metallic blue-green colour and ranges in size from 1.3-1.9 mm (Doane *et al.*, 1989) (Fig. 3.1). Males have very prominent red eyes whereas females have less prominent brownish-black eyes (Doane *et al.*, 1989). *Macroglenes penetrans* is univoltine (having one generation per year) and is well synchronized with its host, with adult parasitoids flying and seeking out hosts shortly after wheat midge lay their eggs in late June and early July (Affolter, 1990).

Figure 3.1. *Macroglenes penetrans* (male) resting on a wheat head in a commercial field near McLennan, AB. Photo credit: Shelby Dufton (AAFC-Beaverlodge)

In North America, parasitism of the wheat midge is dominated by *M. penetrans*. Surveys conducted by Olfert *et al.* (2003) in 2001 found an average parasitism rate of 62% for *M. penetrans*. In Montana in 2005, it was found that *M. penetrans* constituted 35% of emerged adult wheat midge and parasitoids (Shanower, 2005). Thompson and Reddy (2016) found that populations of *M. penetrans* in Montana peaked shortly after wheat midge populations. Their study found that *M. penetrans* flight period occurred from late June to late July and abundance for this species significantly increased when fields were irrigated (Thompson & Reddy, 2016). An average parasitism rate of 22% was reported for *M. penetrans* in North Dakota based on soil surveys conducted between 1995 and 2006 (Knodel & Ganehiarachchi, 2016). In Saskatchewan, *M. penetrans* annually controls an average of 20 to 45% of the wheat midge population (Olfert *et al.*, 2009). *Macroglenes penetrans* is typically found wherever its host is reported in North America. This holds true for the Peace River region in northern Alberta. First records of the wheat midge were reported in the Peace River region in 2011, though it was likely in the area at non-economic levels prior to this (WCCP, 2011, 2013). In 2014, adult *M. penetrans* were found easily and in high numbers in sweep samples collected in flowering canola seeded on wheat stubble during early stages of wheat midge emergence (WCCP, 2014). These were reported to have moved to wheat later on in the season (WCCP, 2014).

Another parasitoid species accidentally introduced to Canada from Europe is *Euxestonotus error* (Affolter, 1990; Mason *et al.*, 2017). This univoltine species is black, has a shiny, flat scutellum, and is approximately 1-1.2 mm in length (Affolter, 1990). *Euxestonotus error* attacks the egg stage of the wheat midge, completing its development in the final larval stage of the host (Affolter, 1990). *Euxestonotus error* was first reported in North America in New York in 1861 by Fitch (Gahan, 1933). A recent sweep-net survey found that *E. error* was present in Flathead County in northwestern Montana (Echegaray *et al.*, 2016). This is the first record of the species in the Pacific Northwest and Echegaray *et al.* (2016) suggest that *E. error* may have moved to the area via migration.

A third parasitoid species, *Platygaster tuberosula*, was deliberately introduced from Europe to Langenburg, Saskatchewan in 1993 and 1994 as a biocontrol agent (Johansson, 1936; Olfert *et al.*, 2003). A follow-up survey was conducted from 1996-2001 at Langenburg and in a 5-km radius around the original release site (Olfert *et al.*, 2003). At the original release site, the number of adult *P. tuberosula* recovered annually between 1996 and 2001 ranged from 3 to 21 and increased to 89 individuals (a 5% parasitism rate) in 2001 (Olfert *et al.*, 2003). At two fields adjacent to the release site, parasitism rates of 2% and 4% parasitism by *P. tuberosula* were observed (Olfert *et al.*, 2003). The results of this survey serve as the record for the establishment of *P. tuberosula* in Saskatchewan (Olfert *et al.*, 2003).

Since the wheat midge was first recorded in the Peace River region, soil core surveys have been performed annually (Jennifer Otani, AAFC-Beaverlodge, personal communication, January 27, 2019). Very few wheat midge cocoons were collected between 2012 and 2014 and no parasitoids were found (Jennifer Otani, AAFC-Beaverlodge, personal communication, January 27, 2019). In 2015, two sites near Girouxville, AB yielded cocoons and had parasitism rates of 40% (n = 5 cocoons collected) and 50% (n = 2 cocoons collected) (Jennifer Otani, AAFC-Beaverlodge, personal communication, January 27, 2019). There have been no surveys of wheat midge parasitoid diversity in the Peace River region to date. In this study, the geographic distribution, species diversity, and percentage parasitism of parasitoids of the wheat midge were investigated.

3.3. Materials and Methods

3.3.1. Insect Collections

3.3.1.1. Sticky Card Collections

Yellow sticky cards (14 x 18 cm) (ACP Trap, Manufacturer: Alpha Scents, Inc. [Oregon, USA]) were placed at canopy height to determine presence and estimate peak populations of wheat midge and parasitoids during the growing season. Four sticky cards were placed at the southern replicate of the field plot experiment described in Chapter 2 of this study (Methods 2.3.2.1). Sticky cards were collected weekly from July 2 to August 9, 2016, and July 5 to August 1, 2017. In 2016, the first collection of the cards was delayed due to weather and represents a period of two weeks. Using a dissecting scope (Zeiss Stemi 2000-C, Manufacturer: Carl Zeiss Microscopy, LLC [New York, USA]), wheat midge adults and parasitoids were identified, counted and recorded for each card.

3.3.1.2. Wheat Head Collections

To determine the diversity, parasitism rate, and geographic distribution of parasitoids, 10 and 11 commercial wheat field sites were sampled throughout the Peace River region in 2016 and 2017, respectively (Table 3.1). To increase the likelihood of midge recovery, fields were selected that had been seeded to wheat midge susceptible cultivars (Table 3.1). Wheat heads were collected at 20-metre intervals along an M-shaped path, repeated twice for each field (Fig. 3.2).

The sampling effort differed between years. In 2016 between August 10 and 20, 200 heads were collected at each sampling point (30 sample points/field x 10 fields = 60,000 heads) to ensure sufficient recovery of midge to assess parasitism. However, initial sampling from several fields recovered less than 5 larvae per 400 heads. The remainder of heads from these fields were discarded such that only 24,000 heads were processed. Heads from the remaining fields were processed until at least 500 larvae per field were recovered. In 2017 between August 2 and 31, 100 heads were collected at each sampling point and processed (30 sample points/field x 11 fields = 33,000 heads) because results from 2016 showed more extensive collecting was unnecessary. Collections were timed to coincide when the developing wheat kernels were between early milk and early dough stages, when wheat midge larvae were anticipated to be in their final larval instar. Samples were stored in paper bags at 21 ºC until they could be processed.

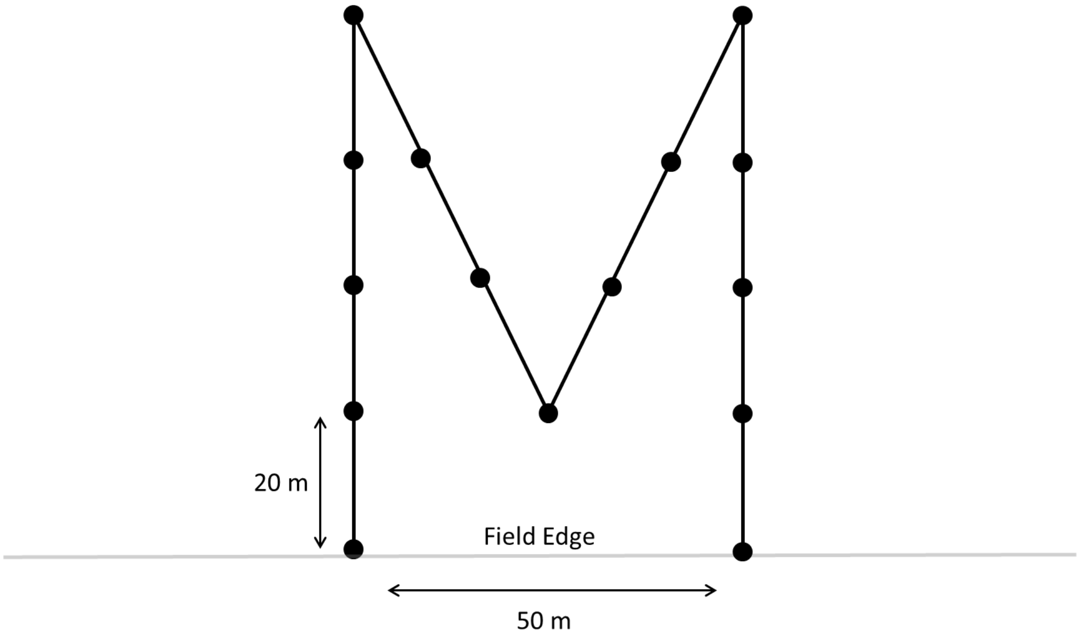

Figure 3.2. M-shaped field collection pattern for wheat head parasitoid survey. Collection points were spaced 20 metres apart. 200 heads were collected at each point in 2016. In 2017, 100 heads were collected at each point. Wheat heads were clipped and placed into paper bags until they could be processed.

Wheat heads from each collection point in the field were run through a single-head thresher (Almaco specialized equipment model SV SRE-2, Manufacturer: Almaco [Iowa, USA]) to separate out the different components of the wheat heads. Wheat heads were run through in groups of 10 so as not to overload the thresher. Air was turned off and chaff vents were covered on the thresher to retain larvae from the sample. The threshed sample was then placed into sieves (Endecotts Ltd. Brass Laboratory Test Sieves, Aperture Sizes: 4.75 mm and 1 mm, Manufacturer: Endecotts Ltd. [London, UK]) to further separate wheat components for sorting. These sieves created three layers of material: large pieces of wheat chaff (awns and stems), kernels and smaller pieces of chaff, and a fine layer of wheat chaff. The first of these layers was determined to contain no larvae and was thrown out. Each of the other layers was kept for sorting by hand. Layers were placed onto trays and sorted using a fine paintbrush to separate and search through material for wheat midge larvae.

All larvae were placed into plastic cups (Solo® 59.1 mL plastic cup) filled with 20 to 30 g of autoclaved soil for rearing. In 2016, soil used for rearing cups was collected from fields at the Agriculture and Agri-Food Canada research farm in Beaverlodge, Alberta (AAFC-Beaverlodge) (55.199, -119.396) in 2016. In 2017 larvae were placed onto autoclaved soil collected from each of their respective collecting sites (Table 3.1). Reverse osmosis (RO) water was applied to rearing cups as needed three times weekly to maintain a soil moisture of about 17.5% (Basedow, 1977a). A solution of 0.1% methyl paraben was applied, as needed, to control mould in the rearing cups.

3.3.2. 2016-2017 Overwintering and Rearing

In 2016, 21,028 larvae were collected and placed into rearing cups (11 to 73 larvae/cup) for overwintering in 2016. Before being placed into overwintering, a majority of the larvae were observed to have buried themselves in the soil. Larvae were overwintered from September 24, 2016 until April 27, 2017. Rearing cups were placed inside small cardboard boxes filled with shredded paper. These were placed into larger boxes filled with more shredded paper to further insulate them. Larvae were placed in two overwintering locations to reduce potential larval mortality; i.e., outdoors in mesh cages insulated with wheat straw (1m x 3m x 1m) sheltered by spruce trees (average temperature inside of insulated boxes of -3.3° C ± 7.0 SD) and a walk-in refrigerator (average temperature inside of insulated boxes of 2.5° C ± 0.9 SD). Onset^{TM} TidbiT v2 data loggers were placed inside the boxes and were set to record temperature every 15 minutes. Rearing cups were brought indoors every three weeks to check moisture and mould levels. At this time, cups were exposed to 22° C for up to 4 hours.

Between April 27 and May 7, 2017, rearing containers were removed from overwintering conditions. Wet sieving was used to determine larval overwintering mortality at each site (Doane *et al.*, 1987). Soil from rearing cups was placed into the top layer of the sieves. Running temperate water over the sieves, soil was gently massaged through each layer. Fine organic material in the bottom layer was floated and collected in large plastic Petri dishes (diameter: 10 cm). Using a dissecting scope (Zeiss Stemi 2000-C), wheat midge cocoons were sorted out from organic material and recorded. Larvae inside cocoons with tears or ruptures in their cuticle were determined to be dead. Live larvae were retained and placed back onto autoclaved soil collected from AAFC-

Beaverlodge individually or in groups (115-500 per container). Some larvae were housed individually to determine if the ratio of parasitoid to host was greater than 1:1. Group densities were determined by the number of larvae obtained from each site. A portion of larvae from each site was retained (n=4,754 in total) and not wet-sieved to determine the effect that wet-sieving had on larval mortality.

Rearing cups were maintained in the lab under natural ambient light (intensity of 2 lumens and room temperature (19.7 ° C ± 1.2 SD). Reverse osmosis (RO) water was applied to rearing cups as needed three times weekly. Rearing cups were maintained at a moisture level of 17.5% (Basedow, 1977a). Moisture levels were determined by weight and RO water was added as required. A solution of 0.1% methyl paraben was applied as needed to control mould in the rearing cups. All containers were checked each day for emergence of adult wheat midge and parasitoids. All emerged individuals were mouth-aspirated and preserved alive in 95% EtOH.

3.3.3. 2017-2018 Overwintering and Rearing

In 2017, 28,276 larvae were collected and placed into rearing. Larvae were placed onto soil individually or in groups (47 to 153 per container). Larvae were overwintered from December 13, 2017 to April 27, 2018, using the same methodology and the two outside locations (average temperature inside of insulated boxes of -5.1° C ± 7.6 SD) and walk-in refrigerator (average temperature inside of insulated boxes of 3.2° C ± 1.0 SD) as was used in 2016. Moisture and mould checks were excluded in 2017, because sufficient moisture was added to rearing cups prior to overwintering (Sheila Wolfe, AAFC-Winnipeg, personal communication, October 28, 2017).

In 2018, soil from rearing cups was not wet-sieved to determine overwintering mortality in order to reduce mortality from the process of sieving itself. Larvae from the same sites and same overwintering locations were combined into larger containers in groups of 300 to 863. Rearing cups were maintained in the lab under natural ambient light (intensity of 2 lumens) and room temperature (20.4 °C ± 1.9 SD). Rearing containers were placed into clear plastic bins (35 cm x 82 cm x 49 cm) with lids to maintain humidity. Moisture was maintained at the same level using the same procedure discussed in Section 3.3.3. RO water was applied to rearing cups as needed once weekly. A solution of 0.1% methyl paraben was applied as needed to control mould in the rearing cups. All containers were checked daily for emergence of adult wheat midge and parasitoids. All emerged individuals were mouth-aspirated and preserved in 95% EtOH.

Unless otherwise stated, all values are reported as mean ± SD. Larval parasitism rate was calculated as emergent parasitoids/(emergent parasitoids + emergent adult midges) x 100.

3.3.4. Insect Identification

Identifications of wheat midge larvae were determined using Barnes (1928). Identifications of *Macroglenes penetrans* were determined using Gibson *et al.* (1997) and by referring to voucher specimens held at AAFC-Beaverlodge.

Table 3.1. Commercial wheat field sites monitored in 2016 and 2017. All sites were seeded to wheat midge susceptible cultivars. Wheat heads were collected when the wheat midge were believed to be in the final, L3, larval stage. Mean (±SD) density, overwintering mortality, and parasitism resulting from larval rearing summarized below. Larvae collected in 2016 and 2017, were overwintered from September 26, 2016 to April 27, 2017 and from December 11, 2017 to April 27, 2018, respectively.

Year	**Nearest Town**	**Province**	**Latitude**	**Longitude**	**Variety**	**Larvae/100 Heads**	**Larval Overwintering Mortality (%)[1]**	**Larval Parasitism (%)[2]**
2016	Beaverlodge	AB	55.514	-119.403	Stettler	45.8 ± 22.7	61.5 ± 21.2 (n = 5)	54.3 ± 9.3 (n = 2)
	Fort Vermilion	AB	58.340	-116.073	Stettler	79.0 ± 31.2	54.6 ± 11.7 (n = 6)	38.9 ± 6.6 (n = 5)
	Girouxville	AB	55.715	-117.287	Stettler	.	.	.
	Girouxville	AB	55.773	-117.286	Stettler	82.3 ± 54.9	68.6 ± 6.5 (n = 6)	No emergence
	Guy	AB	55.553	-117.183	Roblin	93.2 ± 91.0	77.6 ± 6.2 9 (n = 5)	46.7 ± 13.3 (n = 3)
	Hythe	AB	55.316	-119.463	CDC Go	18.7 ± 11.3	64.4 ± 1.1 (n = 2)	No emergence
	Jean Côte	AB	55.970	-117.394	Stettler	278.2 ± 70.0	59.0 ± 3.9 (n = 14)	63.2 ± 28.8 (n = 6)
	La Crete	AB	58.224	-116.133	AC Intrepid	.	.	.
	McLennan	AB	55.659	-117.053	Superb	71.3 ± 19.2	50.9 ± 4.7 (n = 5)	71.0 ± 3.6 (n = 2)
	North Star	AB	55.708	-117.389	Stettler	.	.	.
	Notikewin	AB	55.970	-117.678	Alsask	.	.	.
2017	Bonanza	AB	56.053	-119.931	Alsask	14.1 ± 8.0	.	No emergence
	Fort Vermilion	AB	58.355	-116.041	Stettler	284.8 ± 146.1	.	71.3 ± 12.3 (n = 7)
	Fort Vermilion	AB	58.340	-116.043	Stettler	168.3 ± 103.6	.	No emergence
	Fort Vermilion	AB	58.307	-115.933	Alsask	41.3 ± 19.5	.	No emergence
	Girouxville	AB	55.773	-117.286	Stettler	186.0 ± 88.2	.	45.5 ± 5.2 (n = 7)
	Girouxville	AB	55.743	-117.389	Harvest	100.3 ± 47.8	.	66.6 ± 4.2 (n = 3)
	Guy	AB	55.558	-117.206	Roblin	15.2 ± 12.2	.	65.5 (n =1)
	Jean Côte	AB	55.956	-117.420	Stettler	5.1 ± 3.4	.	No emergence
	La Crete	AB	58.228	-116.204	AC Intrepid	52.6 ± 27.3	.	No emergence
	McLennan	AB	55.659	-117.053	Superb	72.2 ± 39.3	.	35.5 ± 13.4 (n = 3)
	Rolla	BC	58.872	-120.192	Thorsby	45.7 ± 24.1	.	No emergence

[1] n = total number of samples removed from overwintering (90-600 larvae/sample); [2] n = total number of samples with emergence following overwintering (emergence ranged from 1-223 adult wheat midge + parasitoids/sample)

3.4. Results

3.4.1. Sticky Card Collections

Sticky cards in the plot trial caught very few adult wheat midge (2016: n=3, 2017: n=4) which precludes drawing conclusions regarding peak adult activity. All parasitoids collected in 2016 were identified as *Macroglenes penetrans*. Parasitoid counts were highest on August 9, 2016 (Fig. 3.3). It is likely the population of parasitoids in the plot trial peaked in mid-August, though collections did not occur after August 9 (Fig. 3.3). In 2017, counts indicate that wheat midge adult populations peaked on July 25 in the plot trial (Fig. 3.3). Parasitoids collected in 2017 were all identified as *Macroglenes penetrans*. Parasitoid populations also peaked on July 25 in 2017 (Fig. 3.3).

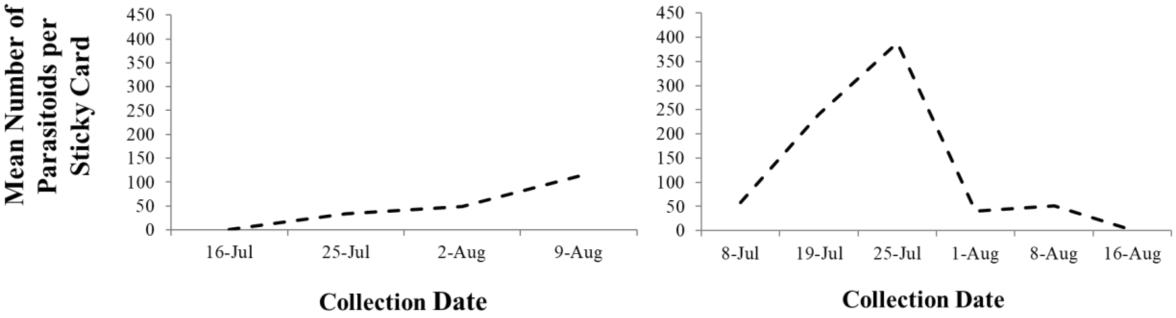

Figure 3.3. Seasonal activity of parasitoids in the plot trial seeded at the Beaverlodge Research Farm in 2016 and 2017.

3.4.2. 2016-2017 Parasitoid Survey

In 2016, wheat head samples were collected in 11 fields. Due to low rates of larval infestation (<5 larvae per 400 heads), heads from four fields were discarded. From the remaining seven fields, a total of 21,028 wheat midge larvae were collected from

24,000 wheat heads. From these latter fields, wheat midge larval densities ranged from 19 to 278 larvae per 100 heads (Table 3.1).

3.4.2. 2016-2017 Overwintering Mortality

Overwintering mortality was relatively high for all sites with an average of 61.2±11.9% for all sites (Table 3.1). Overwintering mortality ranged from 50.9% to 77.6% at these sites (Table 3.1). Following the wet-sieving process, 6,124 viable cocoons were recovered out of the 16,274 larvae that went into rearing.

3.4.3. 2016-2017 Parasitoid Rearing

In 2016, emergence was very low for all sites that were collected from. Thus, emergence data for larvae that were sieved following the overwintering period were pooled with those that were not. A total of 518 wheat midge adults and 627 parasitoids emerged from material collected in 2016 that was bulk reared. Individual rearing yielded only 13 parasitoids and 16 wheat midge. There was no emergence from the material collected near Hythe and Girouxville, AB (Table 3.1). Parasitism ranged from 38.9% to 71.0% (Table 3.1).

3.4.4. 2017-2018 Parasitoid Survey

In 2017, a total of 28,951 wheat midge larvae were collected from 33,000 wheat heads. From these fields, wheat midge larval densities ranged from 5.1 to 284.8 larvae per 100 heads.

3.4.5. 2017-2018 Parasitoid Rearing

In total, 1,063 wheat midge adults and 1,513 parasitoids emerged from the bulk-reared larvae collected in 2017. Individual rearing yielded only 14 parasitoids and 10 wheat midge. Parasitism was highest at the site near Fort Vermilion, AB (site one), with a parasitism rate of 71.1±12.3 % (Table 3.1). The lowest parasitism rate, 35.5±13.4%, was observed near McLennan, AB (Table 3.1). There was no emergence from the material collected from Bonanza, AB, Fort Vermilion (sites two and three), AB, Jean Côte, AB, La Crete, AB, and Rolla, BC. Parasitoids that emerged from each site were determined to be *Macroglenes penetrans* except for one individual. This parasitoid emerged from material collected near Guy, AB. This parasitoid is a shiny, black wasp that is 0.9 mm long (Fig. 3.4). It bears a horn on the first abdominal segment that reaches all the way towards its head. It has been tentatively identified as *Inostemma walkeri* Kieffer by Dr. Peter Buhl (IT University of Copenhagen; Copenhagen, Denmark). (Fig. 3.4).

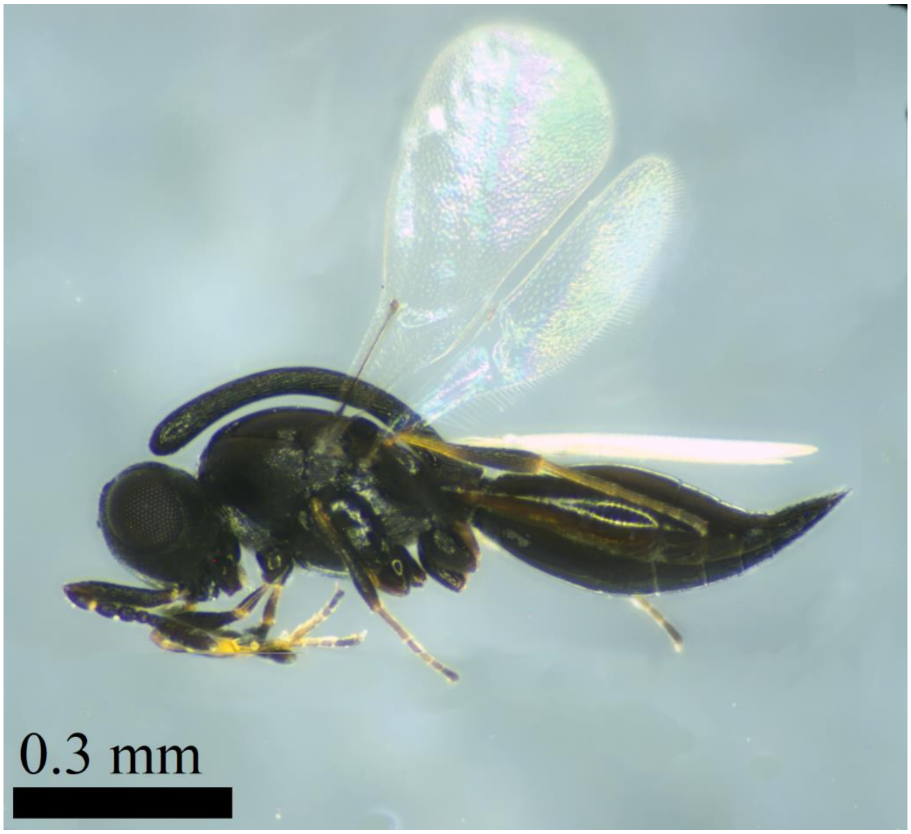

Figure 3.4. Female *Inostemma* sp. that emerged from wheat midge larvae collected at a commercial wheat field near Guy, AB. Photo credit: Shelby Dufton (AAFC-Beaverlodge)

3.5. Discussion

Relatively high densities of wheat midge larvae were collected from around the Peace River region during both years of the parasitoid survey. Despite this, relatively low emergence of parasitoids and wheat midge adults was observed in the material that was reared. Larval longevity may be an important reason explaining low emergence in the rearing that took place both years. Barnes (1952) found that adult wheat midge continued to emerge up to 12 years after larvae were originally collected in the field. Affolter (1990) indicated that this prolonged diapause might also occur when conditions to break it are not met. These include a cold-period of at least 120 days at low temperature (below

10 ºC), an undefined period at normal temperature (above 0 ºC), and a 5-week period during which soil moisture should be extremely high. All three of these conditions must be met for the wheat midge larvae to emerge from their diapause. In 2016, the cold-period experienced during overwintering was broken periodically when moisture and mould levels were checked.

In keeping with the conditions that Affolter (1990) indicated were required to break diapause, other studies have cited inconsistent moisture in rearing containers as a possible reason explaining low emergence (Hinks & Doane, 1988). Moisture levels in rearing containers during this study remained fairly consistent each year, with the soil being moistened at regular intervals. However, moisture was maintained at a level of 17.5% (Basedow, 1977a), whereas more recent studies have found optimal conditions to be between 30-50% (W. Cheng *et al.*, 2017).

Parasitoids of the wheat midge also experience a prolonged diapause (Barnes, 1952). In Saskatchewan and Manitoba, a second exposure to low temperature is necessary for over half of the *M. penetrans* in some populations to emerge (Wise and Lamb 2004). Both temperature and moisture could be factors resulting in low adult emergence numbers seen for larvae collected in the Peace River region.

A majority of the larvae collected in 2016 were wet-sieved and redistributed into new rearing containers, which may explain the low emergence rates in the first year. Larvae were removed from overwintering during a vulnerable life-stage, when they were emerging from diapause, and may have been more susceptible to mechanical injury. Wet-sieving was not conducted for the second year in an attempt to reduce mortality from this process.

Low emergence from larvae collected in 2017 may be due to the amount of time larvae remained in wheat heads before they were extracted and placed into rearing. The amount of time larvae remained in wheat heads ranged from one to four months. Wise and Lamb (2004) found that larval mortality was over 95% after larvae remained in wheat heads for 13 weeks. Wallengren (1937) found that larvae resisted desiccation for two months under laboratory conditions.

Euxestonotus error and the introduced *Platygaster tuberosula* were not found in either year of this survey. *Platygaster tuberosula* has not been observed outside of Saskatchewan since its introduction there in 1993 and 1994 (Olfert *et al.*, 2003). These surveys indicate that it has not expanded its range into the northern reaches of Alberta.

The results from the wheat head parasitoid surveys conducted in 2016 and 2017 confirm that *Macroglenes penetrans* is the dominant parasitoid of the wheat midge in the Peace River region. These results are similar to other parasitoid surveys conducted for the wheat midge in North America (Knodel & Ganehiarachchi, 2016; Olfert *et al.*, 2003; Olfert *et al.*, 2009; Shanower, 2005; Thompson & Reddy, 2016). This parasitoid is an essential component of the integrated pest management of the wheat midge. From 1991-2000, it was estimated that *M. penetrans* helped to save more than $345 million (adjusted to 2018 CAD) in insecticide costs in Saskatchewan alone (Olfert *et al.*, 2009). The presence of this parasitoid and other natural enemies of the wheat midge provide immense economic savings for growers and producers.

The single *Inostemma* sp. found in the rearing has not yet been identified to species. This genus is typically recognized by the horn-like process on the first abdominal segment in females (Masner & Huggert, 1989). This genus is found worldwide with many of its species still undescribed (Masner & Huggert, 1989). Species in this genus parasitize gall midges (Cecidomyiidae) (Masner & Huggert, 1989). Masner and Huggert (1989) suggest that the characteristic long horn of female *Inostemma*, which houses their long ovipositors, confers them an advantage in attacking hosts that are in areas that are difficult to reach.

Affolter (1990) describes one *Inostemma* species in his report on the parasitoid complex of *S. mosellana* and *C. tritici*, *I. mosellanae*. *Inostemma mosellanae* is described to have a short horn on its first abdominal segment that reaches its scutellum. The species discovered in this study bears a similar horn, characteristic of the *Inostemma* genus, which reaches from the first abdominal segment to its head. To date, there have been no reports of *I. mosellanae* in North America. *Inostemma horni* Ashmead (Hymenoptera: Platygasteridae) was observed to seek and oviposit in wheat midge eggs in Bellingham, WA (Reeher, 1945). The species was confirmed as *Inostemma* sp. by Dr. Lubomir Masner (National Identification Service; Ontario, Canada). The specimen was then sent on to Dr. Peter Buhl (IT University of Copenhagen; Copenhagen, Denmark), who tentatively identified the species as *Inostemma walkeri*. Dr. Buhl indicated that this species has previously been collected in “grass” and “cereali” by F. Walker in 1836. To my knowledge, this is the first report of *I. walkeri* for North America.

CHAPTER 4: GENERAL DISCUSSION

Wheat midge has occurred in Canada since its introduction in the 1800s, though it did not become a major pest until the mid-1980s. This insect is found everywhere wheat production occurs between the 42nd and 62nd parallels (Affolter, 1990; Olfert *et al.*, 2016). Wheat midge was first recorded in the most northern part of its range in 2011 in the Peace River region (WCCP, 2011, 2013). Currently, control of this pest is marked by the use of cultural controls, insecticides, and midge-tolerant varieties of wheat. Because wheat midge is a relatively new pest to the Peace River region, much of the area is still planted with susceptible varieties of spring wheat. Some movement towards the use of tolerant varieties has been observed, but it is important to consider alternative control methods for this pest such as natural enemies.

The overall goal of this project was to provide a more thorough understanding of the natural enemies of the wheat midge in the Peace River region. In 2016 and 2017, I examined five objectives relating to the impacts of cropping systems and natural enemies on wheat midge. (1) The effects of crop type and canopy structure on carabid assemblages in this region. Depending on the nature of the effects, predatory species likely to feed on wheat midge larvae may become more or less dominant in the carabid assemblage. (2) The effect of plant density on wheat midge populations. Seeding at different rates can influence the density of plants in the field and, in turn, the density of wheat midge. (3) The diversity of carabid assemblages found in the region. Given its geographic location, the Peace River region has fairly unique growing conditions compared to the rest of the Canadian Prairies which likely influences the species make up of its carabid assemblages. (4) Whether carabid assemblages had a significant effect on wheat midge populations. Dominant carabid species in this region may be able to provide some level of control for wheat midge populations. (5) The geographic distribution, species diversity, and percentage parasitism of parasitoids of wheat midge in the region. Wheat midge parasitoids have been reported but little is known about the species diversity and their impact on wheat midge populations for this region.

Few effects of crop type and canopy coverage on carabid assemblages were observed in this study. This was inconsistent with much of the established literature on the topic. Typically, carabid beetles are reported as being particularly sensitive to changes in crop type, vegetation density, and canopy coverage (Honek, 1988; Perfecto *et al.*, 1986; Thomas *et al.*, 2006). It has been hypothesized that these factors influence carabid beetles due to their effects on the light and moisture availability at the soil surface (Cárcamo & Spence, 1994; Honek, 1988; Lindroth, 1961-1969; Lovei & Sunderland, 1996; Rivard, 1966; Thomas *et al.*, 2006; Varis *et al.*, 1984). In the second year of the study, lower wheat densities did result in higher carabid activity densities and species richness. This could be because there was less vegetation at the soil surface to impede carabid movement or because a large portion of the carabid assemblage was comprised of species that prefer dry, open grasslands (Lindroth, 1961-1969; Thomas *et al.*, 2006).

Based on pitfall trap catches, my results indicate that the carabid assemblage in the Peace River region is different compared with much of the Canadian Prairies. Twelve species made up 90% of the catch with ten of those species preferring open grasslands (Lindroth, 1961-1969). *Poecilus lucublandus*, a native, predatory species, dominated the catch in both years of the study. Very few introduced species were collected in this study and made up a small percentage of the total catch. This is unusual in the Canadian

Prairies, where the introduced species *Pterostichus melanarius* has typically been reported to comprise 5-50% of the total catch in many studies. Studies currently underway are finding that this species comprises 70-80% of the total catch in southern and central Alberta (H. Catton, AAFC-Lethbridge, personal communication, January 22, 2019; H. Carcamo & P. Reid, AAFC-Lethbridge & AAFC-Lacombe, personal communication, January 22, 2019). Given the Peace River region's northern latitude and relative isolation from the rest of the Prairies, it is unsurprising that *P. melanarius* and other introduced species are found in low abundances in the region. It is likely these populations are relatively new to the area and it will be interesting to observe changes in their relative abundance in the coming years.

Higher tiller densities resulted in higher larval populations in the first year in the study. This trend is commonly reported in the literature, where increased tillering extends the period during which plants are susceptible to wheat midge and higher numbers of larvae survive to maturity (Elliott *et al.*, 2011b). In each year of the study, wheat midge populations were very low, most notably in 2017. It is possible that effects would be difficult to observe due to small population sizes in the second year.

Mean carabid activity density and midge density were neither positively nor negatively correlated in this study. However, a number of species were present that are known to feed on wheat midge; i.e., *Agonum placidum*, *Bembidion quadrimaculatum*, *Bembidion rupicola*, *Bembidion timidum*, *Pterostichus adstrictus*, *Pterostichus femoralis*, and *P. lucublandus* (Floate *et al.*, 1990). Of these species, *P. lucublandus* made up a large portion of the total catch in the study. Captures of this species peaked in early to mid June, likely coinciding with the presence of wheat midge larvae on the soil surface. This species is a promising candidate for the control of wheat midge in the Peace River region. Further investigation into this species' effect on wheat midge in this region would be beneficial.

Larval densities were relatively low during both years of the plot trial and did not affect wheat biomass or yield. The presence of wheat midge larvae increased the percent protein found in wheat in the first year of the study. This has been shown previously by Dexter *et al.* (1987), who noted that wheat midge damage typically results in higher protein content.

No effects of carabid abundance were detected on wheat biomass or yield in paired treatments of wheat, of wheat and pea, or of wheat and lambsquarters. Similarly, Cárcamo *et al.* (1995) did not detect correlations between carabid abundance versus crop biomass or grain yield. However, in the second year of the current study, carabid abundance was correlated with total plant biomass in paired wheat and canola treatments. In these treatments, biomass increased in wheat when carabid abundance was high. Previous literature has suggested that reduced predation pressure on aphids could explain decreases in grain yields (Helenius, 1990). It is possible that carabids were reducing the presence of pests in our study and thus resulting in an increase in biomass. However, given that there was no effect of carabid activity density on wheat midge and there were no other pests monitored in this study, further investigation would be necessary. The opposite trend was observed in canola, where carabid abundance resulted in lower biomass. There is no established literature that shows this trend.

Macroglenes penetrans was the dominant parasitoid recovered in our study. *Platygaster tuberosula* and *Euxestonotus error* were not recovered. This is in keeping with much of the literature, which reports *M. penetrans* as the dominant parasitoid of wheat midge in North America (Knodel & Ganehiarachchi, 2016; Olfert *et al.*, 2003; Olfert *et al.*, 2009; Shanower, 2005; Thompson & Reddy, 2016). The presence and parasitism rate of this parasitoid in this region is a very positive sign for growers. *Macroglenes penetrans* can reduce insecticides costs and provide savings for growers by limiting the need for insecticide applications against wheat midge (Olfert *et al.*, 2009).

One other parasitoid species was recovered in this study, a single *Inostemma* sp. female. *Inostemma mosellanae* parasitizes wheat midge in Europe, but has not been reported in North America (Affolter, 1990). *Inostemma horni* has been reported to oviposit in wheat midge eggs in the United States (Reeher, 1945). Based on morphological differences, the specimen recovered in the current study has been identified as *Inostemma walkeri*. This is the first report of *Inostemma walkeri* species from wheat midge in North America.

Though wheat midge has one of most well developed set of insect pest management strategies, no single form of control completely mitigates its impact on wheat production. As such, there are still gaps in knowledge relating this pest and its biological controls. Natural enemies are a valuable resource and we should continue to optimize their impacts on pest insects. This study is the first report on the diversity of wheat midge parasitoids and carabid species in agricultural landscapes in the Peace River region. Further exploration into these groups should be undertaken for this region, specifically focusing on species such as *P. lucublandus*.

The diversity of the carabids observed in this region are also somewhat unique when compared to the rest of the Canadian Prairies, which may reflect the unique conditions seen in the region. Because of its northern latitude, the region experiences a short growing season, long summer days, and tight crop rotations. The Peace River region contributes greatly to wheat production in Canada. In 2018, wheat production in the Peace River region totaled 1,400,800 metric tonnes, approximately 14% of the total for Alberta (Statistics Canada, 2019). Based upon the findings of this study and the importance of wheat production in this area, natural enemies may have a very important role in the control of wheat midge in the Peace River region.

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