Examining the Biology and Monitoring Tools of *Sitodiplosis mosellana* in the Peace River region, Alberta

by

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Abstract

Wheat midge, *Sitodiplosis mosellana* Géhin (Diptera: Cecidomyiidae), is an invasive pest of wheat that has spread into the Peace River region of Alberta, Canada. The biology and monitoring of wheat midge has not previously been examined in this region. Wheat midge overwintering density and parasitism rates were examined by rearing field collected soil, and overwintering mortality was determined by rearing wheat midge in soil in different conditions. Parasitism rates were high (>50%). Rates of adult emergence were higher when soil was held under controlled conditions overwinter. Patterns of adult wheat midge emergence in the field were compared to published bioclimatic models. The differences between adult emergence in the Peace River region were great enough that development of a regionally-optimized model is necessary. In the Peace River region, adults emerged 300 GDD base 7 °C after a rainfall event. Multiple peaks of wheat midge emergence and capture on pheromone-baited traps were observed. Capture of adult wheat midges on different delta traps (orange or green), and pheromone lures (Scotts™ flex, Scotts™ septa, or Great Lakes IPM™ septa) were compared. In 2017, delta traps with a larger surface area captured more midges, and traps baited with the Scotts™ flex lure captured more midges than the Great Lakes IPM™ septa lure. Different un-baited yellow sticky cards (Great Lakes IPM™, Alpha Scents™, or Alpha Scents™ rolled into a cylinder) were compared for wheat midge capture. More midges were captured on the smaller, Great Lakes IPM™ unbaited yellow sticky cards than the two, larger Alpha Scents™ cards. We examined relationships between capture of adult midges on pheromone or yellow sticky card traps to larval density by extracting larvae from wheat heads at the same sites. We found no relationships between pheromone-baited traps and larval densities and were unable to determine relationships between yellow sticky cards and larval densities. To compare harvest metrics to trap capture, we collected harvest samples (1 m^2) at the same sites. We found no relationships between harvest metrics and pheromone-baited traps and were unable to determine relationships with yellow sticky traps. We examined the longevity of adult females in the lab and found no differences in longevity between females held individually or held with males. In commercial wheat fields, we conducted hourly counts of active female wheat midges to test circadian flight patterns. Peak wheat midge flight occurred at twilight. Soil core samples were collected from sites before monitoring to determine relationships with adult midge densities. No relationships were found between soil core samples and captures of adult midges in the spring.

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Chapter 1: Introduction

Integrated pest management (IPM) programs rely on the combined use of predictive, monitoring, and control tools to provide long-term management of insect pests. The overall goal of most IPM programs is to maintain economic viability with decreased insecticidal inputs (Stern et al., 1959), but modern implementations aim for overall sustainability and increased productivity. Meeting these goals requires well-researched tactics that apply throughout the growing season and across years. These strategies also need to combine best practices across disciplines: entomology, pathology, botany, agronomy and ecology. Another requirement of effective IPM strategies is the ability to move past reactionary tactics without use of prophylactic tactics. To do this, we need a thorough understanding of pest biology, population dynamics, and effective tools to monitor the changes in pest density and activity in the field.

Sampling pest populations forms the basis of many IPM strategies. Most commonly, populations are sampled to determine pest activity or presence in an area, or pest densities to guide control action (Cohnstaedt et al., 2012; Morris, 1960; Strickland, 1961). When sampling to detect pest presence and activity, strategies should prioritize sensitive and efficient monitoring tools (Witzgall et al., 2010). Attractive traps incorporate visual cues such as shape and colour, or chemical cues such as food baits, plant volatiles, or pheromones to lure the target pest (Cohnstaedt et al., 2012; McNeil, 1991; Witzgall et al., 2010). Optimization of attractive traps requires research to promote trap sensitivity, efficiency and exclusion of bycatch. Sampling pest densities to guide control action needs to reflect damage potential of the target pest (Onstad, 1987; Stern, 1973; Weersink et al., 1991). Sampling pest densities can include the use of attractive or passive traps such as sticky cards, pan traps or pitfall traps, if the target is mobile (Prokopy et al., 1982; Ragsdale et al., 2007; Southwood, 1978). Often more direct measures of pest density such as counts of pest insects on plants are conducted *in situ* (Bode and Calvin, 1990; Boeve and Weiss, 1998; Buntin et al., 2004). Sampling can also involve insect removal from the host plant by beating or shaking plants, or collection with sweep-nets or suction traps (Allison and Pike, 1988; Way et al., 1981; Wise and Lamb, 1998; Wise and Lamb, 1998). If feeding damage is diagnostic, damage symptoms can be quantified to measure insect activity (Näsi et al., 2015; Riedell and Blackmer, 1999). Approaches are also combined so that trap capture can indicate timing to initiate field scouting (Bracken and Bucher, 1977; Eller et al., 1994; Walker et al., 2003; Wise et al., 2009). Monitoring tactics to determine damage risk must relate the number of sampled insects to damage. Correct timing of in-field placement can increase the effectiveness of both sampling tactics, which can be improved by the application of bioclimatic models (Strand, 2000; WMO, 1988).

Incorporation of meteorological data into different pest management programs (Higley et al., 1986; Strand, 2000; WMO, 1988) makes the inclusion of detailed climatic models into regional and on-farm management programs practical. Forecasts of pest population density based on climatic suitability, pest introductions, or migration can indicate risk levels that producers can use for decision-making, such as crop species and cultivar selections, timing of planting, irrigation and harvest activities (Strand, 2000; WMO, 1988). During the growing season, use of climatic models can decrease pesticide inputs through accurate estimates of pest activity periods, population densities, and timing of control tactics (Strand, 2000; Way and Van Emden, 2000; WMO, 1988).

Phenological models of the relationship between insect development and temperature have been widely researched (Damos and Savopoulou-Soultani, 2012; Danks, 2006; Pruess, 1983). Growing degree days are one of the most common approaches to modelling insect development. Growing degree days are based on either linear or sigmoidal relationships of insect development rates over increased temperature and are bounded by lower and upper thresholds (Allen, 1976; Higley et al., 1986). There are various different mathematical approaches to calculating degree days that vary in complexity. Growing degree days accurately describe insect development in many systems but are less accurate at extreme temperature values (Worner, 1992).

The responses of insect pests to meteorological variables differs among species, populations, and life stages. Developmental thresholds, ability to survive at different temperatures, interactions between different meteorological variables, and the relative importance of moisture, humidity, and photoperiod all differ widely among species (WMO, 1988). Developmental thresholds and fatal temperatures vary with life stage for many insects (WMO, 1988). Bioclimatic models need to be validated for local climatic conditions, values and risk attitudes before inclusion in IPM programs (Strand, 2000). A thorough understanding of pest biology is required not only to develop accurate bioclimatic models, but also to implement an effective IPM program in general.

The wheat midge, *Sitodiplosis mosellana* Géhin (Diptera: Cecidomyiidae), is a major pest of wheat (*Triticum spp.*) throughout its native and invasive range. Wheat midge causes damage to wheat grains through larval feeding. Wheat midge can complete development in barley, *Hordeum vulgare* L., rye, *Secale cereale* L., and couchgrass, *Elymus repens* (L.) Gould, (Basedow, 1972; Basedow and Schütte, 1973), but is only an economic pest of wheat. Losses per year (listed in 2018 US dollars) attributed to wheat midge during outbreaks vary: $51 million in north east Saskatchewan in 1983 (Olfert et al., 1985), $105 million in New York in 1954 (Borkent, 1989), and $1.8 million in one county in Montana in 2006 (Thompson and Reddy, 2016). Wheat midge damage tends to be sporadic, with outbreaks occurring when abiotic conditions, such as spring soil moisture, are optimal and population densities are high. In its native range, wheat midge is sporadically a pest, causing $109 million in crop losses in the UK during 2004 (Oakley et al., 2005).

The wheat midge was introduced to North America in 1828 in Québec (Felt 1921 cited in Olfert et al. 1985) and has since spread across North America. Wheat midge can be found in Canada in British Columbia (Reeher, 1945), Alberta (Lamb et al., 2002), Saskatchewan (Wright and Doane, 1987), Manitoba (R J Lamb et al., 2000), Ontario, Québec, New Brunswick, and Nova Scotia (Borkent, 1989). In the United States, it has been recovered in Montana (Olfert et al., 2009), North Dakota (Knodel and Ganehiarachchi, 2001), and New York (Felt, 1908). Originally Palearctic in origin (Borkent, 1989), wheat midge is found in most wheat-growing regions including China (Miao et al., 2013), the UK (Bruce et al., 2007) and Europe (El-Wakeil et al., 2013).

Wheat midge has a univoltine life cycle throughout its range (Mukerji et al., 1988). Third instar larvae overwinter in cocoons found mostly within the top 0-6 centimetres of soil, however, some cocoons are found as deep as 15 cm in the soil profile (Doane and Olfert, 2008). Third-instar larvae undergo obligatory diapause within the cocoon that lasts at least 70 days below 2 °C (Hinks and Doane, 1988). After diapause, larvae travel to the soil surface and pupate. Pupation occurs without a puparium (Borkent, 1989). Both temperature and moisture regulate emergence of larvae from the cocoon. In Saskatchewan, an average of 218 Degree days (GDD) above a threshold of 3 °C is required for larvae to break diapause (Doane and Olfert, 2008) and 250 GDD above 3 °C in Belgium (Jacquemin et al., 2014).

Wheat midge pupal development time varies by geographic region. In Saskatchewan, adult midges emerge late June to mid-July (Elliott, 1988a; Elliott et al., 2009). Throughout Europe, adult wheat midges emerge both earlier in the calendar year and with less GDD accumulation (Basedow, 1972; Elliott et al., 2009; Jacquemin et al., 2014) than in Canada. In Finland, however, midges emerge at lower GDD accumulations than in Canada, but at the same point in the calendar year (Kurppa, 1989). In Belgium, adult emergence occurs between late May and the end of June (Jacquemin et al., 2014). In Germany, adults emerge between early and late June (Basedow and Gillich, 1982), and emergence can span 7 weeks (Basedow, 1972). In Finland, adult wheat midges emerge mid-June to early July (Kurppa, 1989). Adult wheat midge emergence is most different from Canadian populations in China, where adult midges emerge from late April to early May (Cheng et al., 2017). Typically, males emerge 2.3 calendar days before females (Pivnick and Labbé, 1992), or a mean of 32 GDD base 5 °C (Elliott et al., 2009).

Females mate at the site of emergence then disperse into wheat fields. Female midges release a pheromone signal that has been identified as 2, 7-nonanediyl dibutyrate (Gries et al., 2000). Females call in late afternoon immediately after eclosion then disperse from the emergence site after mating (Pivnick and Labbé, 1993, 1992). Females emerge with a mean of 40 eggs (Basedow, 1977), but that number can increase to >50 after 5 days of maturation (Basedow and Schütte, 1973). Males remain at the emergence site while females disperse to locate plant hosts (Basedow, 1977; Smith et al., 2007) (Figure 1.1). While Cecidomyiids are weak fliers (Hall et al., 2012), wheat midge females can actively fly more than 1000 m and can be transported on high altitude wind currents (Hao et al., 2013; Miao et al., 2013). Adult females live for 2 (Basedow and Schütte, 1973) to 7.3 days in the field (Pivnick and Labbé, 1992). Females only actively fly when wind speeds are below 7.2 km/ hr (Basedow, 1977) or 10 km/ hr (Pivnick and Labbé, 1993). This period of active flight takes place just before or at twilight, which occurs between 19:30 and 20:00 in Germany (Basedow, 1977) and between 20:00 and 21:45 in Saskatchewan (Pivnick and Labbé, 1993).

Figure 1.1 Wheat midges in situ. A. Male wheat midge. B. Female wheat midge. Photo credit: AAFC-Jorgensen and Dufton

Wheat midge larvae develop within the spikes (inflorescences) of wheat or other cereals. Eggs are laid singly or in clusters of 2-6 on the spikelet, most commonly on the exterior of the wheat floret, on the palea (Mukerji et al., 1988; Smith and Lamb, 2001). The eggs hatch after 4-7 days and larvae move inside the floret to feed (Mukerji et al., 1988). Larvae will not develop from eggs laid on wheat spikes or after anthesis (Basedow and Schütte, 1973; Elliott and Mann, 1996). Adult female midges are more attracted to young wheat spikes than spikes in anthesis or barley spikes (Ganehiarachchi and Harris, 2007). On wheat, larvae mature and finish feeding in 9-10 days (Ding and Lamb, 1999; Gagné and Doane, 1999). Larvae feed on the exterior of kernels developing inside the floret during the first two larval instars. The third instar remains inside the exuvium of the second instar for a period of two to three weeks (Figure 1.2 A) (Gagné and Doane, 1999). The larvae then leave the wheat spike in response to moisture from rain or heavy dew (Mukerji et al., 1988). Third-instar larvae can remain inside the wheat spike for more than 5 weeks and can successfully complete diapause and emerge from excised spikes if to dry conditions prevent larvae from entering the soil (Wise and Lamb, 2004). When larvae leave the spikes, they fall to the soil surface and burrow into the soil (Figure 1.2 B).

Figure 1.2 Wheat midge larvae. A. Third instar wheat midge larva on a damaged wheat kernel. B. Overwintering third-instar larva inside cocoon on soil.

Wheat midge damage decreases wheat yield and quality. Larval feeding on seeds causes shrivelling and cracking (Lamb et al., 2000; Mukerji et al., 1988). Damage to seeds can cause kernel abortion, biomass loss (Figure 1.3), increased risk of sprouting, smut or fungal infection, and yield loss when kernels become too light to be harvested mechanically (Helenius and Kurppa, 1989; Lamb et al., 2000; Mukerji et al., 1988). Quality losses occur due to lower breadmaking quality of damaged kernels (Miller and Halton, 1961). The Canadian Grain Commission decreases wheat grade from No.1 CWRS at 2 % midge damage, from No. 2 CWRS at 5%, and from No. 3 CWRS to feed at 10% midge damage (Commission, 2018). Damage is higher in durum wheat (*Triticum durum*) than in bread wheat. There is no direct relationship between larval density and damage (Lamb et al., 2000), as economic damage depends on the percent of infested kernels (Chavalle et al., 2015; Lamb et al., 2000). Kernels infested with one midge larva lose as much biomass as kernels infested with multiple larvae (Lamb et al., 2000). Economic thresholds for grain infestation for spring and durum wheat by wheat midge are 6% to prevent downgrading and 10% to prevent yield loss based on costs and wheat prices in the 1990's (Lamb et al., 2000). However, larvae are protected from insecticde applications once inside the spikelet (Elliott, 1988b). This means that monitoring and control need to target wheat midge adults.

Figure 1.3 Wheat kernels (variety Roblin) collected from a commercial wheat field. Left: healthy, undamaged kernel. Right: wheat midge damaged kernel displaying damage symptoms: ruptured bran, shrivelling, and white mark.

Different monitoring tools have been developed to monitor adult wheat midge activity and density. Wheat midges captured on yellow sticky cards can predict larval infestation in Manitoba (Lamb et al., 2002). An action threshold of 3 or more midges per 10 traps over 3 nights is recommended for high protein wheat, and 4 or more midges per 10 traps for lower quality wheat (Lamb et al., 2002). Attractive traps baited with the female produced sex-pheromone are widely used to monitor adult wheat midge activity. In the UK, the suggested action threshold is 20 male midges per trap per day (Oakley et al., 2005); however, action thresholds using pheromone traps have not been developed in other regions (Mircioiu, 2004). Currently, in-field counts of adult females dictate decisions on management action in the Prairie Provinces of Canada. The recommended threshold is 1 female midge per 4-5 wheat heads to protect quality, and 2 female midges per 4-5 heads to protect yield (Elliott et al., 2002). In Germany, a “critical number” of 1 female midge per 3 wheat heads is recommended (Basedow and Schütte, 1973).

Integrated control of wheat midge includes suppression of wheat midge densities by the parasitoid *Macroglenes penetrans* Kirby (Hymenoptera: Pteromalidae), which maintains high levels of parasitism in Europe (Basedow, 1972; Chavalle et al., 2018) and in Canada (Shanower, 2005; Wise and Lamb, 2004). *Macroglenes penetrans* was unintentionally introduced with wheat midge to North America. Efforts have been made to introduce two other parasitoids to Canada: *Euxestonotus error* Fitch (Hymenoptera: Platygastridae) and *Platygaster tuberosula* Kieffer (Hymenoptera: Platygastridae) (Doane et al., 2001). While small populations of these species have established where they were introduced, they have not spread across the Prairie Provinces (Echegaray et al., 2016; Olfert et al., 2003). Wheat midge “tolerant” wheat varieties are the main tools used by growers across the Canadian Prairies. Tolerant varieties are protected from wheat midge damage via an antibiosis response induced by larval feeding (Blake et al., 2014;Lamb et al., 2000). Chemical control of midge infestation in susceptible wheat cultivars includes application of the registered compounds, chlorpyrifos and dimethoate (Elliott, 1988a). Cultural control methods have had variable success. Ploughing does not affect overwintering mortality or emergence rates of wheat midge (Wallengren, 1937). Manipulation of seeding date could potentially shift the susceptible period (spike emergence) so that it occurs either earlier or later than midge emergence, causing “incoincidence”, yet wheat midges sometimes fly for up to 7 weeks and can still develop in secondary tillers (Basedow, 1972).

Occurrence and severity of wheat midge damage is strongly dependent on abiotic conditions. The degree of synchrony between wheat spike emergence and midge flight affects outbreak severity regardless of wheat midge density. Wheat midge outbreaks are still unpredictable to reliably forecast, as fecundity and parasitism are not well studied. We still do not fully understand the conditions that affect the phenology of wheat midge emergence and flight. Wheat midge phenology is variable across its large geographic range and has the potential to be different in the most northern part of its range in the Peace River region of Alberta ("WESTERN COMMITTEE ON CROP PESTS," 2011). To date, all studies in Canada have focused on Saskatchewan, Manitoba and southern Alberta. It is unclear how wheat midge biology is affected by the short growing season and long summer photoperiod that characterizes this northern region. Geographically, this region is characterized by rolling hills, abundant woodlands, and widespread clay loam soil, which differs from the geography of the southern prairies and could impact wheat midge biology.

1.1 Objectives

The objectives of this research were to determine aspects of wheat midge biology that are relevant to management in the Peace River region and to optimize available monitoring tools.

1. Determine the overwintering success of wheat midge in the Peace River region. (Chapter 2).
2. Determine the seasonal patterns of wheat midge emergence. (Chapter 2).
3. Determine the longevity of adult wheat midge from the Peace River region. (Chapter 2).
4. Determine the seasonal and diurnal patterns of wheat midge flight (Chapters 2 & 3).
5. Compare the effectiveness of different sex pheromone lures and trap colours for wheat midge capture. Relate capture of midge in pheromone-baited traps to wheat midge density in the Peace River region (Chapter 3).
6. Compare the effectiveness of different spatial distributions, shapes and sizes of yellow sticky cards for wheat midge capture. Determine whether wheat midge capture on sticky card traps can predict wheat midge density in the Peace River region (Chapter 3).

7. Validate the use of fall soil core samples to predict in-field populations of wheat midge the following year (Chapter 3).

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Chapter 2: Biology of *Sitodiplosis mosellana* Géhin (Diptera: Cecidomyiidae) in the Peace River region, Alberta

2.1 Abstract

Wheat midge, *Sitodiplosis mosellana* Géhin (Diptera: Cecidomyiidae), is an invasive pest of wheat in North America, and is found in all wheat growing regions of the world. Wheat midge biology, particularly post-diapause emergence of adults, varies with geographic region. The biology of wheat midge has not previously been examined in the northern-most portion of its range in Canada – the Peace River region of Alberta. The overwintering density and parasitism rate of wheat midge was examined in the field by rearing midge from field-collected soil. Mortality rates of overwintering wheat midge were determined by rearing larvae in soil held under different conditions: inside insulated outdoor cages, within a climate-controlled refrigerator or within a climate-controlled cabinet. In the field, patterns of wheat midge adult emergence were compared to different bioclimatic models of wheat midge emergence developed in other geographic regions. The longevity of newly eclosed, virgin females or females held with males was tested in the laboratory. Circadian patterns of wheat midge flight were tested by hourly counts of active females in commercial wheat fields. Overwintering wheat midge density varied among field sites and years, as did the number of midges remaining in extended diapause. Parasitism rates were high (>50%) overall. Rates of adult emergence were higher when soil was held under controlled conditions overwinter. In-field adult emergence did not match any of the published phenological models. In the Peace River region, adults emerged 300 GDD base 7 °C following a rainfall event. Multiple peaks of wheat midge emergence, up to 20 days apart, were observed. There was no difference in wheat midge adult longevity between females held individually or with a male. In the field, wheat midge flight peaked at twilight, which occurred later in the evening in the Peace River region than in other regions previously examined.

2.2 Introduction

Successful Integrated Pest Management (IPM) programs are based on an understanding of pest and host biology. Pest population dynamics affect sampling, control tactics and economic thresholds, which vary over time and with population fluctuations (Onstad 1987). The life stage(s) of the target pest that causes damage and is susceptible to control will influence selection of monitoring and control techniques. Timing of sample collections and control applications depends on insect phenology and patterns of activity – specifically when insect populations and susceptible stages are present (Chi 1990, Way and Van Emden 2000).

Insect phenology can be modelled over time based on temperature accumulation above a developmental threshold. Over intermediate temperatures, insect development tends to increase linearly with temperature and plateaus or decreases above or below an optimum range (Wagner et al. 1984). Models based on growing degree days (GDD), or the accumulated temperature above a base threshold, are important components of IPM programs for many pests in tree fruit (Riedl et al. 1976, Welch et al. 1978), crops (Esbjerg and Sigsgaard 2014, Hanson et al. 2015), and forest systems (Nealis and Régnière 2014). Phenological models can accurately predict developmental events, even if they can not model density (Welch et al. 1978). Models based on temperature, however, do not always accurately reflect conditions experienced by insects due to behavioural adaptations and microclimates, but over large temporal scales the inaccuracies are minimal (Herms 2004). For additional accuracy, some phenological models include other factors, such as photoperiod (Riedl and Croft 1978, Nielsen et al. 2016) or moisture (Tauber et al. 1998, Hodek 2003).

Many potential mechanisms can drive differences in insect pest biology across sympatric and allopatric populations. When pests are introduced to novel environments, they can experience reduced genetic diversity caused by bottleneck effects and genetic drift (Sakai et al. 2001, Allendorf and Lundquist 2003). Others experience no change or even an increase in genetic diversity. An increase in genetic or epistatic variation may occur due to multiple introduction events across different source populations, intra- or inter-specific hybridization, or gene by environment interactions (Lee 2002, Allendorf and Lundquist 2003). Environmental conditions can differ between geographic regions, and insects respond through local adaptations generated by selection pressure or due to fitness trade-offs (Stoks and de Block 2011). This encourages rapid evolution in invasive species (Sakai et al. 2001). Some pest species may be especially plastic in their reactions to temperature or seasonal cues such as photoperiod (Forrest 2016, Shearer et al. 2016).

Insects may respond to changes in environmental conditions through altered population dynamics. This can occur over a single season through increased growth rates or more generations. For some species, this occurs along a latitudinal cline due to predictable variation in growing season length or temperature (Beck and Apple 1961, Corbet et al. 2006, Zeuss et al. 2017). Many insect species have altered propensity for diapause induction with latitude. Different populations of insects within a species may enter diapause at longer critical day lengths (Riedl et al. 1976, Tauber et al. 1988) or have a different diapause incidence (Schmidt et al. 2014, Hou et al. 2016) in different locations. Voltinism can change independently of latitude due to interactions between temperature and photoperiod (Grevstad and Coop 2015) or due to variation in the availability of host or habitat resources (Blanckenhorn 1994, Corbet et al. 2006). Variation in emergence patterns are often due to both photoperiod and temperature, but some species exhibit local differences independent of these cues (Hodgson et al. 2011). Patterns of population growth and periodicity of outbreaks vary with geography due to habitat structure (Liebhold et al. 2006) or shifts in natural enemy complex composition (Klemola et al. 2002). Longevity, fecundity and development rate can differ among geographically isolated populations (Schmidt et al. 2014).

The wheat midge, *Sitodiplosis mosellana* Géhin (Diptera: Cecidomyiidae), is a major pest of wheat (*Triticum spp.*) in most wheat-growing regions of the world. The wheat midge was introduced to North America in 1828 to Québec, Canada (Felt 1921 cited in Olfert et al. 1985) and has since spread across North America (Knodel and Ganehiarachchi 2008, Olfert 2009). Originally palearctic in origin (Borkent 1989), wheat midge has spread to most wheat-growing regions including China (Miao et al. 2013), England (Bruce et al. 2007) and Europe (El-Wakeil et al. 2013). Wheat midge is univoltine across its range (Mukerji et al. 1988).

Wheat midge biology depends on environmental conditions. In Saskatchewan, wheat midge females fly and oviposit at sunset on warm nights when wind speeds are below 10 km/ hr (Pivnick and Labbé 1993). Wheat midge larvae feed on the exterior of kernels as they develop inside the floret (Gagné and Doane 1999) until the third larval instar when rain triggers them to leave the wheat head and drop to the soil (Mukerji et al. 1988). Wheat midge overwinters as a third instar larva within a cocoon in the soil (Gagné and Doane 1999). In the fall, overwintering larval cocoons are found mostly within the first 0-6 centimetres of soil, however, some cocoons occur as deep as 15 cm in the soil (Doane and Olfert 2008). There is an obligatory diapause period in the third larval instar of at least 70 days below 2 °C in North America (Hinks and Doane 1988) or 3-4 months below 10 °C in Germany (Basedow and Gillich 1982). In Germany, wheat midge larvae experience variable overwintering mortality that ranges from 0 to 64% (Basedow and Gillich 1982). Larvae break diapause and burrow to the soil surface to pupate. In western Canada, an average of 218 GDD above a threshold of 3 °C is required for larvae to break diapause (Doane and Olfert 2008) and 250 GDD above 3 °C in Belgium (Jacquemin et al. 2014). Larvae can remain in diapause for up to 13 years if moisture conditions are inadequate (Basedow and Gillich 1982, Wise and Lamb 2004, Cheng et al. 2017). Emergence of adult midge increases when overwintering larvae are exposed to between 20 and 50 % soil moisture (Cheng et al. 2017). Adult emergence after overwintering decreases at temperatures of below 10 °C (Wise and Lamb 2004) or above 30 °C (Cheng et al. 2017).

Multiple developmental models have been applied to different wheat midge populations. Models from Germany (Basedow and Gillich 1982), Finland (Kurppa 1989), England (Oakley et al.

1998), Belgium (Jacquemin et al. 2014), Manitoba (Wise and Lamb 2004), and Saskatchewan (Elliott et al. 2009) are in the scientific literature (Table 2.1). Different base temperatures used in the various models range from 5 °C to 9 °C (Table 2.1). Base temperatures for most models have been determined by best fit to field data, rather than from empirically tested thresholds for development. Most of the models predict adult emergence with degree day accumulation over the growing season, including the Elliott model (Elliott et al. 2009). The Jacquemin model describes wheat midge adult emergence as "waves" that occur a set number of degree days after inductive rainfall events (Jacquemin et al. 2014). All European emergence models predict earlier adult wheat midge emergence than the Canadian models (Table 2.1).

Wheat midge emergence coincides with susceptible stages of wheat, which also varies across its range. Wheat midge adults typically emerge in mid-June in Europe (Basedow and Gillich 1982, Oakley et al. 1998, Jacquemin et al. 2014), late April to early May in China (Cheng et al. 2017), and early to mid July in Canada (Lamb et al. 1999, Elliott et al. 2009). In their native range, wheat midge infests winter-sown wheat (El-Wakeil et al. 2013). In North America, infestation occurs mainly in spring-sown wheat (Dexter et al. 1987), which emerges and flowers later in the season than winter wheat.

Table 2.1. Available phenological models of wheat midge emergence in the literature.

Model	*Region*	*Event*	*Requirements*	*GDD base*
(Elliott et al. 2009)	Saskatchewan	10% adult emergence	693 GDD since March 1	Base 5 °C
		50% adult emergence	784 GDD since March 1	Base 5 °C
		90% adult emergence	874 GDD since March 1	Base 5 °C
(Jacquemin et al. 2014)	Belgium	Pupation	250 GDD	Base 3 °C
		Adult emergence wave	Inductive rainfall event then 170 GDD	Base 7 °C
(Basedow and Gillich 1982)	Germany	Adult emergence	450 GDD	Base 6 °C
(Kurppa 1989)	Finland	Adult emergence	400 GDD	Base 5°C

Several species of parasitoid utilize wheat midge as a host, and adult emergence of parasitoids varies among species. All parasitoids reported from wheat midge hosts are ovolarval endoparasitoids (Affolter 1990). Three parasitoids, all native to the Palearctic, have been reported to use wheat midge as a host in Canada. *Macroglenes penetrans* Kirby (Hymenoptera: Pteromalidae) is a specialist ovolarval parasitoid of wheat midge, with tightly synchronized phenology with wheat midge. *Macroglenes penetrans* frequently undergoes prolonged diapause of 2 years (Wise and Lamb 2004). Adult emergence of *M. penetrans* varies between 3-9 days later than its host, although a range of 3-5 days is the most commonly cited (Affolter 1990, Olfert et al. 2003, Doane and Olfert 2008, Elliott et al. 2011, Chavalle, Buhl, et al. 2015). The same rainfall or moisture requirements reported for wheat midge exist in *M. penetrans* (Elliott et al. 2011, Chavalle, Buhl, et al. 2015). Wise and Lamb (2004) suggest that *M. penetrans* adults die if they emerge before the midge larva leaves the cocoon. The 2 other parasitoid species reported from wheat midge in Canada, *Platygaster tuberosula* Kieffer (Hymenoptera: Platygastridae), and *Euxestonotus error* Fitch (Hymenoptera:

Platygastridae) have both been reported as either well-synchronized with wheat midge (Affolter 1990), or poorly synchronized with wheat midge (Olfert et al. 2003, Chavalle, Buhl, et al. 2015). The variation in reported synchrony is likely because *P. tuberosula* and *E. error* do not appear to have the same dependence on soil moisture for adult emergence (Chavalle, Buhl, et al. 2015).

Wheat midge biology has not been examined in the northernmost part of its North American range, the Peace River region of Alberta, Canada. The developmental model used in Saskatchewan does not appear to accurately fit patterns of midge emergence observed in this region (Jennifer Otani – pers. Comm.). Additionally, rainfall as an inductive event that results in "waves" of wheat midge emergence has not been tested in Canada. The impact of the environment associated with latitudes of 55 °N and greater on wheat midge overwintering mortality rates, longevity, and seasonal and daily activity patterns have not been examined.

2.2.1 Objectives:

1. Determine the overwintering success of wheat midge in the Peace River region and examine methods of overwintering rearing.
2. Determine the seasonal patterns of wheat midge adult emergence.
3. Determine the longevity of adult wheat midge from the Peace River region.
4. Determine the seasonal and diurnal patterns of wheat midge flight.

2.3 Methods

2.3.1 Overwinter wheat midge density and parasitism

In-field density and parasitism rate of overwintering wheat midge was assessed by rearing overwintering specimens from soil collected at commercial fields. Midge-infested soil was collected in the spring of 2016 and 2017 from sites seeded to midge-susceptible wheat the previous year (Appendix 2.1, Table 2.2). In 2016, soil was collected using both a soil corer and spade, whereas in

2017 only spade-collected soil was used. Soil core samples were initially chosen for congruency with the fall soil core survey conducted each year by Alberta Agriculture and Forestry (https://www1.agric.gov.ab.ca/ $department/deptdocs.nsf/all/prm16761). In May 2016 at each site, a total of 68 cores (each 3 cm diameter x 15 cm deep) were collected using a steel soil-corer. The cores were collected 20 m apart along an “M” pattern beginning 20 m into the field from the edge (Figure 2.1). This pattern was repeated 4 times along the western field margin (Figure 2.1). Soil was collected using a spade from 2 sites in 2016 in an effort to maximize the sample size of live overwintering cocoons to rear. Since no midges emerged from soil core samples, only spade-collected samples were used in 2017. Soil was collected using a spade from 2 sites in June 2016 and 5 sites in May 2017 (Appendix 2.1, Table 2.2). In 2016, the top 2.5 cm of soil in a single 1-m-long by 13-cm-wide sample (1300 cm^2) was collected 1 m into the field from the margin. In 2017, 4 samples each with the same depth, width and length as above were collected from each field. Two samples were collected along the field margin and two samples were collected 80 m into the field from the margin.

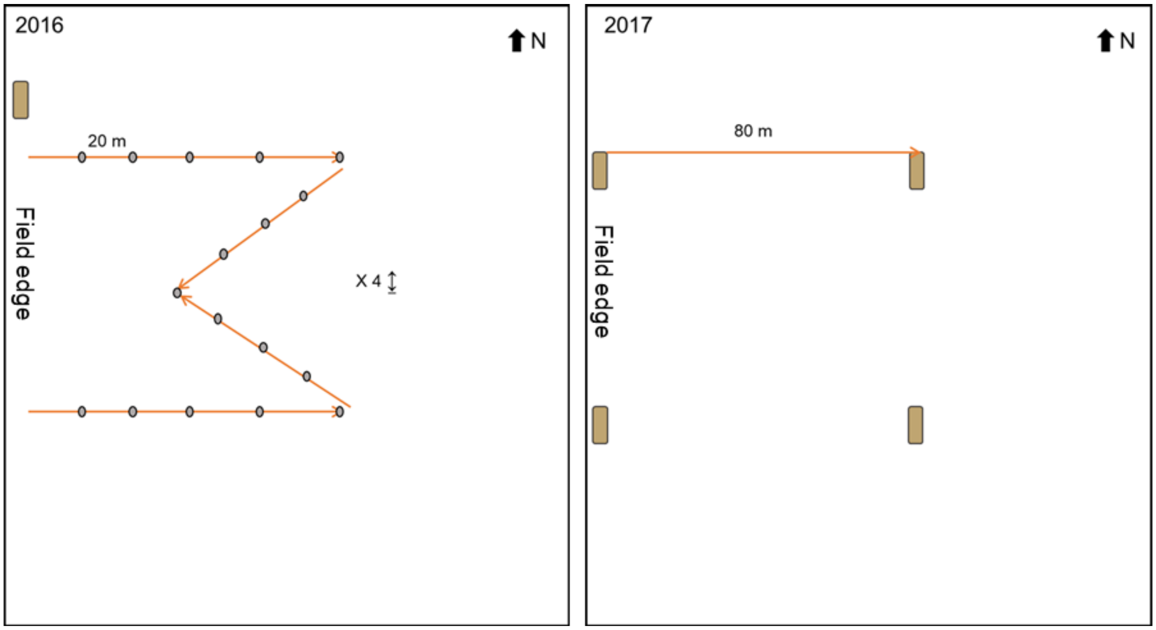

Figure 2.1 Sampling patterns used to collect overwintering wheat midge cocoons from commercial field sites. In 2016, soil was collected both as soil cores and using a spade. N=17 soil cores were collected every 20 m (brown circles) in an "M" pattern. This pattern was repeated 4 times along the western field margin. One 1 m long by 13 cm wide by 2.5 cm deep strip (brown box in figure) was collected from the field margin in 2016. In 2017, the same volume of soil was collected using a spade 4 times in each field: twice along the field margin and twice 80 m into the field.

Samples of soil were housed in 4L ventilated plastic pails. Pails retained soil to a depth of no more than 15 cm (Figure 2.2). Buckets were held indoors at ambient room temperature (20.34 ± 8.63 °C). Soil was moistened using reverse osmosis (RO) water three times weekly. Pails were checked daily for adult midge emergence from the soil throughout the summer growing season, then all soil was wet sieved (Doane et al. 1987) in the fall. Soil was washed through 3 progressively smaller sieves (0.5 cm diameter, 0.25 cm diameter, and finally 1 mm diameter). Organic material retained in the final screen was sorted and cocoons were opened to determine the number of live larvae that remained in extended diapause. Deceased larvae were visibly desiccated.

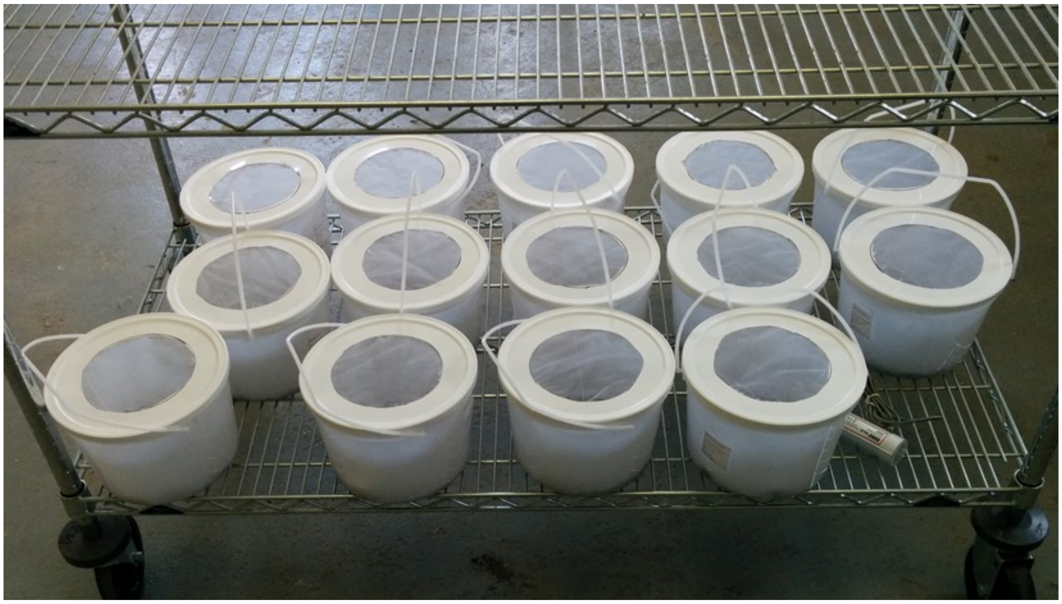

Figure 2.2 Rearing technique to determine wheat midge density and parasitism rate. Soil was collected by spade or n=68 soil cores per site collected from wheat stubble and housed in plastic rearing pails and checked daily for adult midge emergence.

2.3.2 Overwinter wheat midge mortality

Overwinter mortality using different rearing techniques was assessed using larvae collected at 5 sites (Appendix 2.1, Table 2.3). Wheat heads containing larvae were collected in August 2016 when wheat was between early milk and early dough stages (Zadoks et al. 1974). At each site, samples were collected along three North-South transects: along the field margin, and 20 and 50 m into the field from the margin. Eight samples of 50 heads each were collected 50 m apart along each transect (Figure 2.3). Twenty-four samples were collected at each field. Wheat stems were clipped 10-20 cm below the heads and placed into paper bags for transport to the laboratory in a refrigerated container.

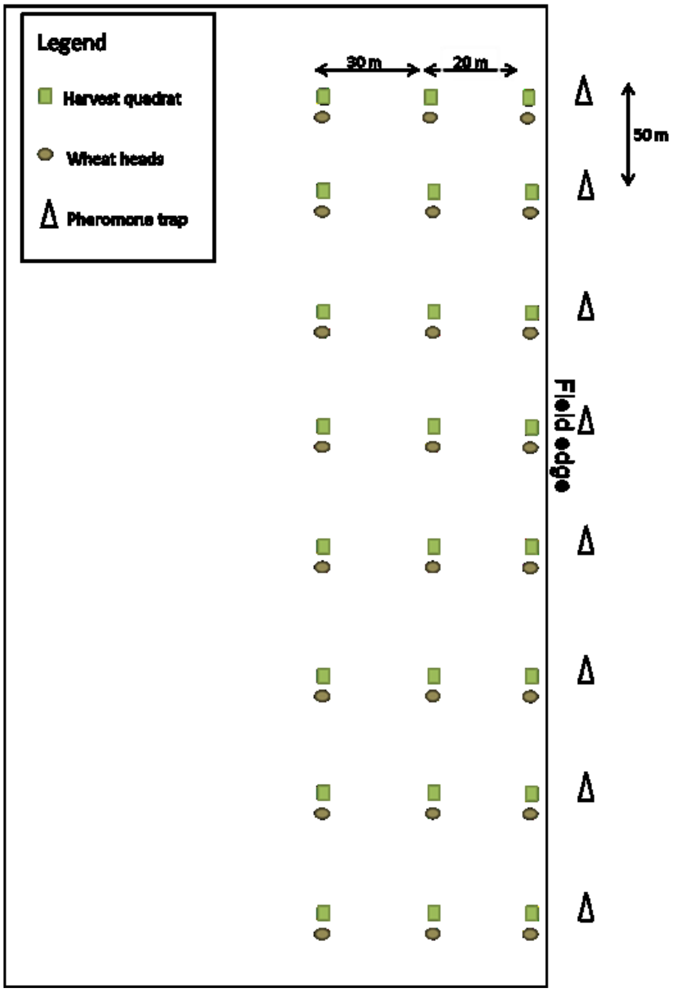

Figure 2.3 Sampling design for collection of wheat head samples from commercial fields. Wheat heads were collected along three North-South transects in each field: along the edge, and 20 and 50 m into the field from the margin. Wheat midge larvae were collected from wheat head samples and reared.

Five heads from each sample were dissected and the number of larvae in each spikelet was recorded. The remaining 45 heads were broken apart using a single-head thresher (Almaco specialized equipment model SV SRE-2). The thresher air was turned off and vents were covered to retain larvae. Samples were hand sorted and the number of larvae per sample counted.

A total of 3986 collected wheat midge larvae were reared under various overwintering conditions from 24 September 2016 until 29 April 2017. Larvae were placed in 20-30 g of autoclaved soil in 59.1 mL Solo™ cups. The soil was obtained from fields located at Agriculture and

Agrifoods Canada (AAFC) – Beaverlodge (55.19, -119.40). Soil was maintained at 17% moisture weight/weight by applying RO water, as needed, and was checked three times weekly. Mould was controlled with applications of 0.1% methyl paraben solution, as needed. Larvae were reared individually (n=324 midges) or at densities of 7 to 111 individuals per cup, depending on the number of larvae present in wheat head samples as they were processed.

Larvae were assigned to one of three temperature regimes for overwintering: 1) cups held outdoors inside cardboard boxes (25 cmx 22 cm x 41 cm and 28 cm x 33 cm x 25 cm) filled with shredded paper within cardboard boxes within a mesh cage insulated with wheat straw (1 m x 3 m x 1 m, constructed of wood and mesh) located within a stand of spruce trees at AAFC-Beaverlodge (mean temperature inside insulation of -1.33 °C ± 3.301 SD) (Figure 2.4); 2) cups held in a walk-in refrigerator (mean temperature of 2.42 °C ± 0.173 SD) inside cardboard boxes (25 cmx 22 cm x 41 cm and 28 cm x 33 cm x 25 cm) filled with shredded paper; or 3) cups positioned in a climate-controlled growth cabinet (mean temperature of 2.65 °C± 0.277 SD). Except for one site, 60-70% of larvae collected from all field sites were placed in the walk-in refrigerator and 17-35% were placed in the climate-controlled growth cabinet. A total of 2019 larvae were collected from the Jean Cote site, and 1542 of those were placed in the outdoor overwintering cages. All overwintering regimes were unlit. Soil was moistened every three weeks, as needed, throughout the overwintering period. Movement of the cups containing larvae from the outdoors or refrigerator indoors to check for moisture and mould resulted in exposure to temperatures up to 22.0 °C for less than one hour, 4 times during overwintering. Larvae in cups maintained in the growth cabinet experienced exposure to temperatures up to 14.4 °C for less than half an hour, 4 times during overwintering. The temperature was recorded every fifteen minutes in each rearing location using Onset ™ Tidbit v2 data loggers.

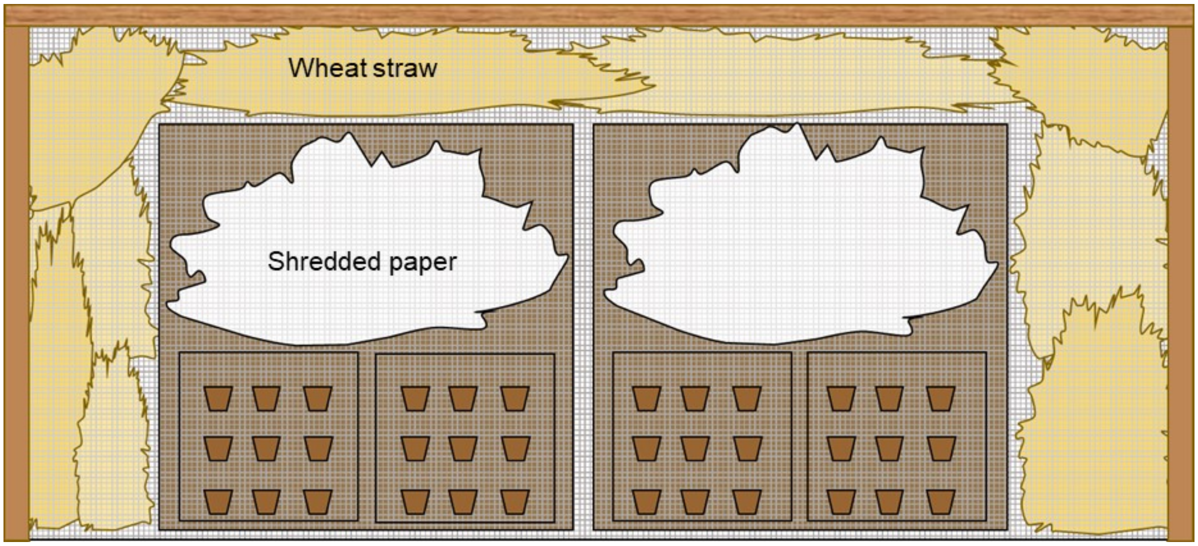

Figure 2.4 Outdoor overwintering cage set up. Cups of soil containing wheat midge larvae were placed inside cardboard boxes (25cm x 22 cm x 52 cm or 28 cm x 33 cm x 25 cm) filled with shredded paper, inside larger cardboard boxes (50 cm x 50cm x 75 cm) also filled with shredded paper. These boxes were held in wood and mesh cages (1 m x 2 m x 1 m) filled with wheat straw. Cages were placed in a stand of spruce trees at AAFC-Beaverlodge.

In April 2017, larvae were moved indoors to encourage adult emergence. Containers were maintained under natural ambient light and temperature (19.69 °C ± 1.216 SD). Soil moisture was maintained at 17.5 % w/w by applying RO water, as needed, three times weekly. Containers were checked twice daily for wheat midge adults and parasitoids. Parasitoids were stored in 95% ethanol.

2.3.3 Statistical analyses for overwinter wheat midge mortality

All analyses were done in R studio 3.13 (R development team 2016). All Linear mixed models were fit with package lme4 (Bates et al. 2015), post-hoc analyses were done with package multcomp (Hothorn et al. 2008), and Wald Chi-square tests were done with package car (Fox and Weisberg 2011). Assumptions of normality and heteroscedasticity were tested visually by examining q-q plots, residual plots, and by conducting Shapiro-Wilks tests on model residuals. Distributions of data were examined visually using histograms and q-q plots against a normal distribution, and different model variance structures were evaluated by comparing AIC values and determinations of residual normality. Non-significant interactions were removed from models if anova comparisons between models with and without the interaction were not significant.

Midge and parasitoid emergence rates among different overwintering treatments were compared with a generalized linear mixed model using lme4 (Bates et al. 2014). Overwintering treatment was a fixed factor, and random intercepts were modelled for different collection sites. Total counts were converted to rates of emerged insects by including an offset of the log of the number of insects originally placed into rearing in the model, as in O'Hara and Kotze (2010). The log value was included, as a Poisson or negative binomial regression model: $\log(y) = x + \log(e)$ simplifies to $\log(y/e) = x$. Data was modelled with a Poisson distribution with a log link. The effect of treatment was determined with a type II Wald Chi-square test.

2.3.4 Wheat midge emergence

The timing of adult wheat midge and parasitoid emergence was tested *in situ* to determine the ability of the existing bioclimatic models to predict midge emergence in the Peace River region. The experiment was conducted at sites seeded to a midge-susceptible wheat cultivar the previous year (Appendix 2.2, Table 2.5). Monitoring was conducted between 20 June – 05 August at 7 sites in 2016, and between 06 June – 09 August at 5 sites in 2017 (Appendix 2.2, Table 2.5).

Emergence timing was determined using plastic emergence traps constructed from 2 L plastic pails (Plastipak™) with the bottoms cut off (Doane and Olfert 2008). Ventilation holes lined with fine mesh were cut in the sides of each bucket (3x 2.54 cm in diameter) and in the lid (1x 5.08 cm in diameter). Each individual trap sampled 174.37 cm^2 of the soil surface. A single clear polyethylene strip coated with Stikem special™ (Scotts™ Canada) (14 cm x 5 cm sticky surface) was secured under the lid of each trap to capture emerging wheat midge adults and parasitoids (Figure 2.5). Traps were placed with the bottom 7.6 cm of each bucket buried in the soil. Traps were positioned 10 m apart along a north-south linear transect within the field along the first row of wheat stubble from the margin. In 2016, 8 traps were placed in each field. Due to low midge capture in emergence traps in 2016, 20 traps were placed in each field in 2017.

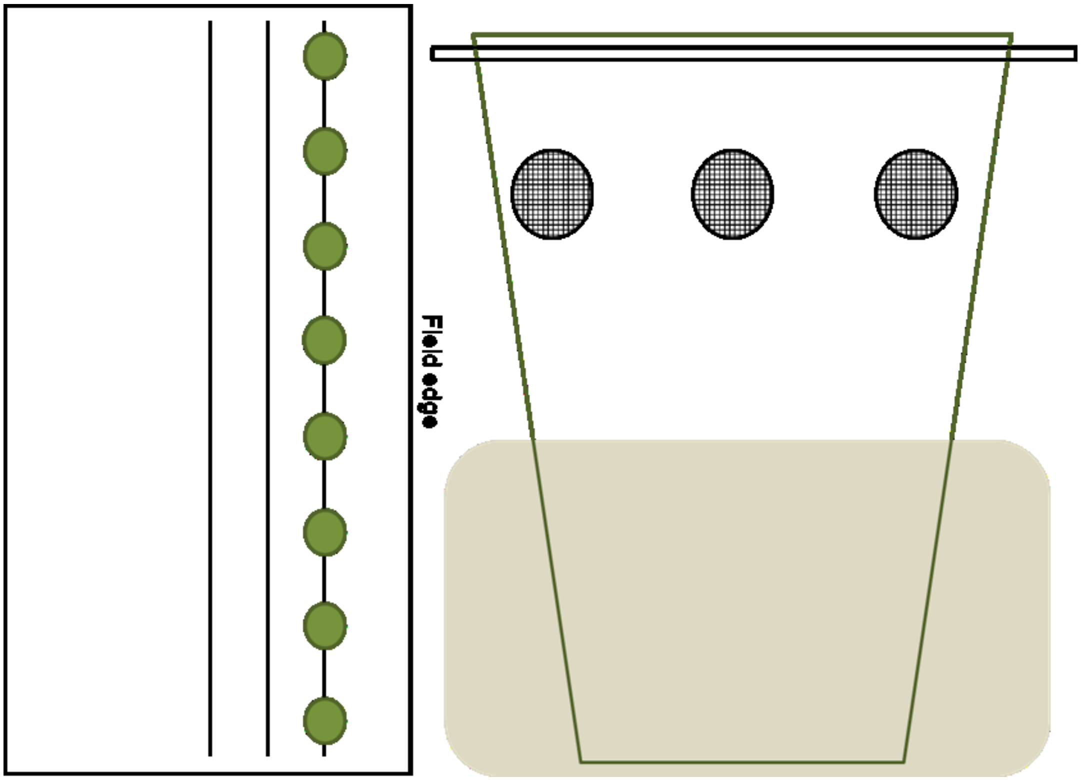

Figure 2.5A. In-field emergence trap experimental design. Green circles represent emergence traps positioned along field edge, 10 m apart. Vertical lines represent rows of wheat stubble. B. Emergence trap, showing bottom portion of the bucket buried within the soil and position of ventilation holes.

Collection strips were removed and replaced every 2-7 days during the flight period. Strips were examined under a dissecting stereomicroscope (Zeiss™ stemi-C) at 8x magnification and wheat midge adults were identified based on antennal morphology (Harris 1966), counted and separated by sex. Specimens of the wheat midge parasitoid *Macroglenes penetrans* Kirby (Hymenoptera: Pteromalidae) were identified using Gibson et al. (1997) and by referring to voucher specimens.

Soil temperature was recorded 1 cm below the soil surface with Onset ™ Tidbit v2 data loggers every fifteen minutes from 13 May until 05 August in 2016, 11 May until 14 August in 2017, and from 08 May until 09 August in 2018. Air temperature was recorded 1 m above the ground with Onset ™ Tidbit v2 data loggers inside Onset RS1 solar shields (Hoskins Scientific™, Unit 100, 18138 105th Avenue Edmonton, AB). In 2018, air temperature was collected from 08 May until 09 August, and from 30 May until 14 August in 2017. In 2016, only soil temperature was recorded. Accumulated rainfall was recorded with Onset™ plastic rain gauges (Hoskins Scientific™, Unit 100, 18138 105th Avenue Edmonton, AB) approximately weekly during the same periods.

2.3.5 Statistical analysis for wheat midge emergence

To test for agreement between dates of adult emergence predicted by the various published models and those observed in this study, observed dates were regressed on predicted dates (Piñeiro et al. 2008). Day of year (DOY) was used in the analyses rather than GDD values in order to include variation in predicted emergence among sites, which was necessary to analyze model fit through a regression approach.

To determine predicted DOY of emergence, dates of GDD accumulation required for 10%, 50% emergence (Elliott model) and for emergence events (Jacquemin model) were determined. GDD values with a base of 3 °C, 5 °C, and 7 °C were calculated from maximum and minimum temperature values using a modified sine-wave method (Allen 1975), and half-day intervals. For the Elliott model, dates of 693 and 784 GDD base 5 °C accumulation were determined for each site. To determine the observed dates of 10 and 50% emergence, cumulative percent of total female emergence (in 2016) and male and female emergence (in 2017 and 2018) was calculated for each site. The dates of 10 and 50 % emergence were estimated by linear interpolation using the approx. function. For the Jacquemin model, dates of accumulated 160 GDD (base 7 °C) after the first and second rainfall events after reaching 230 GDD (base 3 °C) were determined for each site. Dates of peaks of emergence were determined for all sites. Sites with less than 10 midges collected during the season were excluded from analysis. Calendar dates were converted to day of year (DOY) format by subtracting the date from a date stamp of December 31 the year previous.

Linear mixed models were run with lme4 (Bates et al. 2014). Separate models were run for predicted dates determined from soil or air temperature, and for different emergence events (10 and 50% cumulative emergence, or separate emergence peaks) (Appendix 2.2, Table 2.6). For all models, observed DOY was modelled with predicted DOY as a fixed factor, and with year as a random factor. Marginal r^2 values were calculated as in (Nakagawa and Schielzeth 2013) using the MuMIN package (Barton, 2018). To determine whether the predicted DOY significantly impacted model fit, likelihood ratio tests (using the anova function) were conducted between models containing both fixed and random effects (Appendix 2.2, Table 2.6) to models containing only the random effect. Both model accuracy (differences between model prediction and observed values as determined by deviation of regression line of best fit slope from 45 °) and model precision (residual variation between values and the regression line of best fit) were summarized by calculating concordance correlation coefficients (CCC). CCC values can range from -1, indicating perfect disagreement between predicted and observed values, and +1 which indicates perfect agreement between predicted and observed values. CCC values were determined using variance components using the CCRM package (Carrasco and Jover 2003). Interclass correlation coefficients were determined using the psy package (Falissard 2012) for models that would not run using the CCRM package.

2.3.6 Wheat midge longevity

The longevity of adult wheat midges was measured in both 2016 and 2017. In 2016, adults were collected from the soil used in section 1.1 above. Adults were reared in 29.57 mL plastic Solo™ cups under natural ambient light and temperature (20.34 °C ±8.63 SD). Adults were provided 10% (w/w) sugar solution via a cotton wick. Adults were checked daily and the date of death was recorded. The sugar wick caused high mortality in 2016, so data from this experiment were not analyzed.

In 2017, adult wheat midges were collected alive from both field collected soil and from overwintered material (described in section 2.3.2). Adult wheat midges were collected alive then separated by sex. Adults were maintained in plastic 73.93 mL snap-cap vials sealed with foam plugs under natural ambient light and temperature (19.69 °C ± 1.216 SD). Each midge was provided with a single excised wheat head (CVC Roblin) with the cut end submerged in a tray of water for humidity. To test the effect of mating on longevity, adults were maintained either individually (n=67) or in male-female pairs (n=33). Midges were checked twice daily until the caged individuals died. Longevity of unpaired females was compared to females paired with male midges with a student's t-test.

2.3.8 Wheat midge flight

Nocturnal patterns of wheat midge flight were assessed in 2016 and 2017. The number of active adults on wheat heads was counted in commercial fields. One barley and two wheat fields were monitored in 2016 and five wheat fields were monitored in 2017 (Appendix 2.1, Table 2.7).

Counts were done every hour over 2 consecutive nights twice in the season in 2016 and over 1-2 nights in 2017 (Appendix 2.1, Table 2.7). Counts were done after wheat midges were detected on pheromone traps at field sites. The number of female adult midges per 10 heads was counted by one of two observers at five locations in each commercial field. Three counts were done at the field edge (10 m apart) while 2 count locations were within the field (20 m into the field from the margin, 20 m apart). Counts were done under headlamp illumination when it became too dark to observe midges. Use of the headlamp did not appear to influence midge activity.

2.4 Results

2.4.1 Overwinter wheat midge density and parasitism

More midges and parasitoids emerged from field-collected soil in 2016 compared to 2017 (Figures 2.6 & 2.7). In 2016, emergence rates ranged from 30 to 80 %. In 2017, emergence rates varied from 0 to 100 %, but most field sites had between 5 and 25% emergence. There was no emergence of either midge or parasitoids from spring-collected soil core samples, and up to 52 cocoons remained in extended diapause in soil samples after 1 field season.

In both 2016 and 2017, rates of parasitism, as measured by adult parasitoid or midge emergence, were above 50% overall (Figures 2.6 & 2.7). In 2016, parasitism rates were similar at both field sites (Figure 2.6). In 2017, parasitism rates varied from 0% to 100% (Figure 2.7), but very few insects emerged overall.

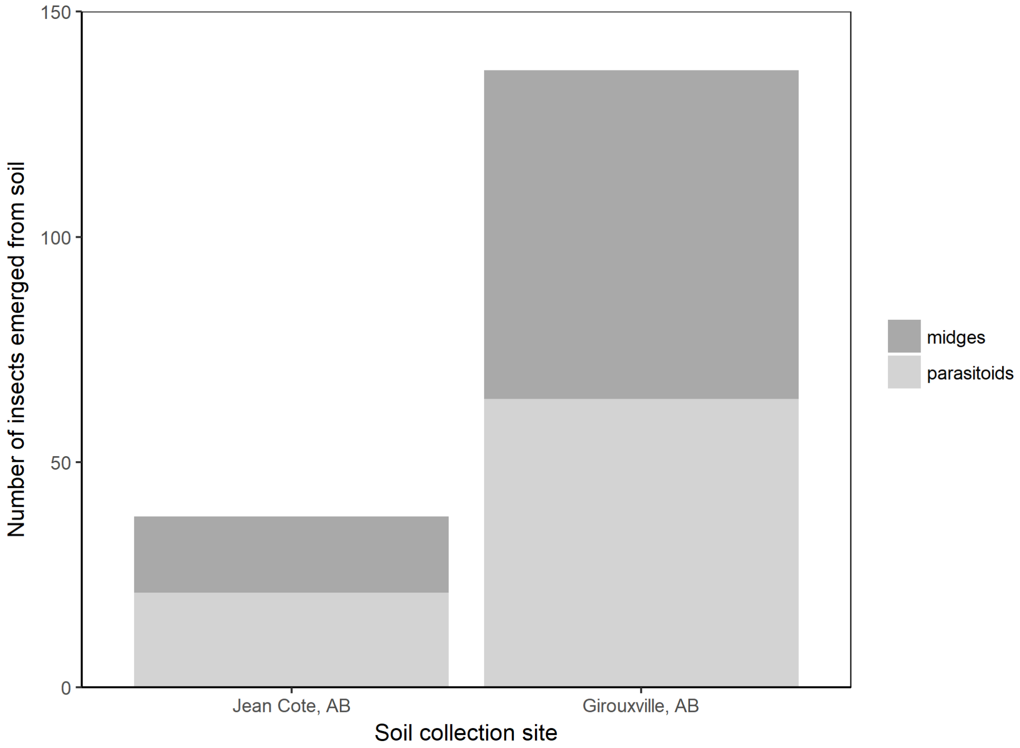

*Figure 2.6 Emergence of wheat midge adults and parasitoid wasps (*M. penetrans*) from soil collected by spade from field sites (1 sample of 1 m x 13 cm x 2.54 cm deep collected at n=2 sites) in June 2016 and maintained in the lab. Soil was moistened three times weekly and emergence was checked daily.*

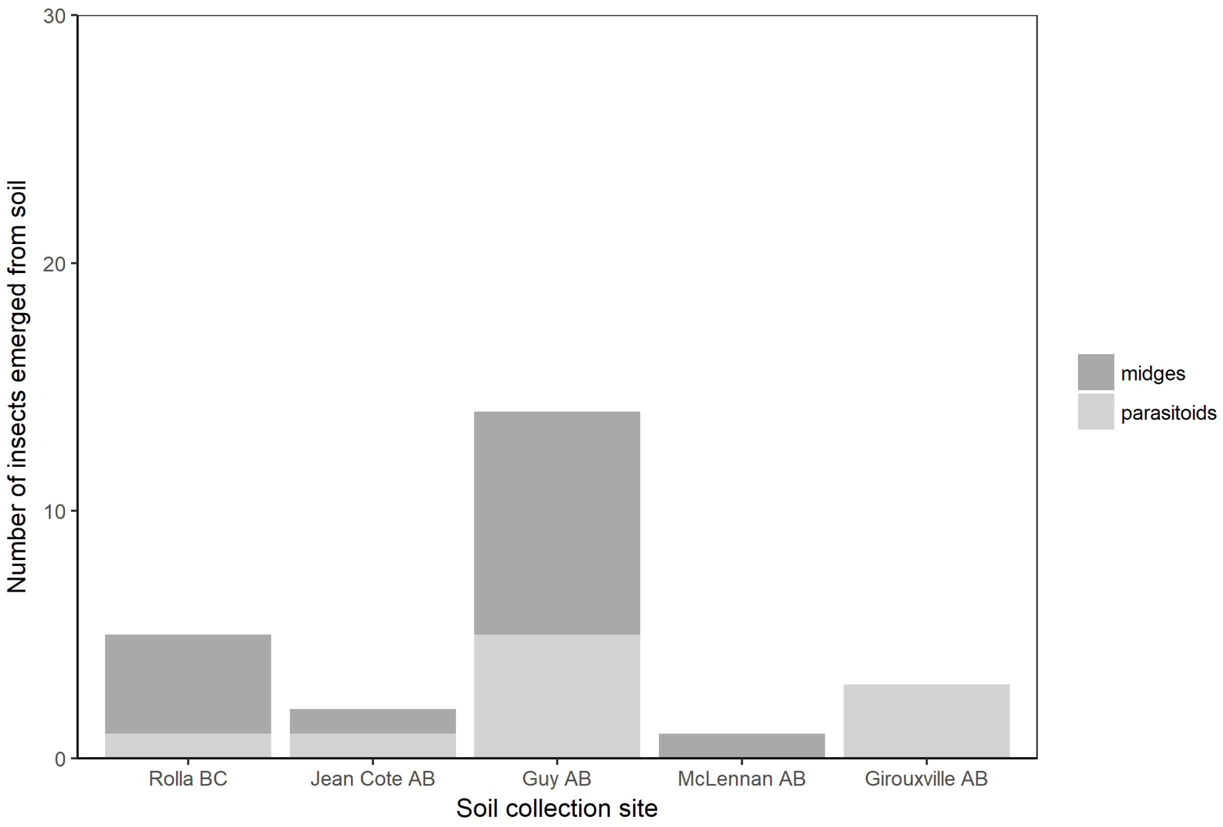

*Figure 2.7 Emergence of wheat midge adults and parasitoid wasps (*M. penetrans*) from soil collected by spade from field sites (4 samples of 1 m x 13 cm x 2.54 cm deep in a square pattern at n=5 sites) in May 2017 and maintained in the lab. Soil was moistened three times weekly and emergence was checked daily. Dark gray portions of bars represent midge emergence and light gray portions represent parasitoid emergence.*

2.4.2 Overwinter wheat midge mortality

The rate of overall insect emergence depended on overwintering conditions (Figure 2.8, χ^2= 105.39, df=2, p<0.001). The rate of emergence was higher for larvae reared in the walk-in refrigerator and in a climate-controlled growth cabinet than for larvae reared outdoors in insulated, cardboard boxes (Figure 2.4). There was no difference in the rate of emergence between the two controlled-climate conditions (Figure 2.8). Emergence rates varied between 0 and 100 % (Figure 2.8).

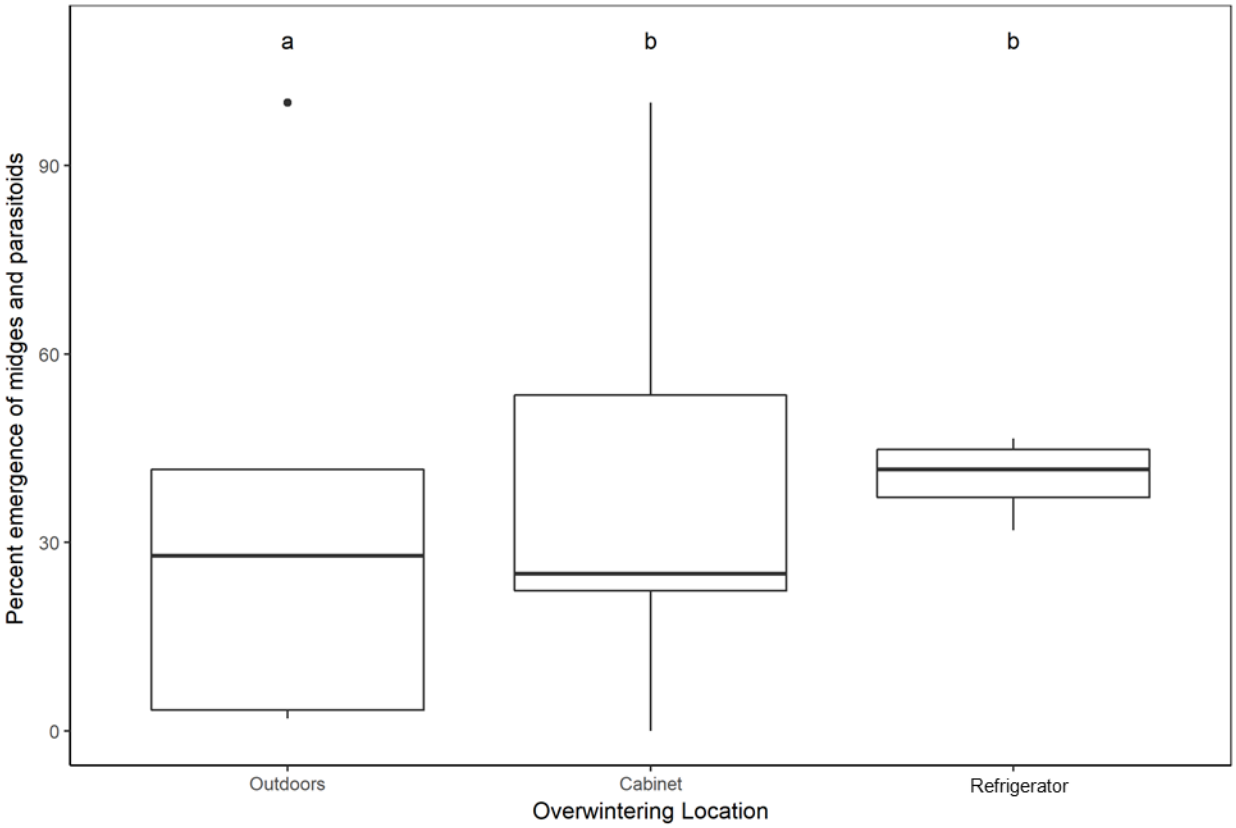

*Figure 2.8 Emergence of wheat midge adults and parasitoid wasps (*M. penetrans*) from different overwintering conditions. Wheat midge larvae were collected from wheat heads August 2016 and placed in autoclaved soil (17.5% moisture) and placed in either: in outdoor cages insulated with straw (n=2067 midges), a climate controlled-cabinet (n=507 midges), a walk-in refrigerator (n=1868 midges) or. The dark midline represents the median and the bottom and top of the box indicate the first and third quartiles, respectively. The vertical lines, or whiskers, indicate the maximum value or 1.5 times the interquartile range, whichever is smaller. Outliers are represented by points above the whiskers. Letters represent significant differences among groups (Post-hoc Tukey test, α=0.05)*

Parasitism rates varied between 50 and 81 % (Figure 2.9), with mean parasitism of 62%. The only parasitoid species collected was *M. penetrans*.

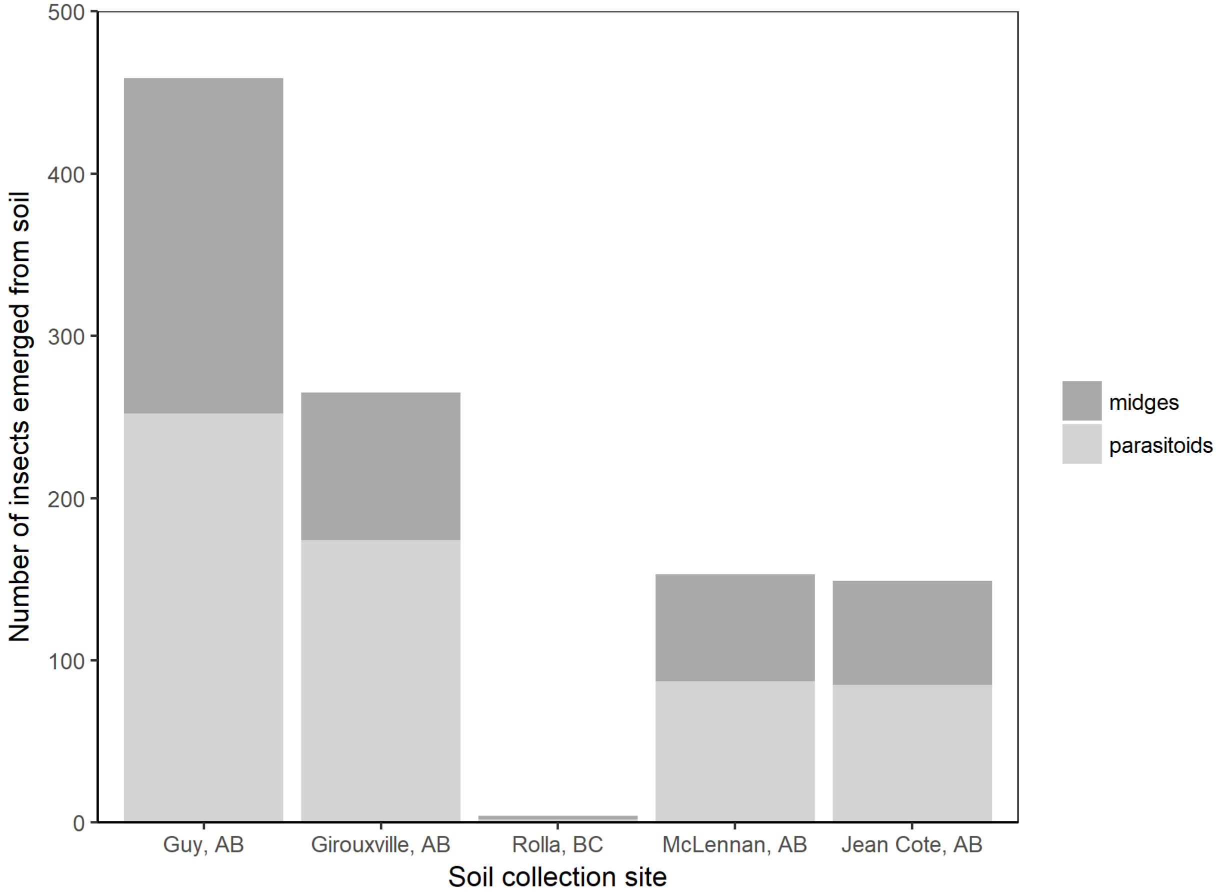

*Figure 2.9 Emergence of wheat midge adults and parasitoid wasps (*M. penetrans*) pooled across different overwintering conditions by field site. Wheat midge larvae were collected from wheat heads August 2016 and placed in autoclaved soil (17.5% moisture) and placed in either: a climate controlled-cabinet (n=507 midges), a walk-in refrigerator (n=1868 midges) or in outdoor cages insulated with straw (n=2067 midges). Dark gray portions of bars represent midge emergence and light gray portions represent parasitoid emergence.*

Midge adults reared in climate-controlled conditions emerged earlier than predicted by the Elliott model. Midge emergence to 10% and 50% occurred 13.87 and 20.61 days earlier, respectively, than predicted by the Elliott model (Table 2.8). Wheat midge adults had two peaks of emergence (Figure 2.10). The first and second peaks of emergence occurred 5 and 4 days later, respectively, than predicted by the Jacquemin model (Table 2.8). The first and second peaks occurred at 234 and 222 GDD (base 7 °C) (Table 2.8).

Table 2.8 Comparison of wheat midge adult emergence in controlled conditions to those predicted under published models (Elliott et al. 2009, Jacquemin et al. 2014). Wheat midge larvae were collected from wheat heads in August 2016 and placed in autoclaved soil (17.5% moisture) and placed in either: a climate controlled-cabinet (n=507 midges), a walk-in refrigerator (n=1868 midges) or in outdoor cages insulated with straw (n=2067 midges). Air temperature was recorded every 15 minutes.

Model	**Base temperature (°C)**	**Difference between predicted date and observed date (DOY)**	**mean GDD**
Elliott 10%	5	13.87	491.8
Elliott 50%	5	20.61	492.3
Jacquemin 1st peak	7	-5	234
Jacquemin 2nd peak	7	-4	222

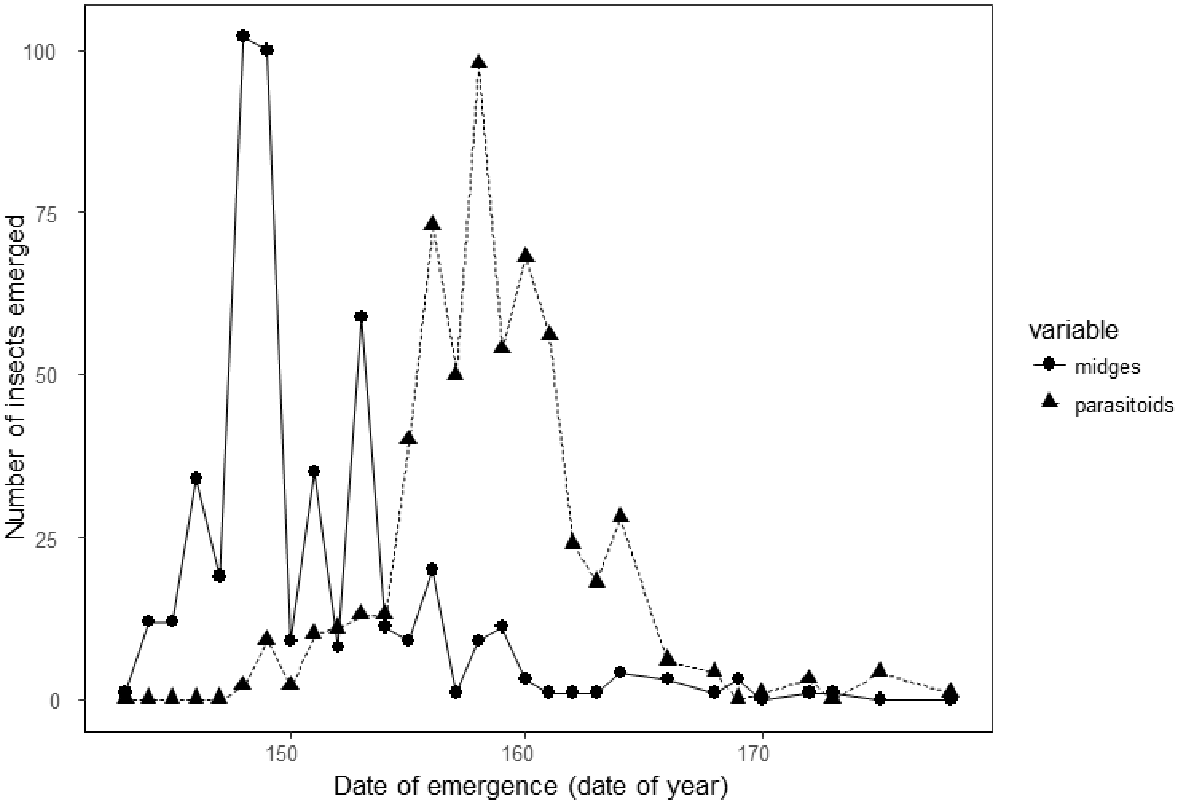

Figure 2.10 Emergence of wheat midge adults and parasitoid wasps (M. penetrans) in controlled conditions over the rearing period. Wheat midge larvae were collected from wheat heads in August 2016 and placed in autoclaved soil (17.5% moisture) and placed in either: a climate controlled-cabinet (n=507 midges), a walk-in refrigerator (n=1868 midges) or in outdoor cages insulated with straw (n=2067 midges). Circles and solid lines represent wheat midge emergence, while triangles and dotted lines represent parasitoid wasp emergence.

2.4.3 Wheat midge emergence

The Elliott model (Elliott et al. 2009) was better at predicting early and mid-season wheat midge adult emergence in the field than the Jacquemin model (Jacquemin et al. 2014), using either air or soil temperature (Table 2.9). An input of air temperature into the Elliott model more accurately predicted early and mid-season adult emergence than an input of soil temperature input. The mean differences between the predicted and observed values were smaller using air temperature. Predictions using soil temperature, however, had higher precision (r^2=0.15 and

0.242) than predictions using air temperature (r^2= 8.46e-5 and 0.009), with values more tightly clustered around the regression line (Figure 2.11). Likelihood ratio tests comparing predictions of emergence dates using soil temperature to null models showed that the relationship between predicted and observed dates was marginally significant for both 10% (χ^2=3.146, df= 1, p= 0.076) and 50% (χ^2= 3.761, df=1, p=0.052) emergence. Models of observed emergence as a function of predictions of emergence date using air temperature were not significantly different from null models for 10% (χ^2=2.11, df=1, p=0.147) or 50% (χ^2=0.082, df=1, p=0.774) emergence. However, using soil temperatures the models consistently predicted earlier emergence than what was observed in the field (Table 2.9). The Elliott model using air temperature predicted 10% emergence that was earlier than observed in 2017 and later than observed in 2018, which caused low overall model precision (Figure 2.11). Models outputs based on soil temperature inputs consistently predicted emergence earlier than it occurred (Table 2.9), and predictions were more consistent between years than with air temperature (Figure 2.11), resulting in more precise models (Table 2.9). Deviation between predicted and observed values was closest for the Elliott model for 50% using air temperature. Mean 50% emergence was 3.97 days later than predicted (SD=4.36) when comparing the observed conditions to the Elliott model. Concordance Correlation Coefficients were low for all models, and all 95% Confidence intervals included 0 (Table 2.9), indicating that agreement between observed and predicted values was low for all models.

Table 2.9 Comparison of wheat midge adult emergence under field conditions to those predicted using published models (Elliott et al. 2009, Jacquemin et al. 2014). Emergence was determined by presence of wheat midge adults on sticky strips placed in plastic emergence bucket traps. Traps (n= 8 per field in 2016, 20 per field in 2017 and 2018) were spaced 10 m apart in the first rows of wheat stubble in fields (n=4 in 2016, 2017 and 2018). Traps were checked 3 times weekly. Temperature was recorded every 15 minutes with Tidbit Hobo™ data loggers at each site. Soil temperature was recorded 2 cm below the soil surface. Rainfall was determined weekly using a rain gauge. Linear mixed models (observed date = predicted date + year) were used. Marginal r^2 values were determined as in (Nakagawa and Schielzeth 2013). Concordance correlation coefficients were determined as in (Carrasco and Jover 2003).

Model	Base (°C)	Temperature source	Mean pred. - obs. (DOY) ± SD	Mean GDD of event	r^2	Slope	CCC	95% CI for CCC
Elliott 10%	5	Air	1.74±6.01	604.8+71.03[a]	$8.467e^{-5}$	-0.021	-0.433[c]	-0.856 — 0.256
Elliott 10%	5	Soil	-7.73±5.17	827.7+242.28[a]	0.150	0.334	0.034	-0.135 — 0.202
Elliott 50%	5	Air	-3.97±4.36	841.4+62.87[a]	0.009	0.133	0.050	-0.281 — 0.371
Elliott 50%	5	Soil	-11.56±5.71	976.5+93.91[a]	0.242	0.343	0.019	-0.093 — 0.131
Jacquemin 1st peak	7 (after 230 GDD base 3 °C and a rainfall event)	Air	-15.37±7.91	313.5+68.60[b]	0.320	-3.112	0.115	-0.140 — 0.357
Jacquemin 1st peak	7 (after 230 GDD base 3 °C and a rainfall event)	Soil	-26±5.20	472.2+97.86[b]	0.404	0.477	$1.772e^{-9}$	$-1.20e^{-6}$ — $1.12e^{-6}$
Jacquemin 2nd peak	7 (after 230 GDD base 3 °C and a rainfall event)	Air	-21.75±8.73	388.2+99.96[b]	0.110	-1.420	0.015	-0.030 — 0.059
Jacquemin 2nd peak	7 (after 230 GDD base 3 °C and a rainfall event)	Soil	-29.2±6.18	516.4+91.07[b]	0.219	1.503	0.014	-0.070 — 0.099

[c] Calculated as ICC value

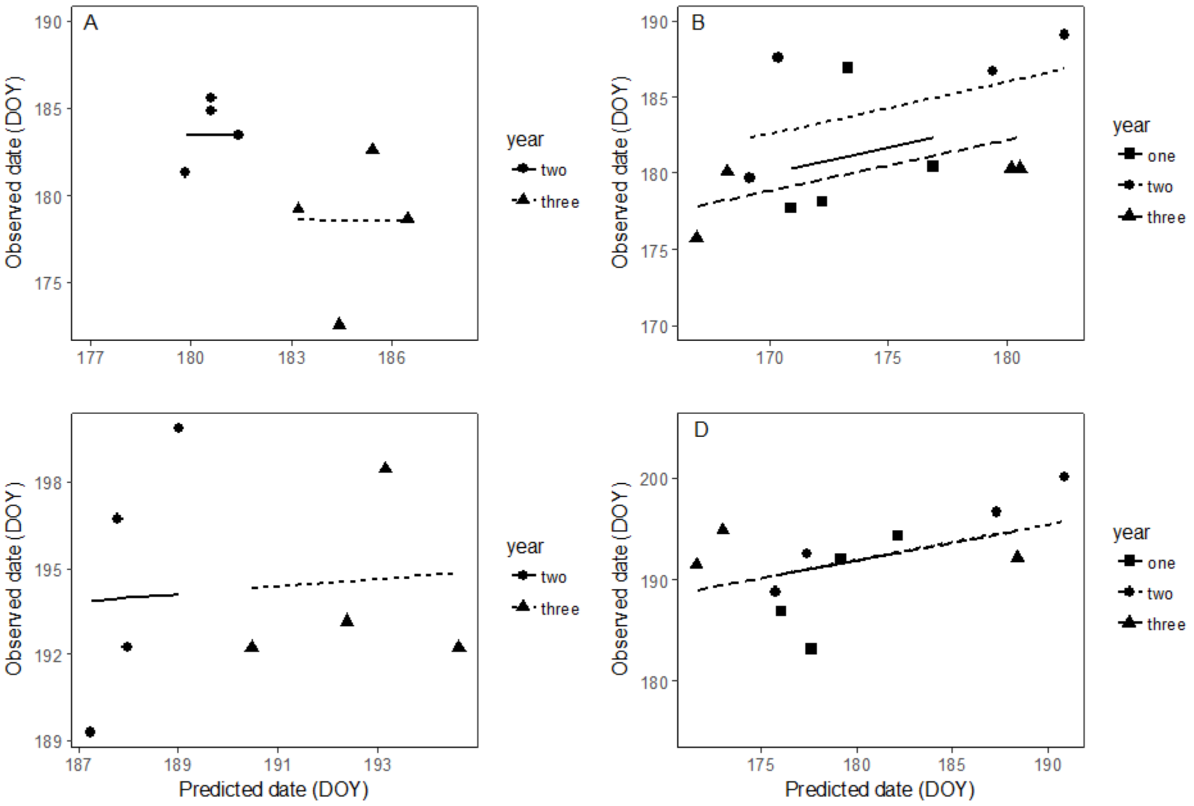

Figure 2.11 Linear regressions of observed adult wheat midge emergence dates (date of year-DOY) on dates (DOY) of necessary GDD accumulation predicted by the Elliott model for: (A) 10% male and female wheat midge emergence using air temperature (n=8 field sites), (B) 10% female wheat midge emergence using soil temperature (n=12 field sites), (C) 50% male and female wheat midge emergence using air temperature (n=8 field sites), and (D) 50% female emergence using soil temperature (n=12 field sites). Dates of 10 and 50% observed emergence were determined using linear interpolation based on cumulative counts of midge emerged in plastic emergence traps (n=20 traps per site for 2017-2018, n=8 traps per site for 2016). Black lines are linear regression lines for each year. Different symbols represent different years.

In all years, the Jacquemin model predicted earlier peaks of emergence than observed for both first (Figure 2.12 A, B) and second (Figure 2.12 C, D) waves of emergence when soil or air temperatures were used in the model. Likelihood ratio tests indicated that predicted values were only significantly related to observed dates for 10% emergence using soil temperature (χ^2= 8.792, df=1, p=0.003). The model predicted earlier emergence than observed in all years (Figure 2.12 B, intercept -105). Null models of observed emergence were not significantly different than models between predicted and observed dates of 10% emergence using air temperature (χ^2=0.026, df=1, p=0.871), or 50% emergence using either air (χ^2=0.651, df=1, p=0.420) or soil temperature (χ^2=0.912, df=1, p=0.340).

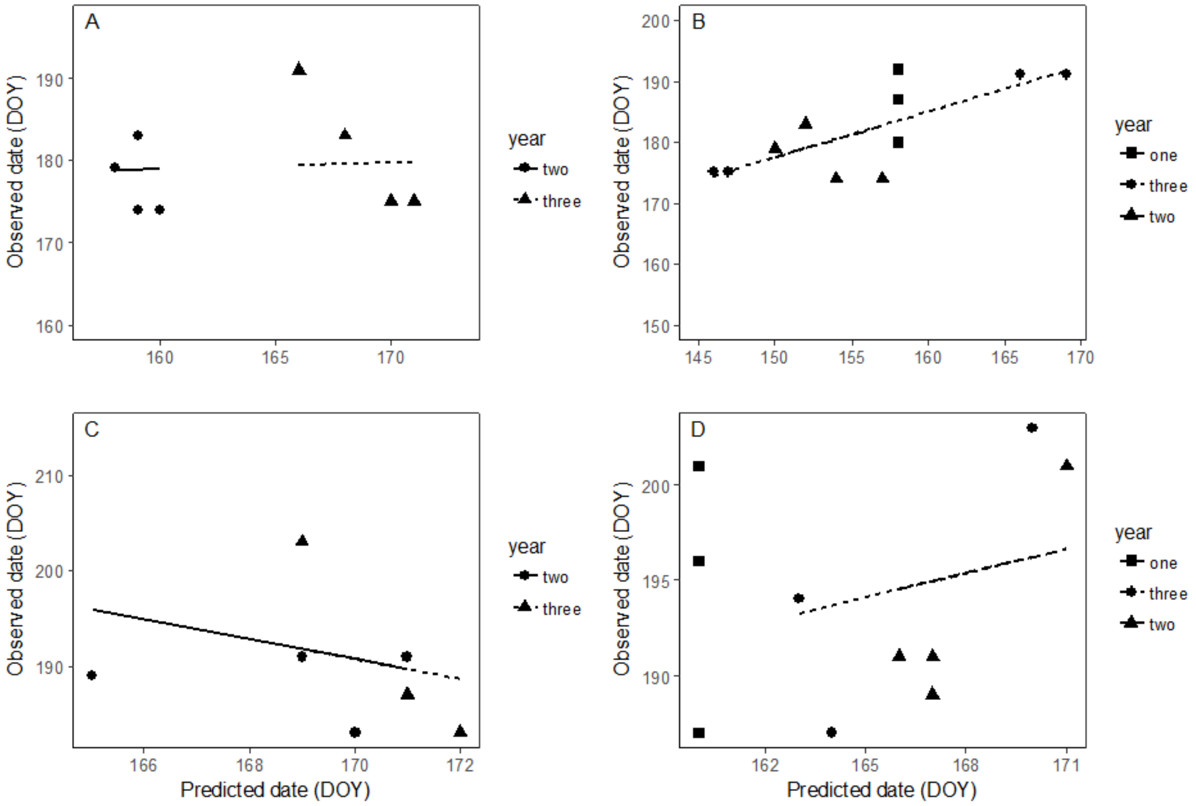

Figure 2.12 Linear regressions of observed adult wheat midge emergence dates (date of year-DOY) on dates (DOY) of necessary GDD accumulation predicted by the Jacquemin model for: (A) first peak of male and female wheat midge emergence using air temperature (n=8 field sites), (B) first peak of female wheat midge emergence using soil temperature (n=12 field sites), (C) second peak of male and female wheat midge emergence using air temperature (n=8 field sites), and (D) second peak of female emergence using soil temperature (n=12 field sites) using linear interpolation based on cumulative counts of midges emerged in plastic emergence traps (n=20 traps per site for 2017-2018, n=8 traps per site for 2016).Black lines are linear trend lines for each year. Different symbols represent different years.

At most field sites, wheat midge emergence occurred in multiple peaks (Figure 2.13). Multiple peaks were observed consistently at the McLennan site and in 2016 and 2018 at the Guy site

(Figure 2.13).The first peak of midge emegence occurred at 313.5+68.60 GDD above base 7 °C after a period of 230 GDD base 3 °C and a rainfall event.

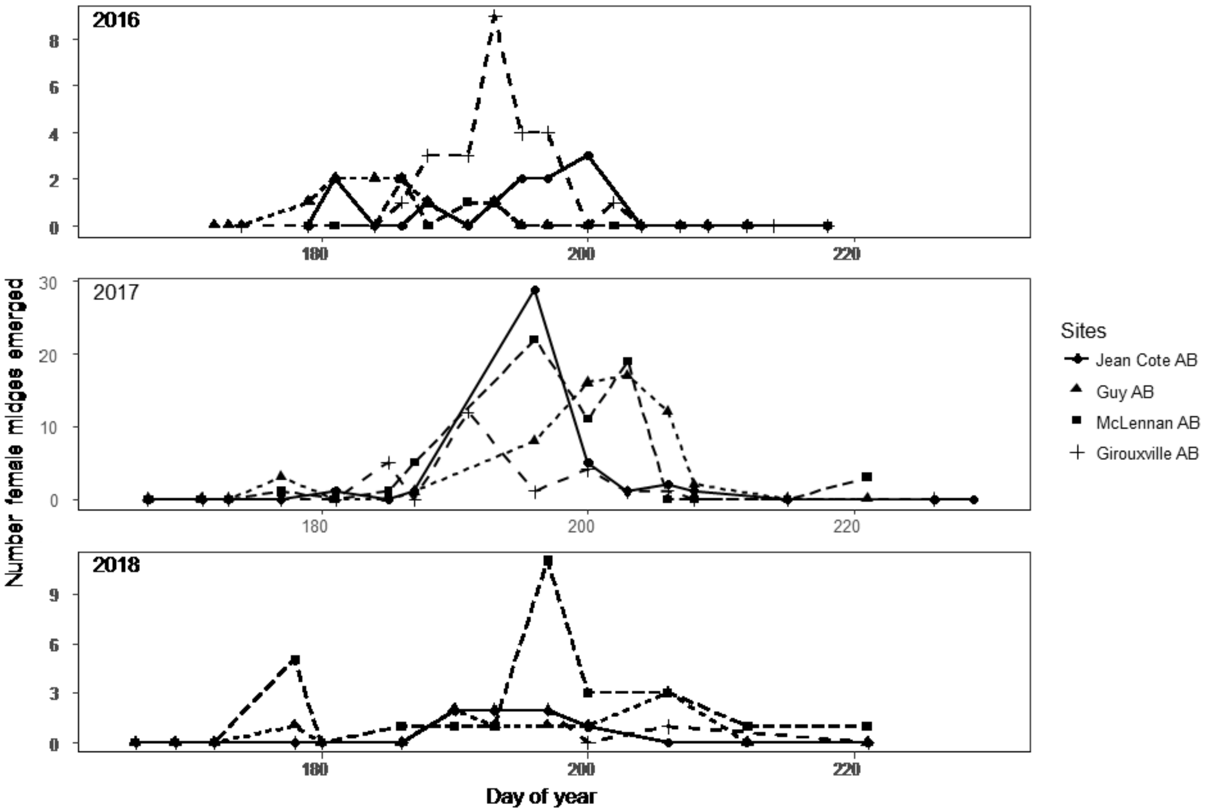

Figure 2.13 Emergence of wheat midge adults on sticky strips placed in plastic emergence bucket traps deployed in wheat fields. Traps (n= 8 per field in 2016, 20 per field in 2017 and 2018) were spaced 10 m apart in the first row of wheat stubble in fields (n=4 in 2016, 2017 and 2018). Traps were checked three times weekly. Different line types and symbols represent different field sites.

2.4.4 Wheat midge longevity

There was no difference in the longevity between paired and unpaired adult females (t = 0.44494, df = 61.161, p-value = 0.6579). Both paired and unpaired females lived for a mean of 2.7 ±1.5 (SD) days.

2.4.5 Wheat midge flight

In 2016 near Girouxville, AB, (sites located between 55.71 to 55.78°N and -117.29 to -117.31° W) peak flight occurred between 21:00 and 0:00 (Figure 2.14). In 2017, at the more northerly site near Fort Vermillion, AB (sites located at 58.35°N and -116.04° W) peak flight occurred at 22:00 (Figure 2.14), although midge numbers were low in all counts. In 2017 near Girouxville, AB, peak flight occurred at 21:00 (Figure 2.14).

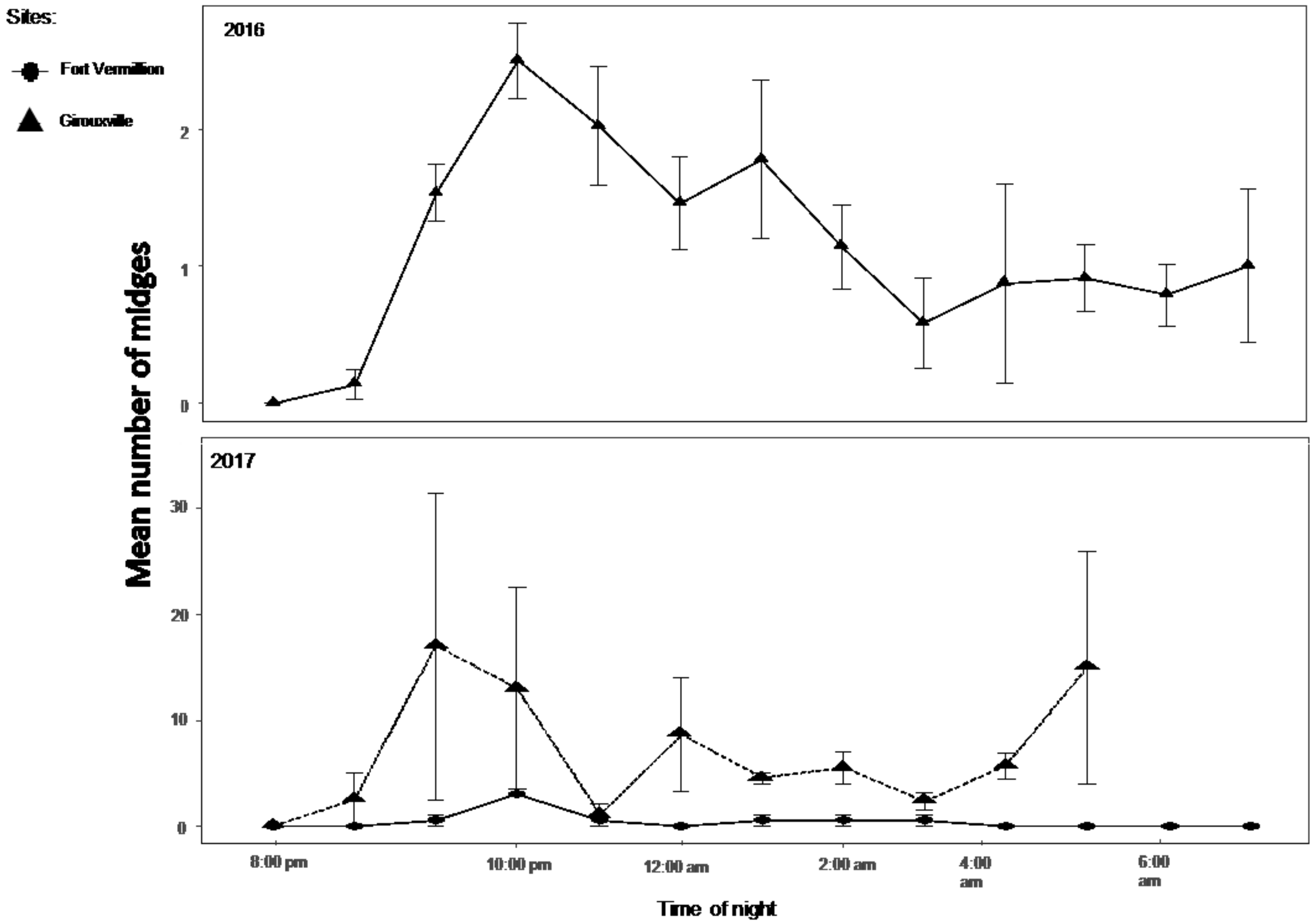

Figure 2.14 Hourly counts of adult wheat midge in commercial wheat fields. Total counts per hour per site (counts on n=10 wheat heads in n=5 locations in each field) were averaged across field sites (n=3 field sites in Girouxville, AB in 2016 and 2017 and n=2 field sites in Fort Vermillion, AB) and count nights (n=4 nights in 2016, n=1 night in 2017). Data points represent means ± SE. Different shapes and line types represent different regions. Graphs for 2016 & 2017 have different y-axis scales

2.5 Discussion

Ecological polyphenisms in host use or phenology have important implications for pest management. Different host strains may be differentially resistant to chemical or biological control methods, especially those associated with their predominant crop host (Silva-Brandão et al. 2018).

Differences in phenology can impact timing of monitoring and the timing of control methods. Wheat midge in the Peace River region displays different emergence than in other geographic regions. Patterns of wheat midge emergence in the Peace River region do not fit any of the published bioclimatic models developed and tested in western Canada (Elliott et al. 2009) or in Europe (Jacquemin et al. 2014). Adult midge reared under controlled conditions emerged after only 450 GDD base 5 °C, or 234 GDD base 7 °C, which is 213 GDD base 5 °C earlier than predicted by the Elliott model and 74 GDD base 7 °C later than predicted by the Jacquemin model. There was high precipitation in May and June of 2016 (>145 mm), but precipitation in May and June of 2017 (87.7 mm – 115.7 mm) and 2018 (44.5 mm – 87.5 mm) resulted in the optimum moisture range for wheat midge adult emergence suggested by Elliott et al. (2009). Adult wheat midge emergence across regions varies and different phenological models and threshold temperatures are required in different areas. While the Jacquemin model did not accurately predict wheat midge emergence in the Peace River region, the GDD values for laboratory-reared and in-field emergence were more consistent with the observed data than values generated by the Elliott model which was developed in western Canada. The experiment was unable to determine exact dates of rainfall events or midge emergence due to the frequency of data collection events. For the purposes of testing the model, midpoints of rainfall checks (weekly in May) and trap checks (approx. 3 times weekly, depending on weather conditions) were used as dates. This may have impacted the accuracy of estimates using the Jacquemin model. For practical IPM purposes, the Elliott model comes the closest to predicting wheat midge emergence in Northern Alberta. Differences between the observed wheat midge emergence patterns in the Peace River region and the published models tested suggest that development of a model that accurately reflects emergence phenology in the Peace River region is necessary. This study was unable to develop and test a new phenological model due to low numbers of field sites. Development of a model predicting “waves” of wheat midge emergence based on a temperature accumulation more appropriate to Northern Albertan wheat midge, in conjunction with inductive rainfall events, warrants further investigation.

It is likely that wheat midge management requires region-specific bioclimatic models. Wheat midge has shifted its development rates regionally to match host plant phenology. In Germany and the UK, it mainly infests winter wheat (Basedow 1972, Oakley et al. 2005, Chavalle, Censier, et al. 2015). In Finland, wheat midge infests winter and spring wheat equally depending on abiotic conditions and has adapted to emerge 3 weeks earlier than midge in Germany, at 400 GDD base 5 °C (Kurppa 1989). In North America, wheat midge has adapted to mainly infest spring wheat (Olfert et al. 1985). This host plant association is reflected by an overall shift in adult emergence from late May- early June to late June- early July. This shift in calendar date also represents a shift in GDD, as midge in Western Canada require 693 GDD base 5 °C for adult emergence while midge in Germany require 500 GDD base 6 °C (Basedow 1972, Elliott et al. 2009). In China, wheat midge adults emerge in April or early May, infest winter wheat and late in May they begin diapause in the soil that lasts through the winter (Cheng et al. 2009, 2017). Wheat midge collected from rye emerged as adults earlier than larvae collected from wheat, even when reared under the same conditions (Basedow 1972). High variation in emergence periods occurs even in wheat-infesting midges in Germany, with some midges emerging early and others emerging later in the season (Basedow 1972). This has been attributed to extended diapause (Wise and Lamb 2004), although it has not been previously reported in Canada. Wheat midge exhibits Type C polymodal emergence (Waldbauer 1978), or emergence that occurs over multiple years. In this study, we saw distinct bimodal emergence patterns consistently at some field sites, as well as high rates of extended diapause. It is possible that the bimodal emergence was due to prolonged diapause in a proportion of the population, or that some wheat midges exhibit a Type A polymodal (or emergence in multiple periods within a field season) response as well as type C. A Type A polymodal emergence pattern would fit the Jacquemin et al. (2015) model concept of "waves" of emergence. The mechanism behind polymodal emergence for wheat midge is unknown, but likely is due to genetics since some midges require two cold periods to emerge under the same rearing conditions (Wise and Lamb 2004). Pre-existing genetic heterogeneity in phenological and diapause responses likely promotes phenological adaptations for alternate host use.

In this study, up to 100% of cocoons collected from the field remained in extended diapause. Whereas, in other studies, only 5% of wheat midges and 12-57% of parasitoids required two winters to emerge in controlled conditions with optimum moisture (Wise and Lamb 2004). Moisture conditions dictate this extended diapause, as up to 89% of wheat midge and parasitoids remain in extended diapause if soil moisture content is outside of the 20-50% optimal range (Cheng et al. 2017). Field-collected soil was kept moist, but soil moisture was not quantified during our rearing study. The soil collected from the field in early May (2017) and June (2016) was dry which may have triggered the initiation of the extended diapause. More research is needed to understand how and when moisture levels trigger an extended diapause in wheat midge larvae. All studies that have examined the effects of moisture on wheat midge diapause maintain consistent moisture levels throughout development (Basedow and Gillich 1982, Hinks and Doane 1988, Cheng et al. 2017). While it is suggested that moisture is required for midge to leave overwintering cocoons (Doane and Olfert 2008), this has never been directly tested.

In our study, parasitism rates of field-collected midge were variable. This variation also occurs in geographically disparate populations of wheat midge, and it is unclear what drives this variation. Parasitism rates in wheat midge vary both within and among populations, but generally are very high: 19.5-48 % in Belgium (Chavalle, Censier, et al. 2015, Chavalle et al. 2018), 0-80% in Saskatchewan (Olfert et al. 2009), 9.3-74.4 % in Germany, and 0-40% in the first year but 0-99% in the second-year emergence in Manitoba (Wise and Lamb 2004). Parasitism rates found in this study were likewise high, reaching up to 100%. *Macroglenes penetrans* was the only parasitoid found in samples collected in the Peace River region of Alberta. Two other wheat midge parasitoids have been confirmed in North America. *Platygaster tuberosula* was introduced in Saskatchewan in 1993-1994 (Doane, Braun, et al. 2001, Olfert et al. 2003), and *E. error* has been recovered from wheat midge samples in Montana (Echegaray et al. 2016). In Belgium, *M. penetrans* is often the predominant parasitoid of wheat midge accounting for 37.5-65.8% of all parasitoids recovered, although up to 8 parasitoid species have been reared from wheat midge in Europe (Chavalle, Buhl, et al. 2015, Chavalle et al. 2018).

Overwintering conditions influence adult wheat midge emergence. A greater proportion of midges emerged as adults when overwintering larvae are reared at a constant temperature regime of 2 °C than in outdoor conditions (Figure 2.8). At constant temperatures, wheat midge cocoons are able to survive at -10 °C with no differences in mortality for up to 120 days (Wise and Lamb 2004). Wise and Lamb (2004) concluded that soil temperatures during the winter are unlikely to be a source of mortality to midge or parasitoids. Fluctuations in temperature likely decreased wheat midge emergence, as fewer midges emerged when larvae are reared in insulated overwintering cages (-1.33 °C ± 3.301 SD) than when housed in controlled environments. In Germany, overwintering mortality varies from 0-64%, and mortality factors could be soil moisture or other environmental conditions (Basedow and Schütte 1973).

Longevity of wheat midge females collected in the Peace River region was slightly shorter than in other regions. Virgin female wheat midge in Manitoba have a mean longevity of 5.4 days in the laboratory (Pivnick and Labbé 1993). In Germany, virgin females live for a mean of 5-7 days (Basedow and Schütte 1973). In this study, both virgin females and females held with males lived for a mean of 2.5 days. The shorter lifespan may be due to lower humidity in our experimental set up, as adult wheat midges are prone to desiccation under low humidity conditions (Hinks and Doane 1988). Although adult midges were held with excised wheat heads to increase humidity, this may not have been sufficient.

Wheat midge are reported to fly during twilight, which occurs at different times in different regions. Peaks of wheat midge flight occurred later at night in the Peace River region than in other regions. Peak wheat midge flight occurs between 20:00 and 21:45 in Saskatchewan (Pivnick and Labbé 1993), which is 1-3 hours earlier than what was observed in the Peace River region. Earlier reports of wheat midge flight suggest that midge fly in the early evening in Europe (Barnes 1932). In-field scouting for wheat midge that are active later in the evening will require producers to scout wheat fields between 21:00 and 0:00 hours. Other monitoring tools that are more user-friendly, will need to be optimized for use in the Peace River region.

Improved management of wheat midge can be achieved by understanding regional variations in biology. The high and variable rates of parasitism emphasize the importance of quantifying populations of natural enemies before applying control decisions. Economic thresholds that include predictions of pest population suppression by natural enemies present in the field are becoming more common (Giles et al. 2003, Chen and Tang 2004, Tang et al. 2008). When parasitoid densities are high, it is unlikely that additional control measures will be necessary. The high rates of extended diapause and variable rates of overwinter mortality suggest that monitoring larvae in the soil will not give accurate indications of adult populations in the spring. In other regions, fall soil sampling is also unreliable (Basedow and Schütte 1974, Oakley et al. 1998, Lamb et al. 1999). It is therefore critical that producers and agrologists have modelling tools enabling the prediction of wheat midge emergence, so that in the spring monitoring and management can be accurately timed. If model outputs suggest earlier or later emergence than occurs in the field, then producers may underestimate midge population densities or miss the critical window for control. Emergence models need to be optimized for the phenological shift that appears to apply to the Peace River region. Accurate timing of monitoring is especially critical given that wheat midge females in the Peace River region may have a shorter longevity than in other regions. Further research is needed to determine how the differences in wheat midge biology that occur in the Peace River region can be applied to IPM strategies.

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Appendix 2.1

Tables referring to site information.

Table 2.2 Commercial wheat fields where soil was collected in spring 2016 and spring 2017 to determine overwintering wheat midge density and parasitism rates.

		Site GPS			
Nearest town	Province	Latitude (degrees)	Longitude (degrees)	Collection methods	Year monitored
Girouxville	Alberta	55.76	-117.29	Core & spade	2016
Jean Cote	Alberta	55.96	-117.39	Core & spade	2016
Girouxville	Alberta	55.71	-117.38	Core	2016
Girouxville	Alberta	55.75	-117.29	Core	2016
Girouxville	Alberta	55.77	-117.29	Spade	2017
McLennan	Alberta	55.66	-117.05	Spade	2017
Guy	Alberta	55.55	-117.18	Spade	2017
Jean Cote	Alberta	55.97	-117.39	Spade	2017
Rolla	British Columbia	55.85	-120.19	Spade	2017

Table 2.3. Commercial wheat field locations where wheat head samples were collected in 2016. Wheat midge larvae were extracted from samples and reared on autoclaved soil.

		Site GPS		
Nearest town	Province	Latitude (degrees)	Longitude (degrees)	Wheat cultivar
Girouxville	Alberta	55.77	-117.29	Stettler
McLennan	Alberta	55.66	-117.05	Superb
Guy	Alberta	55.55	-117.18	Roblin
Jean Cote	Alberta	55.97	-117.39	Stettler
Rolla	British Columbia	55.85	-120.19	Thorsby

Table 2.5 Site locations for testing the timing of adult wheat midge and parasitoid emergence in situ. Commercial fields were selected that had been seeded to midge-susceptible wheat the year before monitoring. Monitoring was conducted between June and August in 2016 and 2017. Fields were monitored with emergence traps.

		Site GPS			
Nearest town	Province	Latitude (degrees)	Longitude (degrees)	Year monitored	Monitoring year crop species
Girouxville	Alberta	55.76	-117.29	2016	Canola
McLennan	Alberta	55.66	-117.05	2016	Canola
Guy	Alberta	55.55	-117.18	2016	Wheat
Jean Cote	Alberta	55.96	-117.39	2016	Canola
Elmworth	Alberta	55.12	-119.44	2016	Canola
Hythe	Alberta	55.32	-119.46	2016	Canola
Girouxville	Alberta	55.77	-117.29	2017	Canola
McLennan	Alberta	55.66	-117.05	2017	yellow peas
Guy	Alberta	55.55	-117.18	2017	alsike clover
Jean Cote	Alberta	55.97	-117.39	2017	Canola
Rolla	British Columbia	55.85	-120.19	2017	Peas

Table 2.7 Site locations for in-field nocturnal wheat midge flight monitoring. Adult midges on wheat plants were counted at 5 locations in each field every hour during the night.

Site GPS location

Nearest town	**Province**	**Latitude (degrees)**	**Longitude (degrees)**	**Year**	**Dates monitored**	**Wheat cultivar**
Girouxville	Alberta	55.77	-117.29	2016	5,6,13, &14 July 2016	Stettler
Girouxville	Alberta	55.71	-117.29	2016	5,6,13, &14 July, 2016	Stettler
Girouxville	Alberta	55.78	-117.31	2016	5,6,13, &14 July, 2016	Barley
Fort Vermillion	Alberta	58.35	-116.04	2017	28 & 29, June 2017	Stettler
Fort Vermillion	Alberta	58.34	-116.04	2017	28 & 29, June 2017	Thorsby
Girouxville	Alberta	55.76	-117.29	2017	10, July, 2017	Stettler
Girouxville	Alberta	55.74	-117.39	2017	10, July, 2017	Harvest
Girouxville	Alberta	55.78	-117.39	2017	10, July, 2017	Harvest

Appendix 2.2

Tables summarizing models used in statistical analyses.

Table 2.4 Linear mixed model used for analysis of emergence from different overwintering treatments. Midge were reared in groups of 7-111 individuals in autoclaved soil (17.5% moisture) in insulated boxes outdoors, in a climate-controlled refrigerator or a climate-controlled growth cabinet. Collection site was included as a random factor in all models.

Experiment	**Model**		**Variance distribution**
Overwintering treatment	Glmer (Insects emerged) ~ Overwinter treatment + offset (log (insects in rearing)) + (1|site)	Random intercept	Poisson – log link

Table 2.6 Summary of linear mixed model used for analysis of observed dates (Day of year) of emergence events as determined by linear approximation (for 10 and 50%) from cumulative counts of emergence in plastic emergence traps (n=10 traps in 2016, 20 traps in 2017& 2018) as a function of predicted dates (Day of year) determined from model values of GDD accumulation at field sites in air or soil. Air temperature was only recorded in 2017 & 2018, so analyses using air temperature include n=8 field sites, while analyses using soil temperature include n=12 field sites. Year was included as a random factor in all models.

Experiment	**Model**		**Variance distribution**
Adult emergence – 10% Elliott model (°C in air)	Lmer (Obs. DOY) ~ Pred. DOY + (1|year)	Random intercept	Normal

Adult emergence – 50% Elliott model (°C in air)	Lmer (Obs. DOY) ~ Pred. DOY + (1|year)	Random intercept	Normal
Adult emergence – 10% Elliott model (°C in soil)	Lmer (Obs. DOY) ~ Pred. DOY + (1|year)	Random intercept	Normal
Adult emergence – 50% Elliott model (°C in soil)	Lmer (Obs. DOY) ~ Pred. DOY + (1|year)	Random intercept	Normal
Adult emergence – 1st peak Jacquemin model (°C in air)	Lmer (Obs. DOY) ~ Pred. DOY + (1|year)	Random intercept	Normal
Adult emergence – 2nd peak Jacquemin model (°C in air)	Lmer (Obs. DOY) ~ Pred. DOY + (1|year)	Random intercept	Normal
Adult emergence – 1st peak Jacquemin model (°C in soil)	Lmer (Obs. DOY) ~ Pred. DOY + (1|year)	Random intercept	Normal
Adult emergence – 2nd peak Jacquemin (°C in soil)	Lmer (Obs. DOY) ~ Pred. DOY + (1|year)	Random intercept	Normal

Chapter 3: Assessment and comparison of available tools for monitoring of wheat midge, *Sitodiplosis mosellana* Géhin (Diptera: Cecidomyiidae) in the Peace River region, Alberta

3.1 Abstract

Wheat midge, *Sitodiplosis mosellana* Géhin (Diptera: Cecidomyiidae), is an invasive pest of wheat that has spread throughout the Peace River region of Alberta, Canada. If conditions favour midge outbreaks, wheat yield and quality loss can exceed millions of dollars. Tools available to monitor wheat midge include: sex-pheromone baited traps, yellow sticky cards and a soil core sample survey. The applicability of these tools for the northern Alberta wheat agroecosystem has not been assessed. Different commercially available Delta traps, pheromone lures, and yellow sticky cards have been used historically for wheat midge monitoring, without any direct comparisons. In this study, two pheromone Delta traps (green and orange) and 3 pheromone lures (flex lure from Scotts™, red septa lure from Scotts™, and a red septa lure from Great Lakes IPM™) were compared. The efficacy of three yellow sticky cards (a 7x12 cm card from Great Lakes IPM™, a 14x 18 cm card from Alpha Scents™, and the Alpha Scents™ card rolled into a cylinder) were compared. Larvae were extracted from wheat heads to determine relationships with adult trap capture. Harvest samples (1 m^2 quadrats) were collected and yield, thousand seed weight, percent protein, and wheat midge damage were compared to trap captures. In 2017, both trap surface area and pheromone lure affected adult midge capture. More adult midges were captured in traps with a greater surface area and in traps baited with the Scotts™ flex lure than the Great Lakes IPM™ septa lure, which had higher and more variable pheromone release rates. We found no relationship between the number of adult midges captured in pheromone traps and either larval density or harvest metrics. The smaller, Great Lakes IPM™ unbaited yellow sticky cards captured more midges than the other two larger, yellow sticky cards. We were unable to determine relationships between

the number of female midges on yellow sticky cards and larval densities and harvest metrics measured at the same sites. No relationships were found between soil core samples and captures of adult midges the following spring.

3.2 Introduction

Integrated Pest Management programs have been developed in agricultural, forestry and urban systems (Hobbs and Humphries, 1995; Kogan, 1998; Lewis et al., 1997; Swanton and Weise, 1991; Thomas, 1999; Van Lenteren and Woets, 1988). One of the most cited definitions of Integrated Pest Management (IPM) (Kogan, 1998) is the integration of population dynamics, abiotic and biotic environmental factors with the judicious use of multiple control tactics into a pest management system (Stern et al., 1959).

If economic or action thresholds exist for a given pest in a managed ecosystem, they can be used in control decisions. Stern et al. (1959) defined economic thresholds as the population level at which management should occur to prevent densities from reaching a level that will cause economic loss. Economic loss is the point at which the cost of control will be equal to or less than losses due to decreased crop yield or quality (Mumford and Norton, 1984; Onstad, 1987). Economic thresholds are determined based on an understanding of pest biology and the interactions of pest population densities with plant responses (Pedigo et al., 1986; Stern, 1973).

A critical component required to establish economic thresholds is the accurate estimation of pest population density. Due to cost, pest density needs to be extrapolated from a restricted number of samples. Binns and Nyrop (1992) review the process to mathematically determine the precision and bias of different sampling tactics. The number of samples collected depends on specific relationships between mean population estimates and sample variance (Binns and Nyrop, 1992; Taylor, 1984). Sampling tactics vary depending on sampling goal(s) (e.g. detection or to make control decisions), target pest species, and the developmental or physiological state of the target pest (Cohnstaedt et al., 2012). The number of samples collected, physical distribution or patterns of samples and the specific monitoring tool are determined for each specific system.

Common sampling tools in insect pest management include sweep net samples, stationary traps, along with visual scouting. Traps coated with an adhesive agent are relatively inexpensive monitoring tools that capture target insects as they move through the environment. Unbaited sticky traps have been used to develop action thresholds for western flower thrips, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), (Shipp et al., 2000), lygus bugs, *Lygus lineolaris* Palisot de Beauvois (Hemiptera : Miridae)(Prokopy et al., 1982), apple maggots, *Rhagoletis pomonella* Walsh (Diptera: Tephritidae), (Stanley et al., 1987), and sugar beet root maggots, *Tetanops myopaeformis* Röder (Diptera: Ulidiidae), (Bechinski et al., 1989) among others. Some pest attraction or aversion due to trap colour or odour from the adhesive can impact trap catch on unbaited sticky cards (Brach and Trimble, 1985; Kirk, 1984), but they can potentially provide unbiased sampling through passive pest interception (Southwood, 1978). Sticky cards are cheap but can be difficult as management tools due to high bycatch, which interferes with pest identification by growers and pest managers. Increased capture of target insects or exclusion of non-target bycatch can be manipulated by altering colour or trap position (Chavalle et al., 2019; Yee, 2013). Sampling strategies that use sticky traps need to consider trap number, spatial distribution, trap colour, and adhesive properties of the traps.

The sensitivity and specificity of unbaited traps can be enhanced with addition of an attractant. Species-specific semiochemicals, or information-bearing chemicals, can be used to attract target insects in the environment. Semiochemicals used for monitoring include both kairomones, or semiochemicals intercepted and exploited by an individual of a different species than the emitter, (Cohnstaedt et al., 2012; Light et al., 2001; Meurisse et al., 2008; Weeks et al., 2011), pheromones for intraspecific communication, such as aggregation pheromones (Leskey and Hogmire, 2005; Weeks et al., 2011) and sex pheromones (McNeil, 1991; Roelofs W. L. Carde, 1977;

Witzgall et al., 2010). Sex pheromones only attract reproductively active individuals of a single sex. Semiochemical-baited traps are useful to detect target species at low population densities and when sensitive detection of phenology or presence is necessary for patchily distributed individuals (Witzgall et al., 2010). Sex pheromones have been used to develop economic thresholds, for timing of control methods and as control methods themselves (e.g. pheromone-based mating disruption, mass trapping) (Witzgall et al., 2010). Many sex pheromones are female-produced, and capture of males may not accurately indicate the breeding population and reproductive potential of an insect population (Witzgall et al., 2010).

Several factors impact the effectiveness of pheromone-baited traps in different systems. Trap shape and position in the environment can affect pheromone plume shape, which influences the efficiency of the trap. In pea moths, *Cydia nigricana* Steph. (Lepidoptera: Tortricidae), only 1 in 4 moths that approach pheromone traps are retained (Lewis and Macaulay, 1976), but alteration of trap shape or position within the crop enhances trap capture (Lewis and Macaulay, 1976). Wind direction, trap height and other objects in the environment impact the ability of target insects to follow pheromone plumes (Lewis and Macaulay, 1976; Murlis, 1992). Environmental conditions affect the pheromone plume shape and active space as well as male receptiveness (Witzgall et al., 2008). Some species of lepidopterans are more selective of pheromone blend composition at lower temperatures, while the response of others can be affected by day length (McNeil, 1991). In species that combine visual and semiochemical cues, optimization of trap colour can improve capture. For many herbivorous insects, yellow traps act as a supernormal “foliage” stimulus and are attractive (Prokopy and Owens, 1983). Use of pheromone traps as monitoring tools to determine phenology or economic thresholds requires optimization based on species and geography. Pest species with a wide or expanded distribution require monitoring systems that work well across the range or that are optimized for each region.

Wheat midge, *Sitodiplosis mosellana* Géhin (Diptera: Cecidomyiidae), is an invasive pest of wheat in North America. In North America, wheat midge has been found as far north as 58° (unpublished data) and as far South as California and Indiana, USA (Olfert et al., 2016). Wheat midge has the potential to expand its range above 60° N due to climate change (Olfert et al., 2016). This pest has recently expanded its range into northwestern Alberta ("WESTERN COMMITTEE ON CROP PESTS," 2011). Different monitoring tools are available to monitor wheat midge, but the efficacy of these tools has not been compared, especially in the northern-most region of the wheat midge distribution. The midge is a serious pest of wheat: in Saskatchewan, $50 million in yield losses were attributed to wheat midge in 1983 (Olfert et al., 1985).

Wheat midge larvae reduce yield by feeding on the developing ovaries and seed, which decreases grain weight and causes seed abortion if feeding pressure is strong (Lamb et al., 2000). Wheat midge damage also decreases grain milling quality (Dexter and Edwards, 2012; Miller and Halton, 1961). The Canadian Grain Commission tolerance for CWRS grade 1 is 2 % midge damage (*Official grain grading guide*, 2018). The relationship between larval density and distribution to both yield and quality loss has been quantified to develop an economic threshold: infestation of 11% of seeds will cause yield loss, whereas infestation of 6% of seeds will cause quality loss (Lamb et al., 2000). However, a threshold based on larval infestation cannot be used to make control decisions because larvae feed inside the developing wheat head and cannot be monitored in the field (Ding and Lamb, 1999). An action threshold also includes capture of four adult midges on 10 sticky cards (each 7.5 cm x 12.5 cm) over 3 days (Lamb et al., 2002). Wheat midge monitoring with sticky cards, however, has not been widely adopted by farmers and agrologists. In Belgium, yellow pan traps capture wheat midge at the same efficiency as yellow sticky cards (Chavalle et al., 2019) and are recommended to monitor density. Wheat midge adult phenology in the Canadian Prairie Provinces is currently monitored with sex pheromone-baited Delta traps. A 1 mg racemic mixture of the female-produced sex pheromone, 2,7 nonanediyl dibutyrate (Gries et al., 2000) is used to bait pheromone traps (Birkett et al., 2004; Bruce et al., 2007). Male midge capture in sex pheromone-baited traps reliably indicates peak wheat midge flight and correlates to larval infestation levels in the UK (Bruce et al., 2007). A tentative action threshold of 20-30 midges captured in pheromone-baited traps over 2 days is recommended in the UK (Bruce et al., 2007). Currently, sex pheromone traps are used in the Canadian Prairies to determine timing for in-field monitoring, but not directly as a control action threshold. Control decisions for wheat midge in Canada are based on in-field counts of adult females on wheat heads. Counts are averaged across multiple locations in the field. An action threshold of 1 midge per 4-5 heads will prevent yield loss, while 1 midge per 8-10 heads is the threshold to prevent quality loss (Elliott et al., 2002). In Germany, 1 adult female per 3 heads is the damage threshold (Basedow and Schütte, 1973).

Wheat midge density maps are generated annually for the Canadian Prairies to forecast wheat midge densities. These maps are based on the number of overwintering midge cocoons (Doane et al., 1987) in 17 soil cores (1.9 cm diam. X 10.8 cm depth) sampled from wheat fields (Doane et al., 2000). Doane et al. (2000) recommend 6 larvae per 100 cm^2 of soil sampled as a threshold for potential economic infestation. Soil core sampling of cocoons, however, does not predict infestation in Manitoba (Lamb et al., 1999), the UK (Oakley et al., 1998) or in Germany (Basedow and Schütte, 1974).

Wheat midge larvae can be controlled through use of resistant wheat cultivars or by chemical control. Resistant plants produce high levels of ferulic acid when triggered by wheat midge feeding, which inhibits larval feeding and eventually causes death (Ding et al., 2000; Lamb et al., 2016; Smith et al., 2004; Thomas et al., 2005; Vera et al., 2013). The resistance, however, is based on a single gene (Kassa et al., 2016; Thomas et al., 2005) that may lose its efficacy over time. Additionally, producers may not wish to use prophylactic control methods, such as resistant cultivars because of high variability in wheat midge populations. Foliar insecticides are used to control wheat midge adult females and eggs (El-Wakeil et al., 2013; Elliott and Mann, 1997). Both dimethoate and chlorpyrifos are registered in Canada for wheat midge control and application of either insecticide can decrease kernel damage by 47% and 75%, respectively compared to unsprayed control plots (Elliott, 1988).

Due to the expansion of wheat midge into the Peace River region of Alberta (“WESTERN COMMITTEE ON CROP PESTS,” 2011) and the potential for further northward expansion, current management techniques need to be assessed in northern regions. Daylength, seasonal and daily temperatures, the length of growing season, and growing practices differ across regions and latitudes. These climatic and cultural differences can impact the effectiveness of management tools and the goals of producers. In general, the efficacy of monitoring tools for wheat midge in Canada need to be compared for their potential use in development of an economic threshold. The overall objective of this research is to test the efficacy of multiple monitoring tools for wheat midge in the Peace River region of Alberta, Canada.

3.2.1 Objectives

1. Compare the effectiveness of different sex pheromone lures and trap colours for wheat midge capture and determine whether pheromone-baited traps can be used as predictive tools of wheat midge density in the Peace River region.
2. Compare the effectiveness of different spatial distributions, shapes and sizes of yellow sticky cards for wheat midge capture and determine whether sticky card trap capture can predict wheat midge density in the Peace River region.
3. Determine the ability of fall soil core samples to predict in-field populations of wheat midge the following year.

3.3 Methods

3.3.1 Comparison of commercially available pheromone traps

The effectiveness of different pheromone lures and traps for wheat midge capture was tested in commercial wheat fields. Traps were deployed in fields seeded to susceptible wheat cultivars during wheat midge flight. In 2016, monitoring was done at 8 field sites in the Peace River region and 2 field sites in Saskatchewan (Appendix 3.1, Table 3.1). In 2017, 9 field sites in the Peace River region and 4 field sites in Saskatchewan were monitored (Appendix 3.1, Table 3.1).

Traps were positioned 50 m apart along a north-south transect at the field margin. Traps tested included green and orange Delta traps (Scotts™ and Scentry™) baited with either a flex lure (Scotts™), a rubber septa lure (Scotts™ or Great Lakes IPM™), or no lure. The eight treatment combinations were organized in a full-factorial randomized block design with each commercial site treated as a block.

Traps were collected and replaced, weather permitting, every 4-14 days from 20 June until 17 August in Alberta in both years, and every 6-13 days from 13 June until 08 August in Saskatchewan. Traps were examined under a dissecting stereomicroscope (Zeiss™ stemi-C) at 8x magnification. Wheat midge adults were identified based on antennal morphology (Harris 1966), counted and separated by sex. Specimens of the wheat midge parasitoid, *Macroglenes penetrans* Kirby (Hymenoptera: Pteromalidae), were identified using Gibson et al. (1997) and through reference to voucher specimens.

3.3.2 Statistical analyses for comparison of commercially available pheromone traps

All analyses were done in R studio 3.13 (R development team 2016). All Linear mixed models were fit with package lme4 (Bates et al., 2014). Post-hoc analyses were done with the package multcomp (Hothorn et al., 2008), and Wald Chi-square tests were done with the package car (Fox and Weisberg 2011). Assumptions of normality and heteroscedasticity were tested visually by examining q-q plots, residual plots, and by conducting Shapiro-Wilks tests on model residuals.

Distributions of data were examined visually using histograms and q-q plots against a normal distribution, and different model variance structures were evaluated by comparing AIC values and determinations of residual normality. Non-significant interactions were removed from models if anova comparisons between models with and without the interaction were not significant.

Season total trap capture of male midges in the different pheromone-lure, trap type combinations was compared using generalized linear mixed models. Lure and card type were specified as fixed factors and site was treated as a random factor (Appendix 3.2, Table 3.2). Error distributions were specified as gamma with a log link in 2016 and as negative binomial in 2017. Season-long male midge capture in pheromone-baited traps positioned at sites in Saskatchewan (2017) was analyzed with a generalized linear mixed model. Lure and card type and their interaction were specified as fixed factors and site was treated as a random factor. Error distributions was specified as a Poisson distribution. Data from one site in Saskatchewan was not included due to incorrect trap set up.

Season total trap capture of male midges was additionally analyzed with trap capture standardized for trap surface area. Total counts were converted to rates of capture per cm^2 of trapping surface in each trap type by including an offset of the log of the cm^2 in the model (O'Hara and Kotze 2010). The log value was included, as a Poisson or negative binomial regression model: $\log(y) = x + \log(e)$ simplifies to $\log(y/e) = x$. These models were structured as above with the same error distributions, except the 2016 data was fit with a negative binomial distribution (Appendix 3.2, Table 3.2).

3.3.3 Release rates of pheromone from lures

The pheromone release rate of each of the three lure treatments (Scotts™ flex, Scotts™ septa, and Great Lakes IPM™ septa) was measured through aeration of lures under controlled conditions. Lures (n=3 per treatment) were aerated individually inside glass chambers (5 cm by 10 cm OD) at

23.5 ± 0.5 °C for three days. Air was drawn through the chambers using Dyna pumps (A.O. Smith, Tipp City, OH, USA) at a rate of 0.4 L/minute into a Porapak Q (50–80 mesh; Supelco—part of Sigma-Aldrich, Canada, Oakville, ON) adsorbent material. The Porapak was flushed daily using 2 mL of 50:50 blend of pentane and diethylether with an internal standard of 500 ng Dodecyl-Acetate. Porapak-q extracts were analyzed with a 5890 Hewlett Packard Gas Chromatograph (GC) (Hewlett-Packard, now Agilent Technologies, Santa Clara, CA, USA) with a DB-5 (30 m x 0.32 mm ID) column (Agilent J&W columns, Agilent Technologies, Santa Clara, CA, USA). The GC oven was operated with the following program: 50 °C held for 1 minute, temperature was then increased to 280 °C by a rate of 10 °C per minute. The injector port was set to 250 °C, and the flame ionization detector was kept at 280 °C. Area counts of the peak of the compound of interest were compared to the area counts with the known amount of the internal standard in order to quantify the amount of pheromone in Porapak extracts.

3.3.4 Statistical analyses for release rates of pheromone from lures

Release rate data was log transformed to meet the assumption of normality as based on Shapiro-Wilks test ($W=0.674$, $p<0.001$). Data were analyzed with a repeated measures linear mixed model with lure type as a fixed factor and date and subject as random factors (Appendix 3.2, Table 3.3). Differences in pheromone release rate among the different lure types were subsequently tested with a type II Wald Chi-square test and a post-hoc Tukey test.

3.3.5 Capture of adult wheat midge in pheromone-baited traps as a predictive tool for larval densities

To determine whether adult wheat midge capture in pheromone traps can predict population density, wheat midge larvae in the subsequent generation were sampled at the same field sites where pheromone trapping of adults took place. To sample larvae, wheat heads were collected in mid-August of both study years when plants were between early milk and early dough stages

(Zadoks et al., 1974). Larval sampling occurred between 02 – 18 August at 8 fields, and between 15-31 August at 9 fields in the Peace River region, in 2016 and 2017, respectively. Four fields in Saskatchewan were sampled for larvae between 11-12 August 2017 (Appendix 3.1, Table 3.1).

Samples were collected along three north-south transects at each field: along the field margin in proximity to the pheromone-baited traps, and 20 and 50 m into the field from the margin. Eight samples, each containing 50 wheat heads, were collected along each transect at points parallel to the eight pheromone traps (Figure 3.1) for a total of 24 samples per field. Wheat heads were clipped 5-10 cm below the head and placed into paper bags for transport to the laboratory for processing.

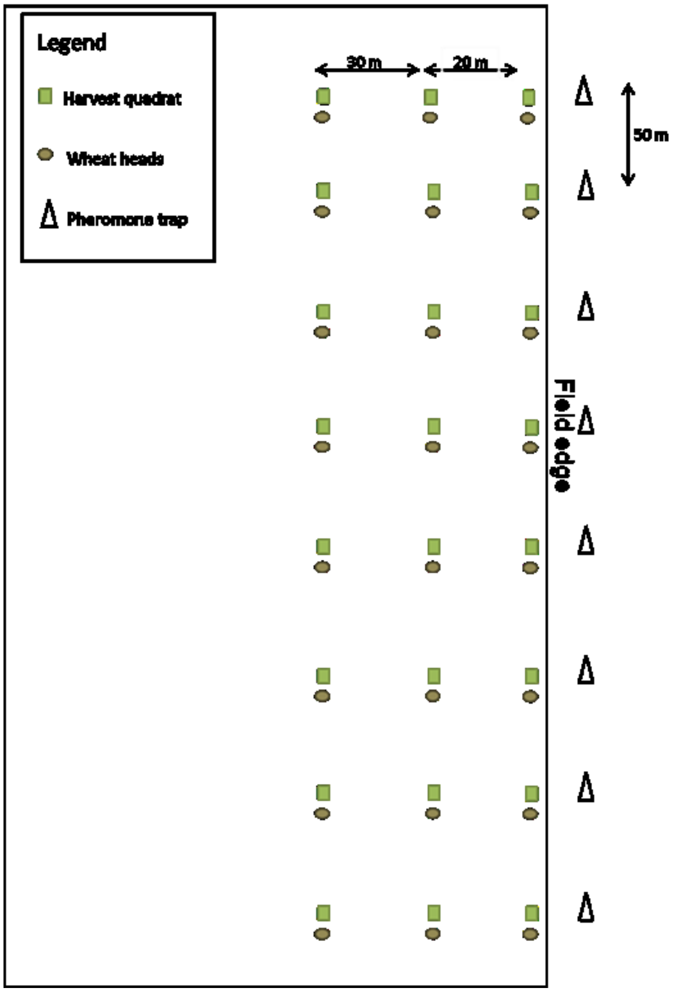

Figure 3.1 Sampling design for collections at commercial wheat fields. Pheromone traps were positioned at the field margin along a north-south transect (8 traps/site). Wheat heads and wheat harvest samples were collected along three parallel north-south transects along the field margin, 20 m and 50 m into the field at 8 points parallel to pheromone trap placement.

Wheat heads were broken apart with a single-head thresher (Almaco specialized equipment model SV SRE-2, 99 M Ave, Nevada, USA), with the air turned off and vents covered to retain larvae. Samples were hand sorted and the number of larvae per sample was counted. Hand-dissections were done to determine the accuracy of samples processed with the single-head thresher. Five heads from each sample were randomly selected for hand-dissection. Since there was no effect of processing method (mixed model ANOVA with method as a fixed factor and site as a random factor, χ^2=1.210, df=1, p=0.271), larval densities used for regression analyses were determined from the remaining 45 heads processed with the single-head thresher.

3.3.6 Statistical analyses for capture of adult wheat midge in pheromone-baited traps as a predictive tool for larval densities

The relationship between adult male midge capture in pheromone-baited traps and larvae in the subsequent generation was examined with repeated measures generalized linear mixed models for 2016 and 2017 (Appendix 3.2, Table 3.4). Male midge capture in all baited traps was calculated cumulatively from the beginning of trapping until the 26th week of year (i.e., early July, when midge emergence usually peaks), from that period until the 29th week of year (i.e., mid-July, when all wheat has completed head emergence), and from that period until the end of trapping. Separate analyses compared the relationship between adult male midge capture in each of the time periods to the total number of larvae sampled for 2016 and 2017 separately. Transect was included as a fixed factor and site was included as a random factor. Conditional and marginal R^2 values were calculated as in Nakagawa and Schielzeth (2013) using the muMIn package (Barton 2018). For 2017 data, location along the north-south was included as a random factor. For 2016 data, the number of larvae was totalled per transect due to overdispersion and poor model fit. Total adult counts were log (x+1) transformed and the model was fit with a Poisson distribution. Differences among transects were analyzed with a post-hoc Tukey test to determine if there was variation in distribution of larvae within fields.

3.3.7 Capture of adult wheat midge in pheromone-baited traps as a predictive tool for harvest metrics

To determine whether pheromone-baited traps can be used as a predictive tool to predict harvest yield or quality loss from wheat midge, wheat samples were collected at the same fields where pheromone monitoring took place. Wheat samples were collected from 8 and 9 sites in 2016 and 2017, respectively (Appendix 3.1, Table 3.1). Wheat was collected when it was ripe (past growth stage 93 Zadoks), between the end of August and the beginning of October. Wheat was collected using the same sampling pattern as described above, but adjacent to the wheat heads that contained larvae (Figure 3.1). All plants within a 1 m^2 quadrat at each sample location were harvested by hand. In 2016, 4 rows were collected, regardless of row spacing, and values were corrected to 1 m^2. In 2017, 1 m^2 samples were collected, and the number of rows varied from three to four depending on row spacing. Plant density estimates could not be determined within each quadrat at harvest due to overlapping plant crowns, each with varying numbers of tillers. In 2016, plant density within the quadrat was estimated during harvest by collecting, cleaning, and counting the plants immediately adjacent to each harvest sample from the field margin transect (Figure 3.1). All intact plants in the outermost seed row along a 1 m transect were collected with a spade so that the number of plants could be counted. Spring plant stand counts were only done at one location in each field in 2016. In 2017, plant density was determined in May by counting the number of plants along a 2 m transect at all sampling locations.

Harvest samples were air dried outdoors (17-36 days in 2016, 15-42 days in 2017) and then in a dryer at 25 °C (9 days in 2016, 15 days in 2017). Dried samples were weighed using a SB12001balance (±0.1 g) (Mettler Toledo™, Argentia Road, Unit 6, Mississauga) to determine above-ground biomass and threshed using a standing thresher (a Wintersteiger™ combine converted to a stationary thresher) with the air turned to the lowest setting. Chaff was removed on an A. T. Ferrell™ seed clipper with a 0.19 cm diameter bottom screen and a 0.32 cm diameter top screen and the air set to 10%. The thousand seed weight was used as the metric to assess seed quality. Two samples of 250 seeds each were weighed using an AX205 analytical balance (±0.001 g) (Mettler Toledo™, Argentia Road, Unit 6, Mississauga) and the mean weight of the two samples was multiplied by 4 to obtain an estimate of thousand seed weight. Percent nitrogen of samples of 120 g per seed was determined using the Dumas method of combustion on a Vario max cube (Elementar™, Elementar-Straße 1, 63505 Langenselbold) with combustion chamber set at 900 °C and oxygen flow rate of 125 mL min −1. Percent nitrogen was converted to percent protein using a conversion factor of 5.7 (Jones, 1941). Wheat midge damage was assigned to samples using a 0-6 scale (Lamb et al., 2000) on samples of 500 seeds.

3.3.8 Statistical analyses for capture of adult wheat midge in pheromone-baited traps as a predictive tool for harvest metrics

The relationship between adult male midge capture in pheromone-baited traps and harvest metrics was examined with repeated measures linear mixed models (Appendix 3.2, Table 3.5). The total number of adults on traps was calculated cumulatively for the three time periods across the season, as described above. The relationship between adult male midge capture and yield (weight of cleaned sample) was analyzed with plant stand density (estimates from plant dissections in 2016, and early season counts in 2017) and cumulative number of male midges as fixed factors and site as a random factor. Individual models were run for cumulative early season, mid-season and season total number of midges for both 2016 and 2017 (Appendix 3.2, Table 3.5). Three seed yield sample outliers with abnormal seed weights (>800 g or <50 g) were excluded, as they were not consistent with the rest of the samples and may have been caused by seeding, nitrogen application errors, or other abiotic field conditions unrelated to wheat midge densities. These outliers were removed to meet the assumption of residual normality, as determined by residual plots and Shapiro-Wilks tests, and removal did not change the interpretation of the analysis. Conditional and marginal R^2 values were calculated as above.

The relationships between the numbers of adult male midge captured on pheromone traps and measures of seed quality were determined with linear mixed models. The relationship between adult male midges captured, and thousand seed weight was examined with repeated measures linear mixed models. The total number of adults on traps for the three time periods was calculated as above. Models of thousand seed weight in both 2016 and 2017 had cumulative adult males captured early and mid-season, and season totals as fixed factors in separate models (Appendix 3.2,

Table 3.5). Models included plant density as a fixed factor and site as a random factor. Models for thousand seed weight assumed a normal distribution. Conditional and marginal R^2 values were calculated as above. The relationship between adult male midges captured and percent protein was examined with repeated measures linear mixed models. The total number of adults on traps for the three time periods was calculated as above. Models of percent protein in both 2016 and 2017 had cumulative adult males captured early and mid season, and season totals as fixed factors in separate models (Appendix 3.2, Table 3.5). Models included plant density as a fixed factor and site as a random factor. Models for 2016 had adult counts log (x+1) transformed (in order to meet assumptions of heteroscedasticity and residual normality as determined by residual plots) and were modelled with a Gamma distribution with a log link. Models for 2017 assumed a normal distribution. Conditional and marginal R^2 values were calculated as above.

The relationship between adult male midges captured in pheromone traps and the degree of midge damage on harvested seeds was examined with a cumulative linear mixed model using the ordinal package (Christensen 2018). The total number of adults in traps for the three time periods was calculated as above. The models were structured with damage (ranked 0-6 as in Wise et al. 2009) modelled with adult trap catch as a fixed factor and site and sample as random factors (Appendix 3.2, Table 3.5). Models were fit with Laplace approximation. The analyses were weighted by the frequency of each damage category for each sample. For the 2016 data, both midge counts and frequencies were log transformed to meet model assumptions and for model convergence. For the 2017 data, only the midge counts were log transformed. P-values for the coefficients were determined with type II Wald Chi-square tests.

3.3.9 Comparison of different commercially available sticky card traps

Unbaited yellow sticky cards were tested as tools for adult wheat midge interception. Field sites were selected that were seeded to wheat midge susceptible wheat cultivars during the monitoring year. Two and four sites were monitored in 2016 and 2017, respectively (Appendix 3.1, Table 3.6). Fields were monitored throughout the wheat midge flight in both years (22 June - 17 August in 2016, and 22 June – 23 August in 2017).

Three different types of yellow sticky cards were compared: 1) a large flat card (Alpha Scents™, 18 cm x 14 cm); 2) a large flat card (Alpha Scents™, 18 cm x 14 cm) rolled into a cylinder; and 3) a small flat card (Great Lakes IPM™, 12 cm x 7 cm). The two types of yellow cards varied visually: the Alpha Scents™ card has a black grid pattern, and the yellow hues differ slightly to the human eye (Figure 3.2). The total surface area available for trapping was equalized by varying the number of traps per treatment in each field. In 2016, a total of 41 traps were placed in each field (Figure 3.3). Two north-south transects were established along the field margin and 10 m into the field from the margin. Ten small flat cards were positioned 10 m apart along each of these transects (Figure 3.3). One large flat and one large rolled card were positioned adjacent to the 3rd, 6th, and 9th small cards from the north end of each transect. Additionally, one trap of each type was positioned at two locations at 50 m and one location at 20 m into the field parallel to the 3rd, 6th and 9th cards of the full transect, respectively (Figure 3.3).

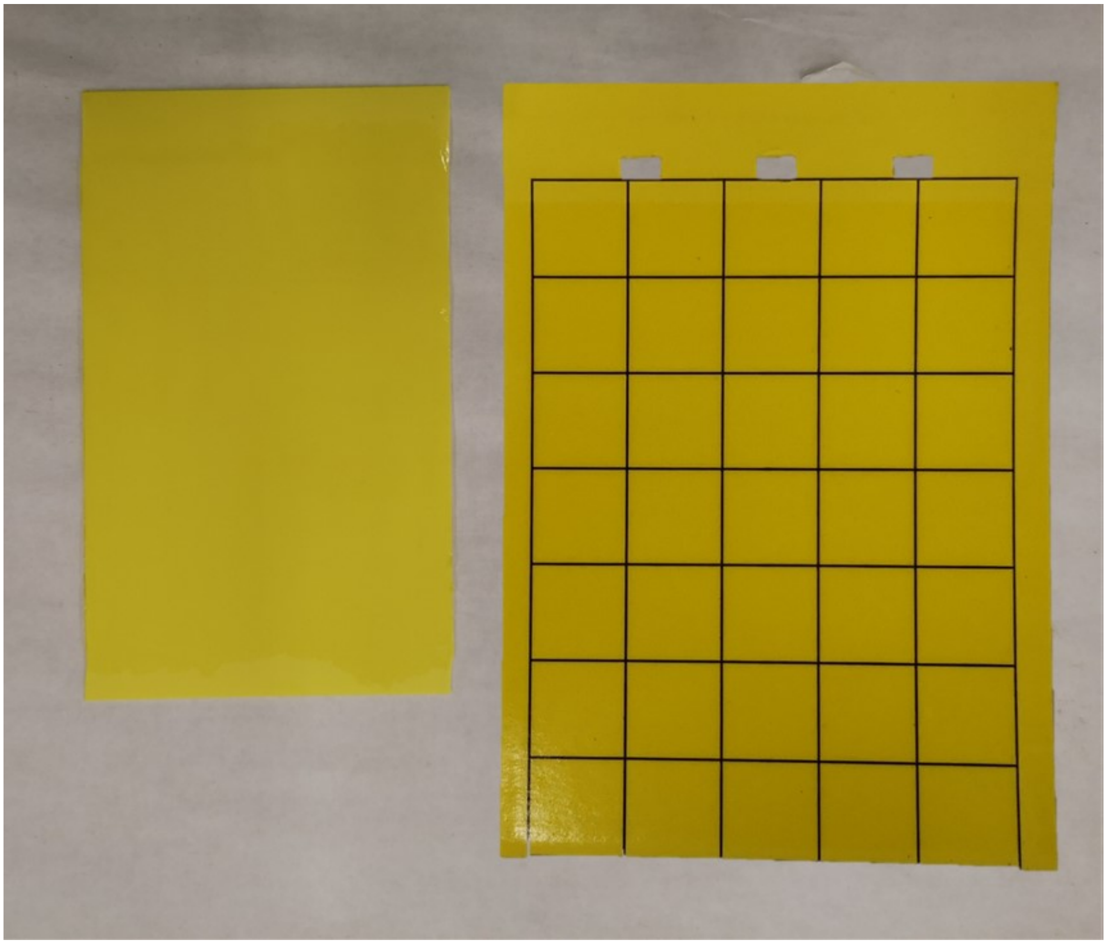

Figure 3.2 Comparison of Great Lakes IPM™ sticky card (12 cm x 7 cm; left) and Alpha Scents™ sticky card (18 cm x 14 cm; right) used in experiments comparing yellow sticky cards for wheat midge monitoring.

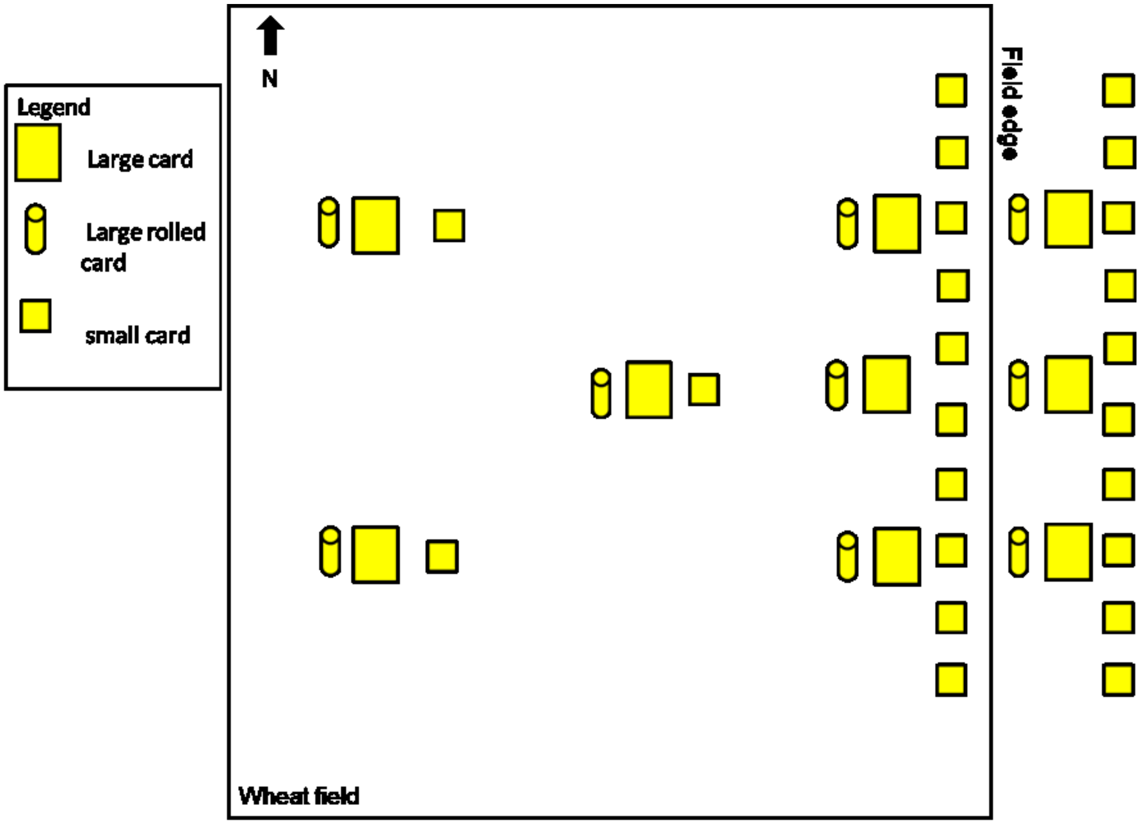

Figure 3.3 Sampling design for sticky card monitoring in 2016. Monitoring was done at commercial wheat fields. Three treatments were tested: a large flat card (18 cm x 14 cm), a large flat card (18 cm x 14 cm) rolled into a cylinder and a small flat card (12 cm x 7 cm). Traps were placed along the field margin, and 10, 20 and 50 m into the field from the margin.

In 2017, yellow sticky cards were positioned along one north-south transect at the field margin (Figure 3.4). Ten small flat cards were positioned 10 m apart along each transect (Figure 3.4). One large flat and one large rolled card were positioned adjacent to the 3rd, 6th, and 9th small cards from the north end of each transect. .

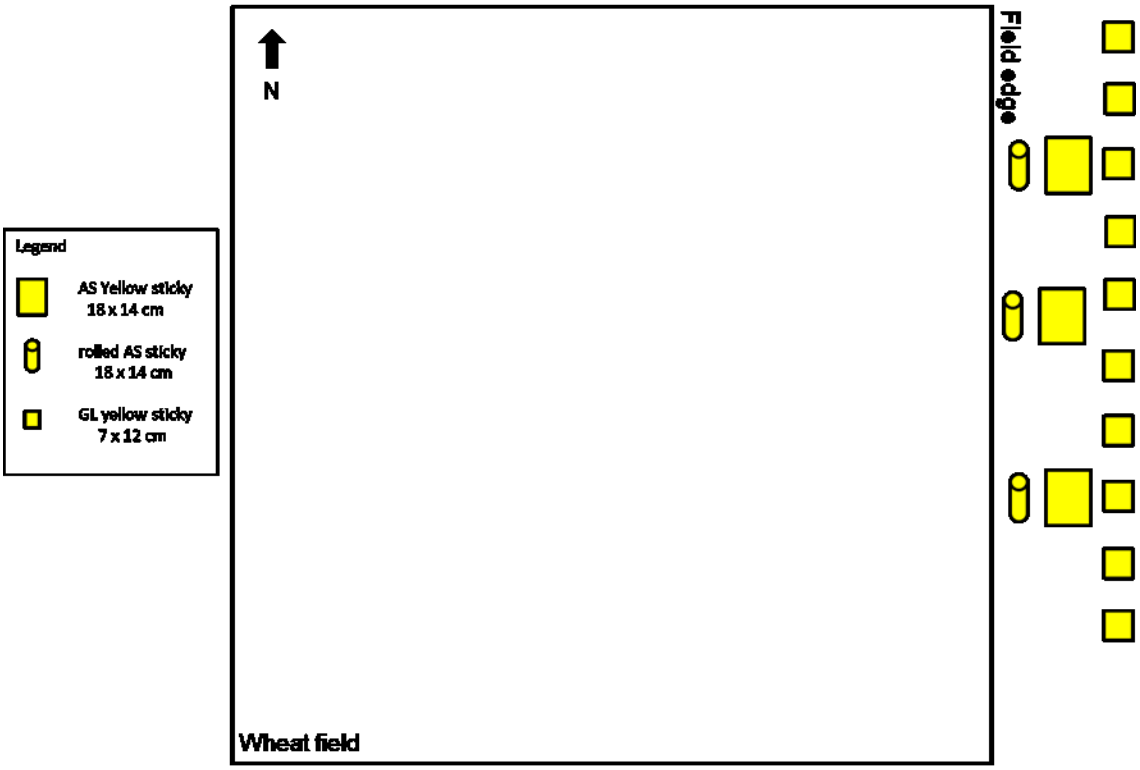

Figure 3.4 Sampling design for sticky card monitoring in 2017. Monitoring was done at commercial wheat fields. Three treatments were tested: a large flat card (18 cm x 14 cm), a large flat card (18 cm x 14 cm) rolled into a cylinder and a small flat card (12 cm x 7 cm). Traps were placed on a north-south transect along the field margin.

Traps were collected and replaced, weather permitting, every 4-10 days between 22 June - 17 August in 2016 and 22 June – 23 August 2017. The number of wheat midges captured on the east- and west-facing sides of the trap was counted separately. Wheat midge adults were identified based on antennal morphology (Harris, 1966), counted and separated by sex. Specimens of the wheat midge parasitoid, *M. penetrans,* were identified using Gibson et al. (1997) and with reference to voucher specimens.

3.3.10 Statistical analyses for comparison of different commercially available sticky card traps

The season total capture of adult midges on the different yellow sticky cards was compared. Data was analyzed with generalized linear mixed models for total male, total female and total parasitoid capture with card type as a fixed factor and site as a random factor (Appendix 3.2, Table 3.7). Data for female midges was fit to a Poisson distribution, while data for males and parasitoids was fit with a normal distribution. Treatment effects were determined with type II Wald Chi-square tests and among group differences were analyzed with post-hoc Tukey tests.

To determine if trap capture differences were due to trap type or the number of traps in a field (10 small flat, and 3 large flat and rolled cards per field), total female capture was modelled at the 3rd, 6th, and 9th traps (from the north end of the trap line, Figure 3.4) for each site. Total counts were converted to rates of capture per cm^2 trapping surface on each trap type by including an offset of the log of the cm^2 in the model (O'Hara and Kotze 2010). The repeated measures generalized linear mixed model included trap type as a fixed factor and trap ID and site as random factors (Appendix 3.2, Table 3.7). The model was fit to a Poisson distribution.

3.3.11 Relationships between adult midges on sticky card traps and larval densities

To determine whether adult wheat midge capture on yellow sticky traps can predict population density, wheat midge larvae in the subsequent generation were sampled at the same field sites where sticky card monitoring took place (Appendix 3.1, Table 3.7). Larvae were sampled at 2 and 4 fields in 2016 and 2017, respectively. Samples were collected at each trap location in 2016. In 2017, five samples, each containing 50 wheat heads, were collected at 20 m intervals along a north-south transect at the field margin in 2017. Wheat heads were clipped and placed into paper bags for transport to the laboratory. Samples were processed as above (Section 3.3.6)

The relationship between the number of female midges on cards and larvae in wheat heads could not be assessed due to the low number of field sites.

3.3.12 Relationships between adult midges on sticky card traps and harvest metrics

To determine whether wheat midge capture on yellow sticky traps can be used as a predictive tool to predict harvest metrics, wheat samples were collected at the same fields where sticky card monitoring took place. Wheat samples were harvested, and yield and quality metrics were measured. Samples were collected from 1 site in 2016 and 2 sites in 2017 (Appendix 3.1, Table 3.7). Samples were collected when wheat had finished ripening and was drying, between the end of August and the beginning of October. Samples were collected adjacent to the wheat head samples collected to assess of larval density. Five samples were collected at 20 m intervals along a north-south transect at the field margin. Wheat stems were hand-harvested within a 1 m^2 sample quadrat. Plant stand counts were done adjacent to the quadrat sampling location in May.

Harvest samples were air dried outdoors for 9 days and then brought indoors and placed in a dryer at 25 °C for 12 days. Dried samples were weighed using a SB12001balance (±0.1 g) (Mettler Toledo™, Argentia Road, Unit 6, Mississauga) balance to determine biomass, threshed, cleaned and assessed as described in Section 3.3.7, above.

The effect of female midges captured on the yield, seed quality and degree of midge damage on seeds could not be assessed due to the low number of field sites.

3.3.13 Comparison of soil core samples to subsequent adult wheat midge in emergence and pheromone-baited traps

Soil core samples to estimate density of overwintering wheat midge cocoons were collected in commercial wheat fields and compared to subsequent adult wheat midge capture in emergence and pheromone-baited traps positioned in the same fields the following spring. In 2016, 8 field sites in the Peace River region and 2 field sites in Saskatchewan were monitored to assess cocoons using soil core samples (Appendix 3.1, Table 3.1). In 2017, 9 field sites in the Peace River region and 4 field sites in Saskatchewan were monitored (Appendix 3.1, Table 3.1). Soil cores were collected following harvest (04 November 2016 in SK, 03-10 May 2017 in AB, and 11 October – 09 November

2017). Soil cores were collected using a steel corer (1.9 cm diam. X 10.8 cm depth). A total of 17 cores (Doane et al., 2000) were collected at each site, positioned 20 m apart along an "M" pattern starting 20 m into the field from the margin. Soil samples were wet sieved (Doane et al., 1987) and cocoons were dissected to determine parasitism rates.

Emergence traps were positioned at these same sites the following spring in the Peace River region (but not in Saskatchewan). Emergence traps consisted of 2 L plastic pails (Plastipak™) with the bottoms cut off (Doane and Olfert 2008). Each bucket had ventilation holes lined with fine mesh cut into the sides 3x 2.54 cm in diameter) and in the lid (1x 5.08 cm in diameter). Emerging midges and parasitoids were sampled using a single clear polyethylene strip coated with Stikem special™ (Scotts™) (14 cm x 5 cm sticky surface) that was secured under the lid of each trap. Traps were placed with the bottom 7.6 cm of each bucket buried in the soil. Emergence traps were checked 3 times weekly between 20 June – 05 August at 7 sites in 2016, and between 06 June – 09 August at 5 sites in 2017. Traps were positioned 10 m apart along a north-south linear transect within the field along the first row of wheat stubble from the margin. In 2016, 8 traps were placed in each field. Due to low midge capture in emergence traps in 2016, 20 traps were placed in each field in 2017. Pheromone traps (n=8) were positioned at adjacent fields to all sites. See section 3.3.1 for details.

3.3.14 Statistical analyses for comparison of soil core samples to adult trap captures

The number of viable cocoons was determined by subtracting the number of parasitized and dead larvae from the total number of cocoons collected at each site. The number of viable cocoons was divided by surface area of the field sampled to determine cocoons per m^2. Cocoons per m^2 were analyzed with linear models with total number of midges per site captured in emergence traps as a fixed factor and separately with total number of midges per site captured in pheromone traps as a fixed factor (Appendix 3.2, Table 3.8).

3.4 Results

3.4.1 Comparison of commercially available pheromone traps

In 2016, there was a marginal effect of trap colour on the number of male midges captured (χ^2= 3.582, df= 1, p= 0.058). Orange traps captured more wheat midges (Figure 3.5), however, this effect was not significant when the surface area of the sticky trapping surface was included as a covariate in the model (χ^2= 0.637, df=1, p= 0.425). As would be expected, more midges were captured in pheromone-baited traps than in unbaited control traps (χ^2=333.213, df= 3, p <0.001), but all lure types attracted a similar number of male midges (Figure 3.5).

In 2017, orange traps captured more midges than green traps, when surface area was not included in the model (χ^2=12.281, df=1, p< 0.001). When trapping surface area was added to the model, there was no effect of trap colour on wheat midge capture (χ^2=0.3645, df= 1, p= 0.546). Lure type significantly affected trap capture in 2017 (χ^2=349.868, df=3, p<0.001). Pheromone-baited traps captured more midges than control traps, and traps baited with the flex lures captured more midges compared to traps baited with the rubber septa lures from Great Lakes IPM™ (Figure 3.5).

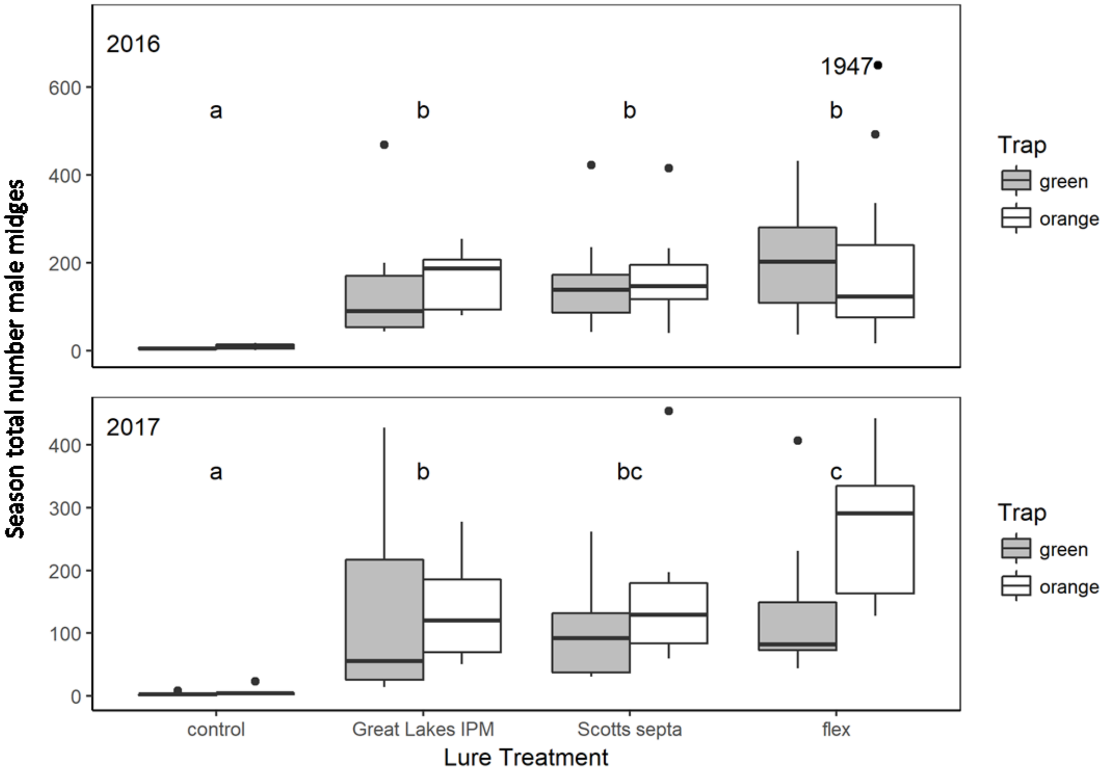

Figure 3.5 Season total number of male midges captured in Delta traps (green or orange) baited with either a rubber septa lure (Great Lakes IPM™ or Scotts™), a flex lure (Scotts™) or no lure (control). Traps were positioned in commercial fields (n=8 in 2016 and 9 in 2017) in the Peace River region of AB during wheat midge flight and replaced weekly. Letters indicate significant differences among lure treatments within year (Post-hoc Tukey tests, α=0.05). The dark midline represents the median and the bottom and top of the box indicate the first and third quartiles, respectively. The vertical lines, or whiskers, indicate the maximum value, or 1.5 times the interquartile range, whichever is smaller. Outliers are represented by points above the whiskers, with the number of midges indicated for extreme outliers. Note: y-axes for 2016 & 2017 have different scales.

In Saskatchewan in 2017, there was a significant interaction between trap type and lure type (χ^2=20.616, df=2, p<0.001) that affected midge capture. Orange traps baited with flex lures captured more midges than green traps baited with flex lures, however, there was no difference in trap capture between trap types in the unbaited control traps or traps baited with the rubber septa Great Lakes IPM™ lures (Figure 3.6).

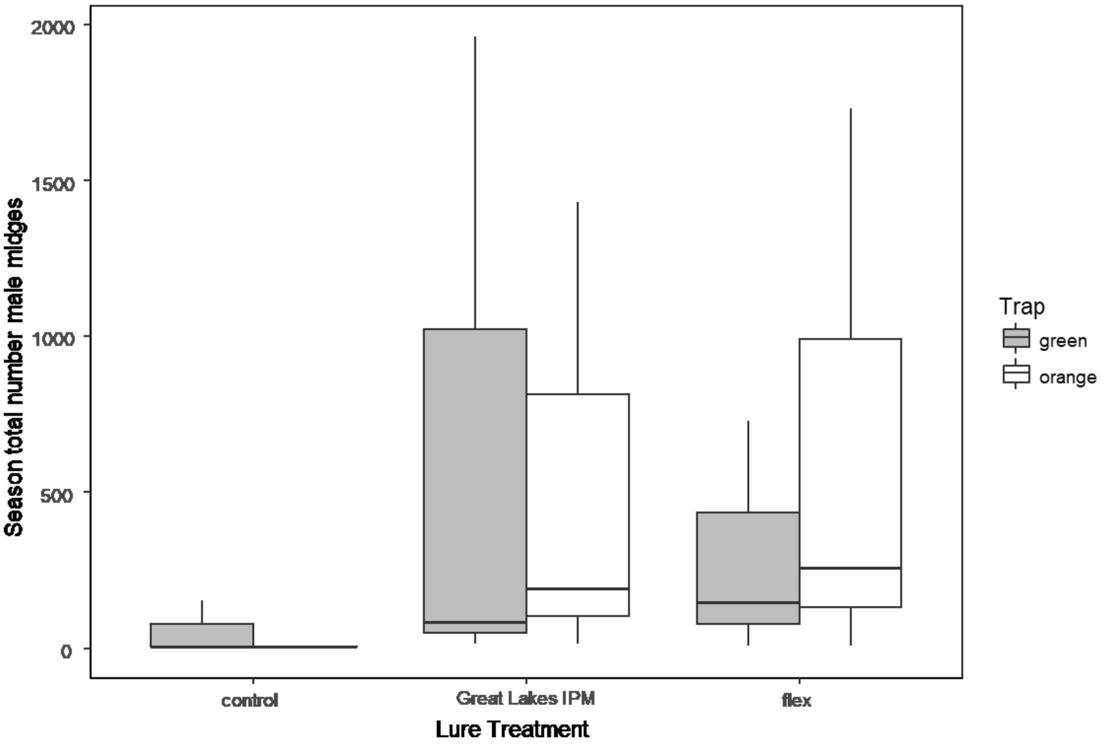

Figure 3.6 Season total male midges captured in Delta traps (green or orange) baited with either a rubber septa lure (Great Lakes IPM™), a flex lure (Scotts™) or no lure. Traps were positioned in commercial fields in Saskatchewan (n=3) in 2017. The dark midline represents the median and the bottom and top of the box indicate the first and third quartiles, respectively. The vertical lines, or whiskers, indicate the maximum value, or 1.5 times the interquartile range, whichever is smaller. When surface area was included in the model there was still a significant interaction between trap type and lure type (χ^2=20.6227, df= 2, p< 0.001). Green traps baited with Great Lakes IPM™ lures captured more midges than orange traps baited with Great Lakes IPM™ lures, however there was no difference between trap types for the other lure treatments.

In 2017 and 2016, some fields in the southeastern Peace River region had two peaks of trap capture during wheat midge flight (Figure 3.7A). Wheat midge flight continued for more than 6 weeks in all sites (Figure 3.7). Peak flight varied among sites but occurred between weeks 25 and 27 at all sites monitored (Figure 3.7).

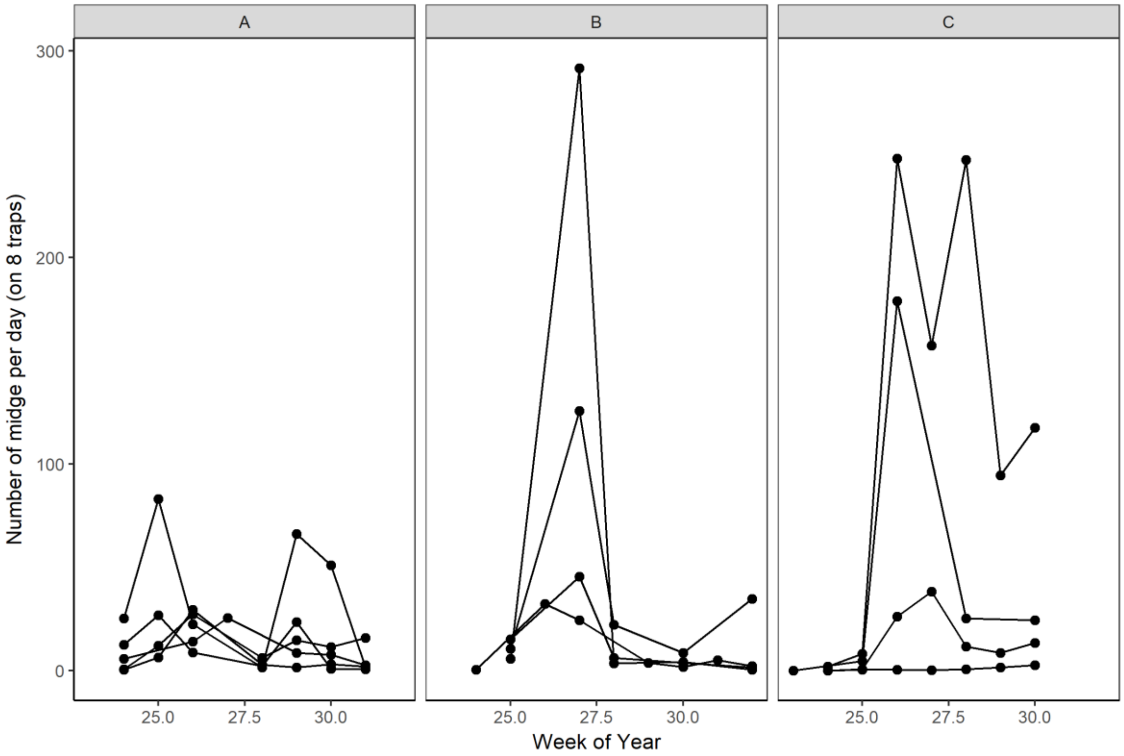

Figure 3.7 Seasonal trends in total male midge trap capture per site in the Peace River Region of Alberta in 2017. Each line represents trap capture from an individual field site. Delta traps baited with either a pheromone lure (Scotts™ flex lure, Scotts™ septa or Great Lakes IPM™) or a no lure control were changed weekly during wheat midge flight. Boxes A,B and C contain data from sites in the (A) Southeastern Peace River region; (B) Southwestern Peace River region; and (C) Saskatchewan trapping regions.

3.4.2 Release rates of pheromone from lures

The red rubber septa lures from Great Lakes IPM™ released more pheromone than Scotts™ flex lures (χ^2=7.262, df= 2, p= 0.026). There was high variability in the amount of pheromone released from the septa lures over 3 days from both Great Lakes IPM™ (mean= 2427 ng ± 2459 SD) and Scotts™ (mean = 1321 ng ± 1670 SD) compared to the flex lures (mean=123 ng ± 43 SD) (Figure 3.8).

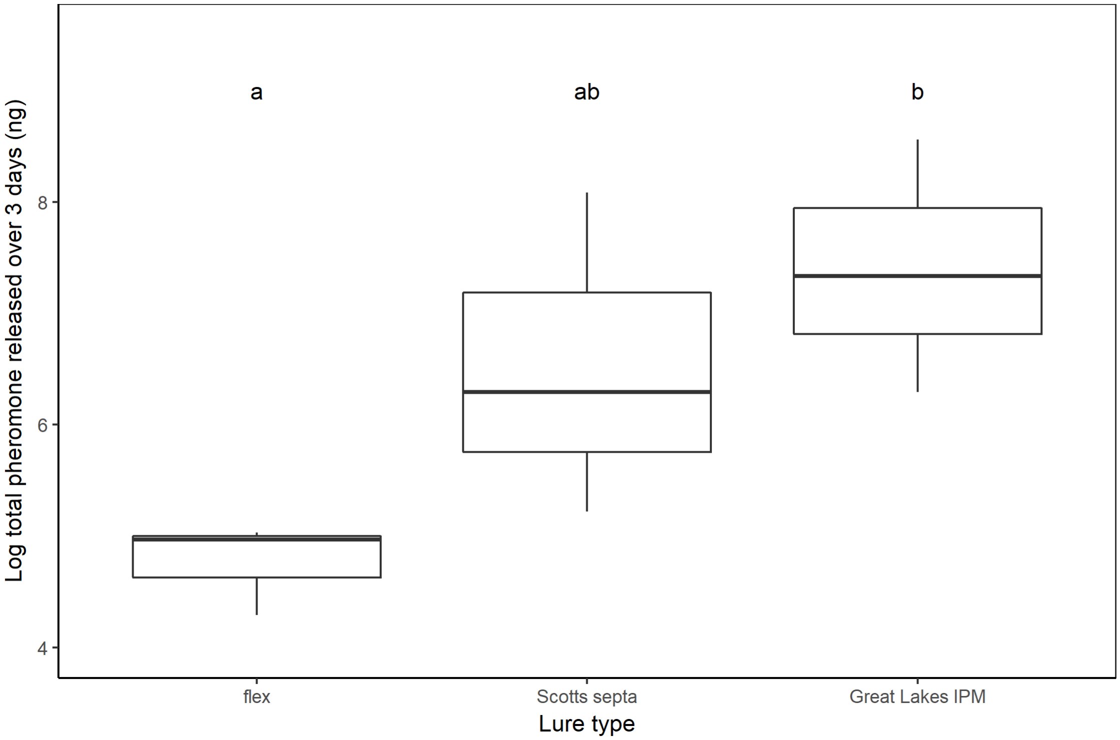

Figure 3.8 Total amount of wheat midge pheromone (2,7-nonanediyl dibutyrate) released (log ng) over three days from: Scotts™ flex lure, Scotts™ septa lure and Great Lakes IPM™ septa lures (n=3 lures per treatment). Letters represent significant differences among lure types (Post-hoc Tukey test, α=0.05). The dark midline represents the median and the bottom and top of the box indicate the first and third quartiles, respectively. The vertical lines, or whiskers, indicate the maximum value, or 1.5 times the interquartile range, whichever is smaller.

3.4.3 Capture of adult wheat midge in pheromone-baited traps as a predictive tool for larval densities

The season total number of adult midges captured in pheromone-baited traps (n=6 per site) did not predict the number of larvae in wheat heads at the same site in 2016 (General linear mixed regression, marginal $r^2 = 0.08$, $\chi^2=0.134$, df= 1, p=0.713) or 2017 (General linear mixed regression, marginal $r^2 = 0.004$, $\chi^2=0.014$, df= 1, p=0.903). This result was consistent with models of 2016 cumulative counts of early (General linear mixed regression, marginal $r^2 = 0.12$, $\chi^2=0.741$, df= 1, p=0.389) and mid-season (General linear mixed regression, marginal $r^2 = 0.09$, $\chi^2= 0.199$, df= 1, p= 0.655) adult midge capture. Similarly, models for 2017 cumulative counts of early (General linear mixed regression, marginal $r^2 < 0.001$, $\chi^2=0.118$, df= 1, p=0.871) and mid-season (General linear mixed regression, marginal r^2 =0.003, χ^2=0.0265 df= 1, p=0.732) adult midge capture were not significant.

The ability of adult midges captured in pheromone-baited traps to predict numbers of larvae in wheat heads could not be analyzed for samples from Saskatchewan due to the small number of field sites. In 2016, there was a significant effect of the location of the sampling transect on the number of larvae recovered in wheat head samples (χ^2=4190, df= 2, $p<0.001$). The number of larvae found in samples decreased with distance into the field (Figure 3.9). In 2017, there was no difference in the number of larvae collected in each of the different sampling transects (χ^2=2.6375, df= 1, p=0.1044), although there was a weak trend for more larvae in samples from the field edge compared to 50 m into the field (Figure 3.9).

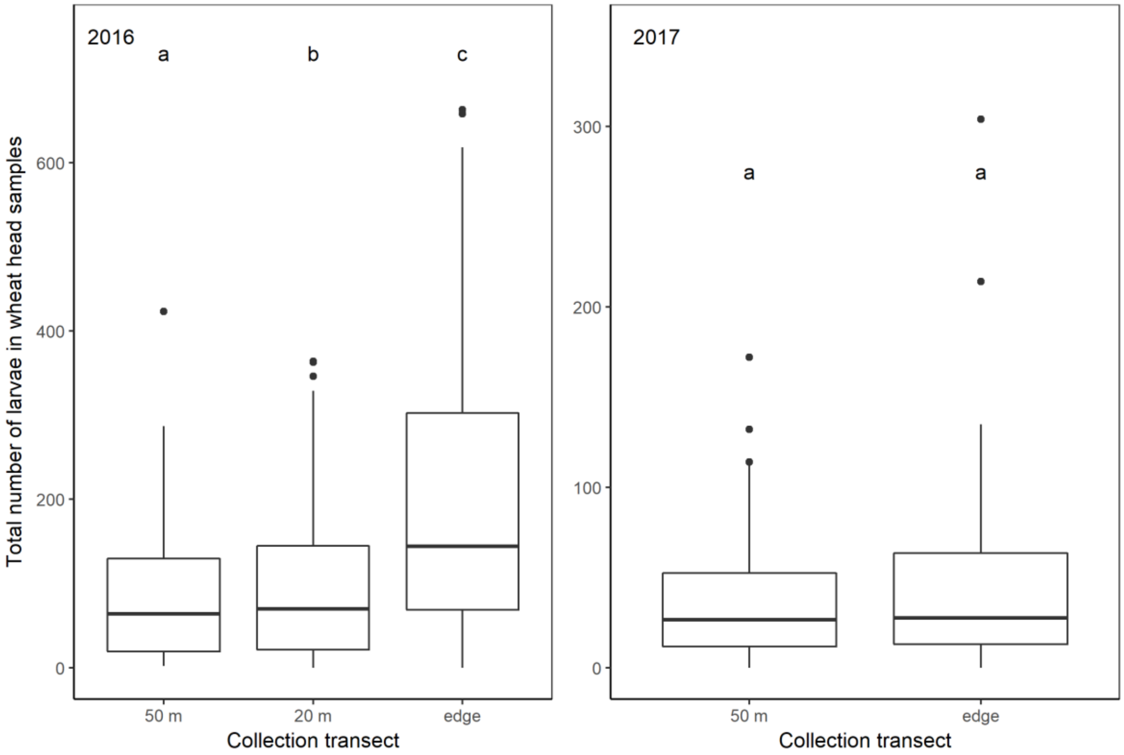

Figure 3.9 Total number of larvae in wheat head samples collected along different transects: along the field edge, 20 m into the field from the edge, and 50 m into the field from the edge. In 2016, 24 samples of 50 heads each were collected from each of 8 fields. In 2017, 16 samples of 50 heads each were processed from each of 9 fields. In 2017, samples from the 20m transect were not processed. Letters above the plot indicate significant differences among groups (post-hoc Tukey test, α=0.05). The dark midline represents the median and the bottom and top of the box indicate the first and third quartiles, respectively. The vertical lines, or whiskers, indicate the maximum value, or 1.5 times the interquartile range, whichever is smaller. Outliers are represented by points above the whiskers. Note: Y-axes have different scales.

3.4.4 Capture of adult wheat midge in pheromone-baited traps as a predictive tool for harvest metrics

In 2016, the number of adult male midges captured in pheromone-baited traps was not related to subsequent wheat yield, thousand seed weight or kernel protein content measured at the same sites (Table 3.9). This finding was consistent whether trap capture from early-season, mid-season, or the season total was used in the analysis. There was a weak trend of lower harvest metrics with higher adult male midge numbers (Figure 3.10). The number of male midges did not significantly affect the damage ratings of seed samples (Table 3.9), although sites with higher midge counts did tend to have fewer seeds in the undamaged “0” category than sites with lower numbers of midges (Figure 3.10 D).

Table 3.9 Summary of parameters of repeated measures linear mixed models used to compare the effect of adult male midges captured in pheromone-baited traps and harvest metrics in 2016 and 2017. A total of 16, 1 m2 quadrats were harvested from each of 8 fields in 2016 and 9 fields in 2017. Weight of 1000 seeds was determined by multiplying the mean weight of 250 seeds per sample by 4 and percent protein was determined by near infrared imaging. Midge damage was assessed on a 0-6 (Lamb et al. 2000) scale for 500 seeds and the relationship between trap capture and damage was analyzed with ordinal regression. All χ2 values were determined from χ2 Wald tests, r2 values are marginal r2 values Z values were determined from the Wald statistic.

Midge counts		Yield			1000 seed weight			Protein			Midge damage	
2016		χ^2	r^2	P	χ^2	r^2	p	χ^2	r^2	p	z	p
	Early-season	0.027	0.076	0.870	0.061	0.008	0.804	0.321	-	0.571	-0.39	0.693
	Mid-season	0.127	0.079	0.910	0.219	0.030	0.639	0.001	-	0.974	1.279	0.201
	Season total	0.028	0.080	0.867	0.262	0.035	0.609	0.001	-	0.969	1.391	0.164
2017												
	Early-season	0.946	0.06	0.331	0.560	0.053	0.454	2.14	0.116	0.144	-0.56	0.575
	Mid-season	1.345	0.075	0.246	1.16	0.102	0.280	1.737	0.098	0.187	-1.04	0.297
	Season total	1.12	0.062	0.299	1.89	0.152	0.170	1.880	0.105	0.170	-1.78	0.755

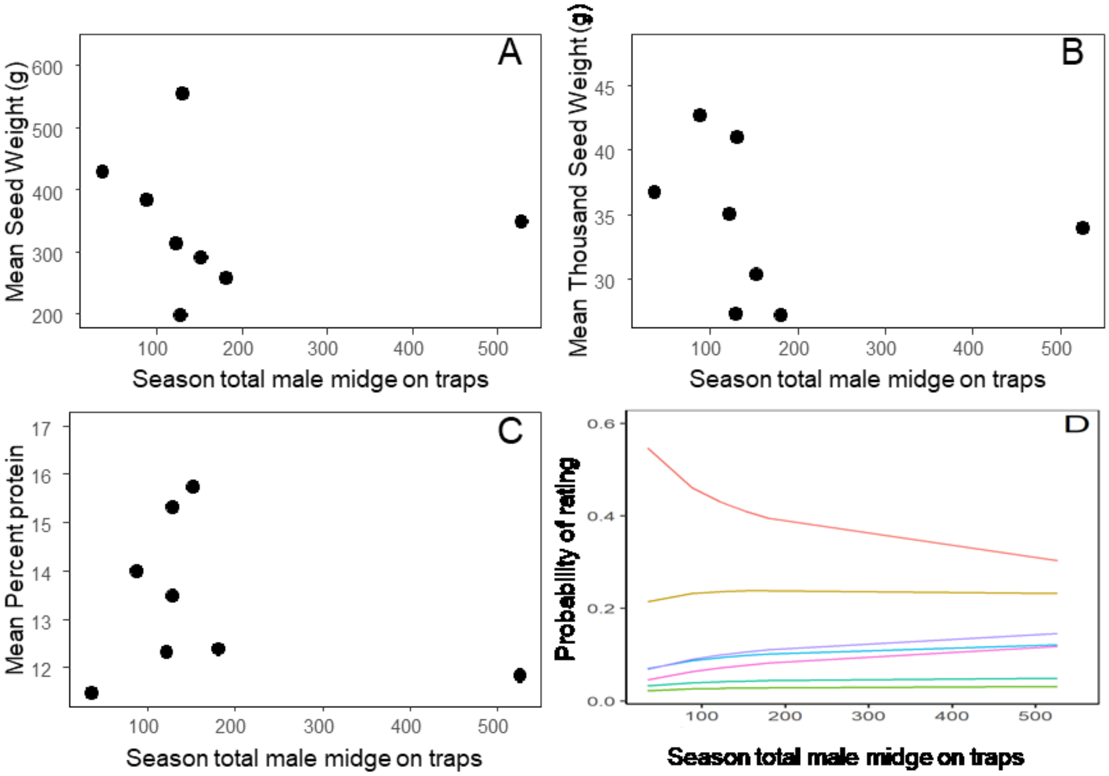

Figure 3.10 Effect of season total male midges captured in pheromone-baited traps at 8 sites in 2016 on subsequent wheat harvest metrics. The male midges captured per trap per day for 6 baited traps were totalled per site. In graphs A-C, black lines represent linear lines of best fit, while dark shading represents 95% confidence intervals. A. Mean seed weight per site was calculated from 16, 1 m^2 *samples per site for illustration. B. The thousand seed weight was calculated by multiplying the mean weight of 2 samples of 250 by 4 for 16 samples per site. Means per site were calculated for illustration. C. Percent protein was determined using Dumas combustion for 8, 120 g samples of seed per site (from the field margin). Mean protein content per site was calculated for illustration. D. Relationship between the probability of seed samples being assigned each damage rating (0-6) and the season total number of male midges captured on pheromone traps. Damage ratings were given for 500 seeds from each of 16 samples collected from the above sites. Damage ratings are from (Lamb et al. 2000). Red= 0, yellow= 1, dark green = 2, light green = 3, light blue = 4, dark blue = 5, pink =6.*

In 2017, there were no significant relationships between the number of male midges captured in pheromone-baited traps and subsequent seed yields, thousand seed weight, or kernel protein content of sampled wheat heads (Table 3.9.9). However, wheat seed yield and protein content tended to decrease with increasing midge capture in traps (Figure 3.11A, C), thousand seed weight appeared to increase with more midges sampled in traps (Figure 3.11B).

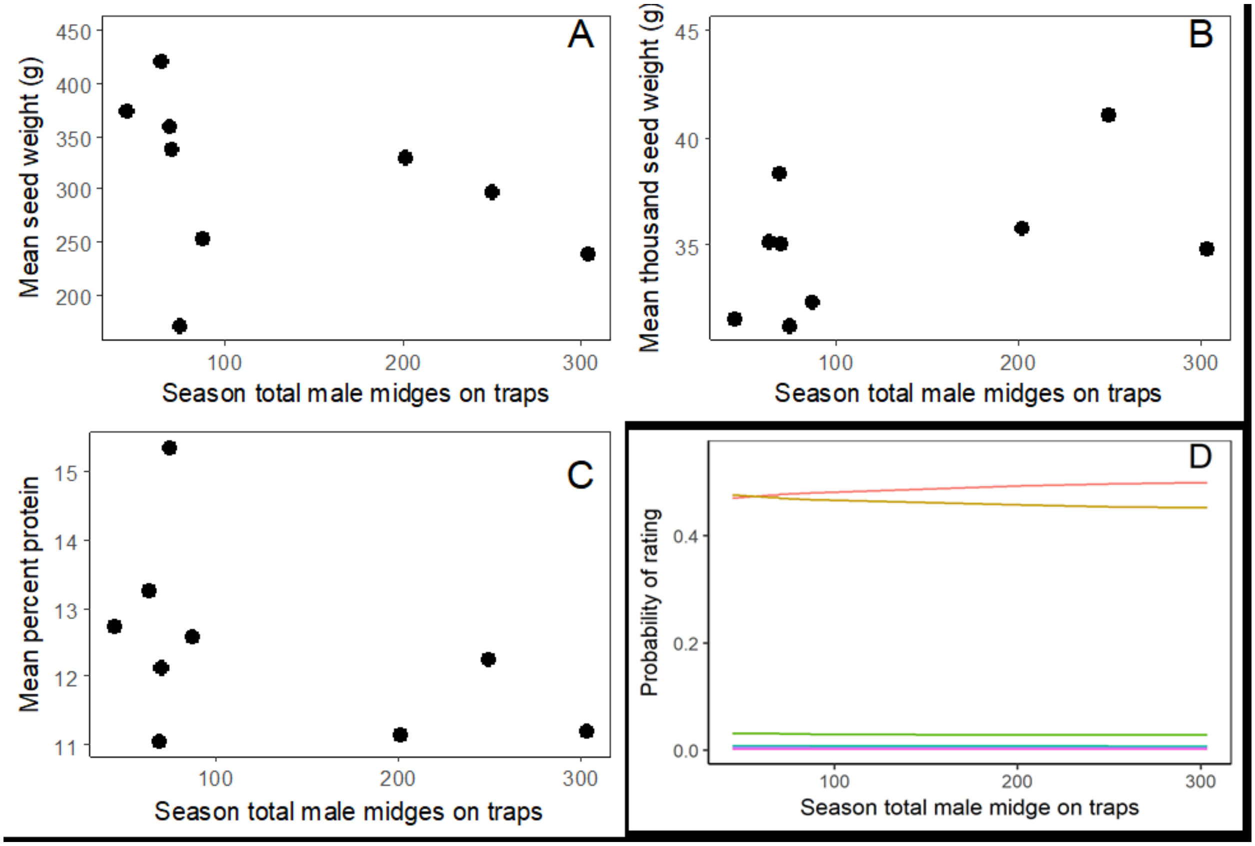

Figure 3.11 Effect of season total male midges captured in pheromone-baited traps on harvest metrics at 9 sites in 2017. The male midges captured per trap per day in 6 baited traps were totalled per site. On all graphs, black lines represent linear lines of best fit, while dark shading represents 95% confidence intervals. A. Mean seed weight per site was calculated from 16 1 m^2 *samples per site for illustration. B. The thousand seed weight was calculated by multiplying the mean weight of 2 samples of 250 by 4 for 16 samples per site. Means per site were calculated for illustration. C. Percent protein was determined using Dumas combustion for 8 samples per site (from the field margin). Mean protein content per site was calculated for illustration. D. Relationship between the probability of seed samples being assigned each damage rating (0-6) and the season total number of male midges captured on pheromone traps. Damage ratings for 500 seeds from each of 16 samples collected from the above sites were used. Legend: Red= rating 0, yellow=rating 1, dark green = 2, light green = 3, light blue = 4, dark blue = 5, pink =6.*

3.4.5 Comparison of different commercially available sticky card traps

In both 2016 and 2017, the type of sticky card influenced season total capture of female midges (χ^2=196.68, df= 2, p<0.001). In both years, the smaller yellow sticky cards captured more female midges than the larger yellow cards arranged in either a flat or rolled configuration (Figure 3.12).

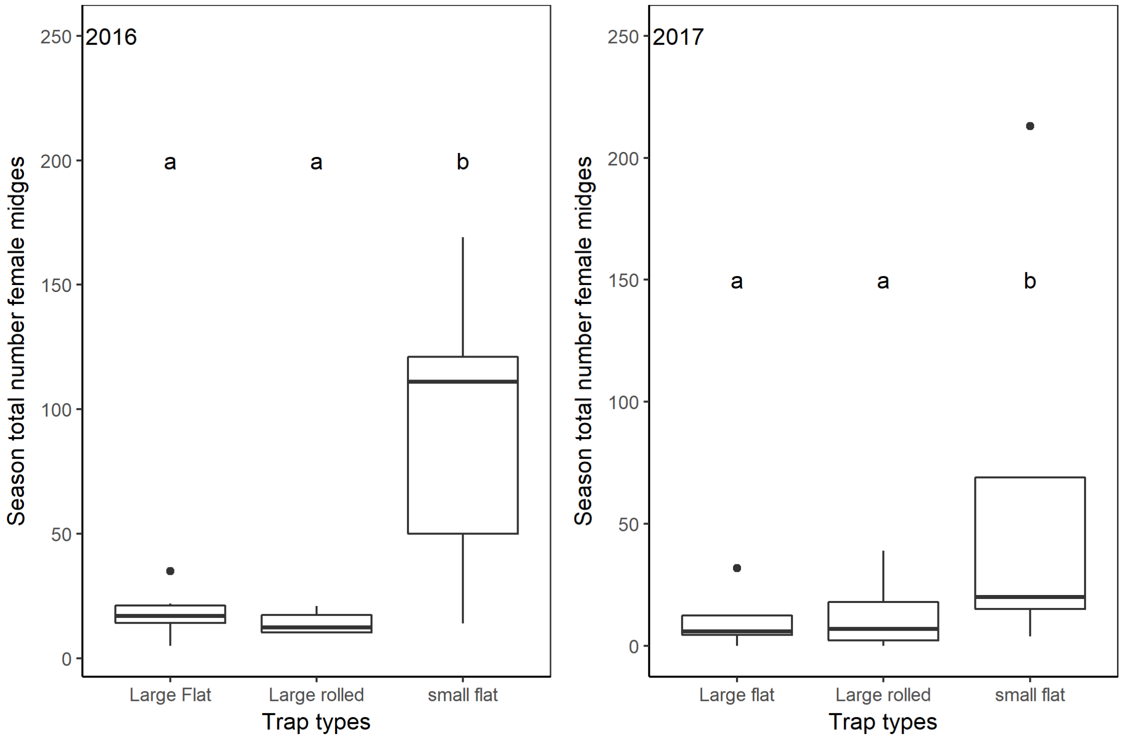

Figure 3.12 Effect of yellow sticky card type on season total number of female midges captured (n=41 cards per site in 2016 and 16 cards per site in 2017). The large flat cards, large rolled cards and the small flat cards were monitored in commercial wheat fields (n=2 in 2016, n=4 in 2017). In 2016, 41 cards were positioned in each field: along the field margin, 10 m into the field and 50 m into the field. In 2017, 16 traps were positioned along the field margin of each field. The dark midline represents the median and the bottom and top of the box indicate the first and third quartiles, respectively. The vertical lines, or whiskers, indicate the maximum value, or 1.5 times the interquartile range, whichever is smaller. Outliers are represented by points above the whiskers. Letters represent significant differences among groups (Post-hoc Tukey test, α=0.05).

To determine whether this difference was due to the number of cards per field, counts from only the three cards adjacent to the larger cards were also compared. Differences in surface area were accounted for by including log surface area in the model as covariate. The analysis using only three small cards indicated that more female midges were captured on small cards than either configuration of the large card (χ^2=20.81, df= 2, p<0.001). Male wheat midge capture was not analyzed due to low total numbers of males captured on yellow sticky cards. There were differences in the number of wheat midge parasitoids captured on the different cards tested

(χ^2=7.28, df= 2, p=0.027). The small cards captured more parasitoids than the large rolled cards and marginally more parasitoids than flat large cards.

3.4.6 Comparison of soil core samples to subsequent adult wheat midge in emergence and pheromone-baited traps

There were no significant relationships between the number of cocoons found in soil cores in the fall of 2016 and the number of adult midges captured in emergence traps (t =0.17, df= 1, p=0.872) or pheromone traps (t=0.27, df= 1, p=0.797) the following year.

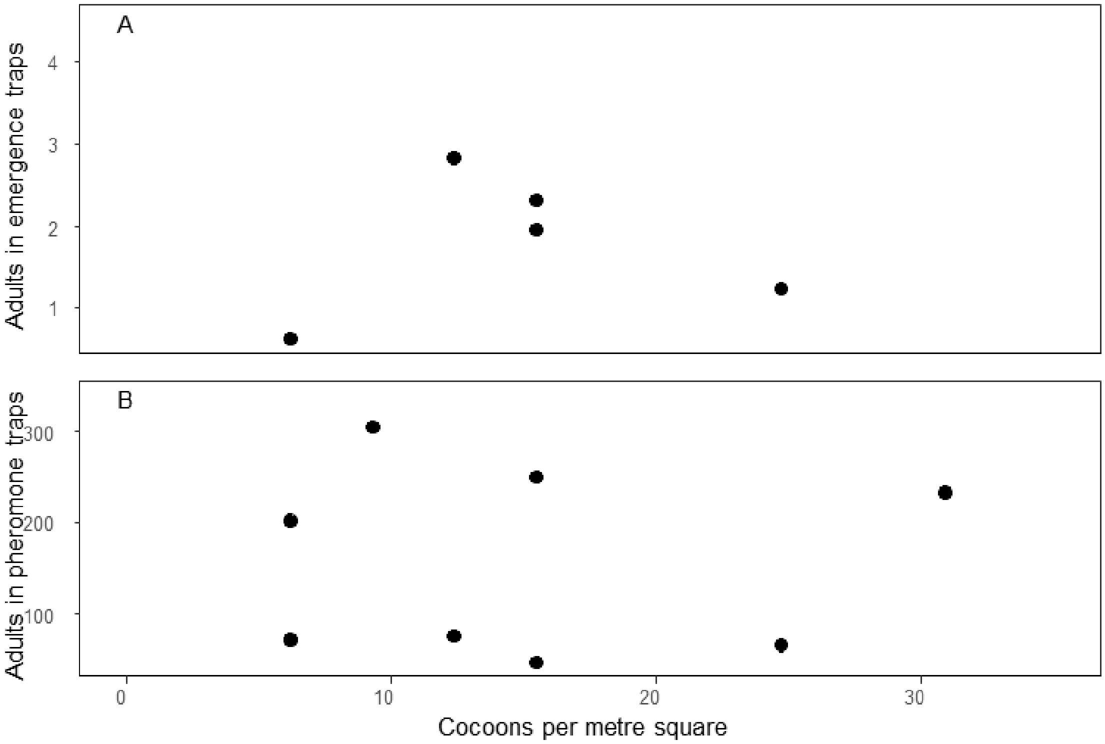

Figure 3.13 Relationships between larval cocoons in soil core samples collected in fall 2016 and subsequent adult midges captured in (A) emergence traps, and (B) pheromone baited traps at sites in the Peace River region in 2017. Dark lines represent lines of best fit and gray areas represent 95% confidence intervals. A. The number of adult midges in 20 emergence traps at each of 5 sites were totalled for traps collected every 2-7 days in 2017. B. The number of adult male midges in 8 pheromone-baited traps at each of 8 sites was totalled for traps collected weekly in 2017.

3.5 Discussion

The tools tested in this study are useful to monitor wheat midge activity but further work is needed to relate midges sampled to economic thresholds. Capture of female midges on yellow sticky cards was indicative of low or high populations of midge larvae. Economic thresholds developed in other parts of the Prairie Provinces, recommend an action threshold of 4 or more adult midges per 10 yellow sticky cards to control populations above the larval threshold of 2 larvae per wheat spike (Lamb et al., 2002). Using this threshold, female midges captured on yellow sticky cards in the current study correctly categorized midge larval populations above the threshold at 1 site and below the threshold at 2 sites. One site, however, was miscategorised as below threshold based on capture of midges on yellow sticky cards. Capture of midges on yellow sticky card traps may accurately indicate the reproductive population of wheat midges as it is biased towards females. Ninety-seven percent of the midges captured on the traps were female. In Manitoba and southeastern Alberta, at least 95% of wheat midges entering wheat fields are female (Smith et al., 2007). While sex pheromone traps capture mostly males (99% of wheat midges captured were male), they were more sensitive than yellow sticky cards and captured a mean of 17 times more midge per cm^2 than yellow sticky cards at nearby sites. In Belgium, pheromone-baited traps captured 100 to thousand times more wheat midges than sticky or pan traps (Chavalle et al., 2019). The pheromone-baited traps, however, did not indicate low or high populations of midge larvae sampled at the same sites. Soil core samples did not reliably predict densities of adult midges that emerged from the soil or male midges on pheromone-baited traps. Further research should be done to determine whether the soil core survey better predicts wheat midge adult emergence over multiple years or in other regions of Alberta.

Yellow sticky cards have been previously studied as a monitoring tool for wheat midge (Chavalle et al., 2019; Lamb et al., 2002), but different sampling patterns, trap sizes and sample sizes have not previously been compared. In this study, small, flat cards captured more wheat midges than the large cards in different configurations. Yellow sticky cards captured high numbers of wheat midge parasitoids, which indicates this tool has the potential to monitor parasitoid populations. This would be valuable as parasitism rates in wheat midge can vary from 3 to 100% (Chavalle et al., 2018), and could be used to refine the action threshold for wheat midge. The small flat traps captured more *M. penetrans* per cm^2 than the large cards whether in the flat or rolled configuration. Yellow sticky cards were sensitive to wheat midge parasitoid populations, as they captured 374 times more *M. penetrans* than pheromone-baited traps.

The large cards have a black grid pattern printed on the plastic while the small cards are plain yellow, and the yellow background hue may differ between the two cards. Other dipteran pests differentiate and have preferences between yellow cards painted different yellow hues. Capture of *Rhagoletis indifferens* Curran (Diptera: Tephritidae) was lower on Alpha Scents™ sticky cards and other painted cards compared to cards with darker and greener yellow paint. However, cereal pests are usually equally attracted to white and yellow traps (Kirk, 1984). It is possible that the smaller yellow cards presented a silhouette similar to that of a wheat plant. This silhouette might attract flying wheat midge, especially as size and edge contours can be more distinguishable visual cues at a distance than colour (Prokopy and Owens, 1983).

Within the family Cecidomyiidae, the importance of colour for orientation differs among species. Trap colour did not affect capture of any *Dasineura* (Diptera: Cecidomyiidae) species tested in pears, black currant and blackberry (Amarawardana 2009). Capture of male and female saddle gall midges, *Haplodiplosis marginata* van Roser (Diptera: Cecidomyiidae), and *Dasineura amaramanjarae* Grover (Diptera: Cecidomyiidae) does not differ among differently coloured un-baited sticky card traps (Skuhravý et al., 1983; Ur Rehman et al., 2014). For these species, olfactory cues may play a more important role in host and mate location. In many *Dasineura* species, females are attracted to host volatiles in wind tunnel and field experiments (Hall et al., 2012), but potential interactions between visual and olfactory cues in these species are unknown. In other Cecidomyiidae, colour affects attraction. Both sexes of the hessian fly, *Mayetiola destructor* Say (Diptera: Cecidomyiidae), are more attracted to green LEDs than red and amber LEDs (Schmid et al., 2017), while sorghum midge, *Contarinia sorghicola* Coquillett (Diptera: Cecidomyiidae), are attracted to yellow, red and white traps (Sharma et al., 1990). In both *M. destructor* and *C. sorghicola*, olfactory cues enhance female attraction to coloured traps (Harris, 1990; Sharma and Franzmann, 2001). The importance of colour differs among stages of host location for *M. destructor*: attractive colours and wheat volatiles increase approaching and landing behaviours, but only wheat volatiles increase oviposition (Harris, 1990).

Although there is evidence that colour impacts orientation of wheat midge adults, the green or orange colour of pheromone traps did not impact wheat midge capture in Alberta. Female wheat midges lay more eggs on wheat heads placed against a contrasting background than a similarly coloured background (Gharalari, 2008). Additionally, male wheat midges are more attracted to yellow than blue un-baited traps (Oakley and Smart, 2002 as cited in Gharalari, 2008). The importance of different colours or hues of colour within the range of green – red spectrum to wheat midge orientation is unknown, as is the relative importance of olfactory and visual cues. Understanding the point at which colour becomes important in host or mate location would benefit trap optimization. For male wheat midges, olfactory cues may be more important than visual cues for mate location, but visual cues may be important in the absence of olfactory cues.

At the sites in Saskatchewan, there was an interaction between trap colour and lure type that affected trap capture. Green traps baited with Great Lakes IPM™ lures captured more midges per cm^2 than orange traps baited with Great Lakes IPM™ lures. There were no differences in capture in traps of different colour for the other lures tested. The same trend appeared in the Alberta data but was not significant. All pheromone traps in Saskatchewan captured more midges than traps in Alberta, so the interaction may not be apparent at lower midge densities. For female coffee berry borers, *Hypothenemus hampei* Ferrari (Coleoptera: Curculionidae), red pheromone-baited traps were more attractive than white traps, especially at the lower pheromone doses tested (Mathieu et al., 1997). Interactions between pheromone release rate and visual cues have also been observed in apple maggots (Epsky and Heath, 1998). Although the pheromone release rate from septa lures was far greater than that from the flex lures, fewer male midges were captured in traps baited with septa than flex lures. The septa lures are in initially loaded with a higher volume of pheromone (1 mg) than the flex lures (100 µg). The high pheromone release rate may actually inhibit or decrease oriented flight response in wheat midge, as occurs in other insect species (Roelofs, 1978), including *Dasineura tetensi* Ruebs (Diptera: Cecidomyiidae) (Amarawardana, 2009), and *M. destructor* (Harris and Foster, 1991). Dose-dependent responses of male wheat midge to pheromone in other studies, however, show no upper threshold (Bruce et al., 2007). Male wheat midges orient to traps baited with lures that release up to 137 µg of pheromone a day, although a release rate of 0.38 µg per day is sufficient for wheat midge monitoring (Bruce et al., 2007). The apparent interaction may be influenced by the variability of release rates among individual septa lures. Individual Great Lakes IPM™ lures in different traps may have had different release rates. The rate of pheromone release depends on temperature, wind, the type of lure, and the loading and chemical composition of the pheromone (McDonough, 1991). The effects of temperature will also vary depending on the molecular weight and polarity of the pheromone (McDonough, 1991). The degree of pea moth pheromone rotational isomerization varies depending on the curing process of different natural rubber septa lures, and there is an interaction between initial loading and septa lure on trap capture (Horák et al., 1989). Increased variability in release ratios of multi-component pheromones from natural rubber septa is related to different solvent types and volume loadings (Kuenen and Siegel, 2015). Daily release rates of the wheat midge pheromone from polyethylene vials increases >3x by increasing lure loadings from 5 mg to 10 mg (Bruce et al., 2007). In the UK, rubber septa lures loaded with 1 mg or 5 mg of pheromone performed as well as polyethylene vials loaded with 5 mg of pheromone over a 1-month period, however rubber septa lures from different sources, in different environmental conditions or over different time periods may perform differently. High variation in release rates among lures could cause inaccurate indications of wheat midge activity, which could delay management activities and cause unnecessary damage. Pheromone traps used for monitoring wheat midge activity need to be sensitive enough to indicate the start of emergence, so that producers can time in-field scouting.

The flight phenology of male wheat midges varied across the study region. Multiple peaks of male wheat midges captured in pheromone traps at sites in the southeastern Peace River region may be due to staggered emergence of adult midges resulting from prolonged diapause in the cocoon stage. Wise and Lamb (2004) suggested that delayed emergence dates in European records may be due to prolonged diapause. Wheat midge can remain in prolonged diapause for up to 13 years, if soil conditions are inadequate for emergence (Barnes, 1956), but rainfall at all sites monitored in this study was in the optimum range (30 -145 mm) for wheat midge emergence (Elliott et al., 2009), except in 2016 when some sites had greater than 145 mm of rain in May and June. Staggered emergence of adult midges within the same cohort can also be caused by abiotic conditions related to timing of rainfall events or temperature accumulation (Elliott et al., 2009; Jacquemin et al., 2014). Two peaks in wheat midge flight activity in some sites in the Peace River region may indicate two periods of wheat midge oviposition in those areas. Wheat heads are only susceptible to wheat midge damage during head emergence and before anthesis (Elliott and Mann, 1996). If peak wheat midge activity occurs twice over a 5-week flight period, the chance of midge emergence synchronizing with wheat heads being at the susceptible stage increases. Use of wheat varieties In Germany, wheat cultivars are used specifically because heads emerge asynchronously to peak midge flight, avoiding damage. (Basedow and Schütte, 1974). Alternatively, multiple pheromone trap capture peaks could also reflect changes in trap efficiency over the season, as occurs in codling moth, *Cydia pomonella* Linnaeus (Lepidoptera: Tortricidae) pheromone monitoring (Riedl et al., 1976). The responsiveness of male insects to female sex pheromones may vary due to competition with females or due to abiotic conditions (McNeil, 1991). Trapping efficiency may decrease across the field season as release rates from pheromone lures decrease. Rubber septa lures used to monitor *H. marginata* last up to 5 weeks under field conditions, but may release >80% of the pheromone load in 10 days under high temperatures and windy conditions (Rowley et al., 2017). Estimates of the field life of pheromone lures for other Cecidomyiidae vary between 2 weeks and 1 year, depending on molecular weight and lure substrate (Hall et al., 2012). The longevity of rubber septa lures loaded with wheat midge pheromone is unknown. Understanding how long wheat midge lures remain effective in the field is critical to implement effective monitoring tactics that capture midges with peak trap efficiency.

There were no significant relationships between pheromone trap capture and the number of wheat midge larvae in wheat heads. Bruce et al. (2007) found a significant relationship between capture of male wheat midges in Delta traps baited with pheromone released from polyethylene vials and larval infestations, but also found high variability in capture among fields only 100m apart. In Saskatchewan, Mirciou (2004) found a significant relationship between male midges captured in green Delta traps baited with pheromone released from rubber septa and larval infestations only in wheat fields seeded on wheat stubble. In the Peace River region, many producers rotate wheat and canola on a two-year cycle. Sex pheromone traps may not detect potential sites of infestation because wheat midge males mate at the emergence site and females travel to oviposition sites (Smith et al., 2007). It is not known if males disperse after mating but male midges fly an average of 400 m on flight mills (Hao et al., 2013). Because pheromone-baited traps only attract males, capture may not indicate the reproductive population. In 2016, more wheat midge larvae were present in samples collected at the field edge than at 20m or 50 m into the field. Higher numbers of midge larvae are found at field edges in Germany (Basedow, 1977), but not in Manitoba (Lamb et al., 1999). In 2017, no differences in larval density occurred between the field edge and 50 m into the field. The differences in distribution may only be apparent at higher wheat midge densities. The effect in this study was not due differences in wheat midge movement into the field owing to crop rotation, as producer-cooperators maintained the same crop rotation patterns for both years. Higher larval density at field margins was not consistent between years, therefore restricting management activities to field margins is not recommended.

There were no relationships between harvest metrics and pheromone trap capture. Decreased wheat yield and quality are related to the percentage of seeds infested with wheat midge larvae (Lamb et al., 2000). Pheromone traps were unable to predict larval densities, and therefore, unable to predict damage caused by larvae. The ability of traps baited with female-produced sex pheromones to predict economic damage in field crops ranges from successful (Tamhankar et al., 2000), to moderately successful (Furlong et al., 2013; Knight and Light, 2005) and unsuccessful (Levine, 1991), when examined across different systems. In this study, a weak trend of increased seed weight with increased trap capture was evident. Increased seed weight, as a result of plant overcompensation, has been observed in wheat infested with *Contarinia triticii* Kirby (Diptera: Cecidomyiidae) but not with wheat midge (Basedow and Schütte, 1973; Lamb et al., 2000).

The number of cocoons recovered from soil cores did not predict the numbers of adult midges that emerged the following spring. No relationships were found between soil cores collected in the fall and assessments of adult wheat midge populations in the same fields or in fields <1km away. Attempts to forecast wheat midge populations from soil samples have been imprecise in Germany (Basedow and Schütte, 1974), the UK (Oakley et al., 1998) and in Manitoba (Lamb et al., 1999). Wheat midge larvae experience variable mortality over winter (Basedow and Gillich, 1982) and require adequate soil moisture to emerge in the spring (Basedow and Schütte, 1971). Soil cores may be inaccurate monitoring tools because 5 (Wise and Lamb, 2004) to 96 % (Basedow and Gillich, 1982) of overwintering midge larvae undergo prolonged diapause. Additionally, identification of Cecidomyiidae larvae in soil samples is difficult and misidentifications can occur even when done by experts (Aitkenhead et al., 1955). Determining risk in agricultural management systems requires monitoring tools that can accurately assess spatial and temporal distributions of pest densities and reliably predict damage. Without accurate tools, growers cannot make decisions about the integration and use of potential control methods.

The development of economic thresholds relies on understanding the relationships between sampled population estimates, population growth, and damage (Onstad, 1987). Relationships between damage and population estimates are complicated and can be related to multiple stages of plant responses; vary among different host, pest, and biological interactions; and vary with environmental conditions (Pedigo et al., 1986). Proposed theoretical and mathematical models for economic threshold development have been criticized alternately as too simple to accurately represent biological processes or too complicated to be of practical use (Bor, 1995; Kogan, 1998; Mumford and Norton, 1984; Pedigo et al., 1986; Saphores, 2000). Developing economic thresholds that are both practical and realistic becomes increasingly critical as the call for whole system and regional management practices increases (Brewer and Goodell, 2012; Lewis et al., 1997). Developing action thresholds for wheat midge that minimize labour intensive in-field scouting would decrease both wheat yield losses and unnecessary insecticide applications. Further examinations of monitoring tools, such as yellow sticky cards, that monitor female midges entering wheat fields are recommended.

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Appendix 3.1

Tables referring to site information:

Table 3.1 Location of commercial wheat field sites monitored for wheat midge with sex pheromone baited traps in 2016 and 2017. Two types of Delta traps and three types of pheromone lures were compared in a full-factorial randomized block design. Traps were placed in fields throughout the wheat midge flight period in both years.

		Site GPS Coordinates			
Nearest town	Province	Latitude (degrees)	Longitude (degrees)	Year monitored	Wheat cultivar
Girouxville	AB	55.77	-117.28	2016	Stettler
McLennan	AB	55.65	-117.05	2016	Superb
Guy	AB	55.55	-117.18	2016	Roblin
Jean Cote	AB	55.97	-117.39	2016	Stettler

Beaverlodge	AB	55.21	-119.40	2016	Stettler
Elmworth	AB	55.11	-119.46	2016	Stettler
Hythe	AB	55.31	-119.47	2016	CDC Go
Rolla	BC	55.85	-120.19	2016	Thorsby
Llewellyn	SK	52.18	-107.50	2016	Plentiful
Saskatoon	SK	52.15	-106.57	2016	Stanley
Girouxville	AB	55.76	-117.28	2017	Stettler
McLennan	AB	55.65	-117.05	2017	Superb
Girouxville	AB	55.74	-117.38	2017	Harvest
Guy	AB	55.56	-117.21	2017	Roblin
Jean Cote	AB	55.96	-117.42	2017	Stettler
Hythe	AB	55.32	-119.44	2017	Eli
Rolla	BC	55.86	-120.19	2017	Thorsby
Elmworth	AB	55.12	-119.43	2017	Stettler
Beaverlodge	AB	55.21	-119.40	2017	Stettler
Radisson	SK	52.43	-107.49	2017	-
Hafford	SK	52.67	-107.37	2017	AC Barrie
Perdue	SK	52.06	-107.66	2017	AC Andrew
Llewellyn	SK	52.18	-107.50	2017	AC Brandon

Table 3.6 Susceptible wheat field sites where adult wheat midge was monitored with yellow sticky cards in 2016 and 2017. Sites were seeded to a cereal crop during the monitoring year. Traps were positioned in fields during wheat midge flight.

		Site GPS Coordinates				
Nearest town	Province	Latitude (degrees)	Longitude (degrees)	Year monitored	crop species	Wheat cultivar
Girouxville	AB	55.72	-117.29	2016	Wheat	Stettler
Girouxville	AB	55.78	-117.31	2016	Barley	-

Girouxville	AB	55.76	-117.29	2017	Wheat	Stettler
Jean Cote	AB	55.96	-117.42	2017	wheat	Stettler
Girouxville	AB	55.78	-117.39	2017	wheat	Harvest
Valleyview	AB	55.08	-117.35	2017	wheat	Stettler

Appendix 3.2

Tables summarizing models used in statistical analyses.

Table 3.2 Summary of generalized linear mixed models used for analysis of season total males captured on different pheromone Delta traps (green or orange) and with different lures (Scotts™ Flex, Scotts™ Septa, or Great Lakes IPM™ septa) at n= 8 field sites in 2016, n= 9 field sites in AB in 2017, and n=3 field sites in SK in 2017.

Experiment	**Model**		**Variance distribution**
Pheromone trap comparison (2016)	Glmer (total males) ~Lure+ Trap+(1|Site)	Random intercept	Gamma – log link
Pheromone trap comparison (2017)	Glmer (total males) ~Lure+ Trap+(1|Site)	Random intercept	Negative binomial
Pheromone trap comparison (Sask.)	Glmer (total males) ~Lure*Trap+(1|Site)	Random intercept	Poisson

Pheromone trap comparison, cm^2 (2016)	Glmer (total males) ~Lure+ Trap+(offset (log cm^2)) +(1|Site)	Random intercept	Negative binomial
Pheromone trap comparison, cm^2 (2017)	Glmer (total males) ~Lure+ Trap+(offset (log cm^2)) +(1|Site)	Random intercept	Negative binomial
Pheromone trap comparison, cm^2 (Sask.)	Glmer (total males) ~Lure + Trap+(offset (log cm^2)) +(1|Site)	Random intercept	Negative binomial

Table 3.3 Summary of linear mixed model used for analysis of log of release rates from 3 different commercially available lure (Scotts™ Flex, Scotts™ Septa, or Great Lakes IPM™ septa). N=3 lures of each type were aerated over 3 days.

Experiment	**Model**		**Variance distribution**
Release rate analysis	Lmer (log (release rate)) ~Lure type+(1|Day) +(1|ID)	Random intercept (for day and ID)	normal

Table 3.4 Summaries of generalized linear mixed models used to examine the relationship between adult counts on pheromone traps (totals of n=6 pheromone-baited traps at each of 8 sites in 2016 and 9 sites in 2017) for early, mid and late season and densities of larvae in wheat head samples (n=45 wheat heads per sample, 24 samples per site were analyzed in 2016 and 16 in 2017).

Experiment	**Model**		**Variance distribution**
Larval densities – early season (2016)	Glmer (total larvae/transect) ~early cumulative adults + transect + (1|Site)	Random intercept	Poisson – log link
Larval densities – mid season (2016)	Glmer (total larvae/transect) ~ log (mid-season cumulative adults) + transect + (1|Site)	Random intercept	Poisson – log link
Larval densities – season total (2016)	Glmer (total larvae/transect) ~ log (season total cumulative adults) + transect + (1|Site)	Random intercept	Poisson – log link

Larval densities – early season (2017)	Glmer(larvae)~early cumulative adults + transect + (1|Site) +(1|location)	Random intercept (site and sample location)	Poisson – log link
Larval densities – mid season (2017)	Glmer (larvae)~ mid-season cumulative adults + transect + (1|Site) +(1|location)	Random intercept (site and sample location)	Poisson – log link
Larval densities – season total (2017)	Glmer(larvae)~ season total cumulative adults + transect + (1|Site) +(1|location)	Random intercept (site and sample location)	Poisson – log link

Table 3.5 Summaries of generalized linear mixed models used to examine the relationships between adult counts on pheromone traps (totals of n=6 pheromone-baited traps at each of 8 sites in 2016 and 9 sites in 2017) for early, mid and late season and harvest metrics determined from n=16 1 m^2 quadrat samples collected from the same field sites. Harvest metrics examined include: total seed weight (yield), thousand seed weight (quality), percent protein (quality), and distribution of damage ranks (0-6 rating).

Experiment	Model		Variance distribution
Yield – early season (2016)	Lmer (seed weight) ~early cumulative adults + plant density + (1|Site) +(1|location)	Random intercept (for both site and sample location)	Normal
Yield – mid season (2016)	Lmer (seed weight) ~ mid-season cumulative adults + plant density + (1|Site) +(1|location)	Random intercept (for both site and sample location)	Normal
Yield – season total (2016)	Lmer (seed weight) ~ season total cumulative adults + plant density + (1|Site) +(1|location)	Random intercept (for both site and sample location)	Normal
Yield – early season (2017)	Lmer (seed weight) ~early cumulative adults + plant density + (1|Site) +(1|location)	Random intercept (site and sample location)	Normal
Yield – mid season (2017)	Lmer (seed weight) ~ mid-season cumulative adults + plant density + (1|Site) +(1|location)	Random intercept (site and sample location)	Normal
Yield – season total (2017)	Lmer (seed weight) ~ season total cumulative adults + plant density + (1|Site) +(1|location)	Random intercept (site and sample location)	Normal
Thousand seed weight – early season (2016)	Lmer (thousand seed weight) ~ season total cumulative adults + plant density + (1|Site) +(1|location)	Random intercept (site and sample location)	Normal

Thousand seed weight – mid season (2016)	Lmer (thousand seed weight) ~ mid-season cumulative adults plant density + (1|Site) +(1|location)	Random intercept (site and sample location)	Normal
Thousand seed weight – season total (2016)	Lmer (thousand seed weight) ~ season total cumulative adults + plant density + (1|Site) +(1|location)	Random intercept (site and sample location)	Normal
Thousand seed weight – early season (2017)	Lmer (thousand seed weight) ~ early season cumulative adults + plant density + (1|Site) +(1|location)	Random intercept (site and sample location)	Normal
Thousand seed weight – mid season (2017)	Lmer (thousand seed weight) ~ mid-season cumulative adults plant density + (1|Site) +(1|location)	Random intercept (site and sample location)	Normal
Thousand seed weight – season total (2017)	Lmer (thousand seed weight) ~ season total cumulative adults + plant density + (1|Site) +(1|location)	Random intercept (site and sample location)	Normal
Percent protein – early season (2016)	Glmer (percent protein) ~early cumulative adults + (1|Site) + (1|location)	Random intercept (site and sample location)	Gamma – log link
Percent protein – mid season (2016)	Glmer (percent protein) ~log (mid-season cumulative adults) + (1|Site) + (1|location)	Random intercept (site and sample location)	Gamma – log link
Percent protein – season total (2016)	Glmer (percent protein) ~log (season total cumulative adults) + (1|Site) + (1|location)	Random intercept (site and sample location)	Gamma – log link
Percent protein – early season (2017)	Lmer (percent protein) ~ early cumulative adults + (1|Site) +(1|location)	Random intercept (site and sample location)	Normal
Percent protein – mid season (2017)	Lmer (percent protein) ~ mid-season cumulative adults + (1|Site) +(1|location)	Random intercept (site and sample location)	Normal
Percent protein – season total (2017)	Lmer (percent protein) ~ season total cumulative adults + (1|Site) +(1|location)	Random intercept (site and sample location)	Normal
Damage ratings – early season (2016)	CLMM (Damage rank) ~log (early cumulative adults) + (1|Site) +(1|location), weights = frequency of rank	Random intercept (site and sample location)	
Damage ratings – mid season (2016)	CLMM (Damage rank) ~ log (mid-season cumulative adults) + (1|Site) +(1|location), weights = frequency of rank	Random intercept (site and sample location)	

Damage ratings – season total (2016)	CLMM (Damage rank) ~ log (season total cumulative adults) + (1|Site) +(1|location), weights = frequency of rank	Random intercept (site and sample location)
Damage ratings – early season (2017)	CLMM (Damage rank) ~log (early cumulative adults) + (1|Site) +(1|location), weights = frequency of rank	Random intercept (site and sample location)
Damage ratings – mid season (2017)	CLMM (Damage rank) ~ log (mid-season cumulative adults) + (1|Site) +(1|location), weights = frequency of rank	Random intercept (site and sample location)
Damage ratings – season total (2017)	CLMM (Damage rank) ~ log (season total cumulative adults) + (1|Site) +(1|location), weights = frequency of rank	Random intercept (site and sample location)

Table 3.7 Summaries of (generalized) linear mixed models used to compare season totals of male, female wheat midges, and parasitoids on different yellow sticky cards in n=4 field sites. Captures were totalled across n=10 small cards, n=3 large cards, and n=3 large rolled cards for the first 3 models. Captures were totalled across n=3 of each card type for the last model of female capture.

Experiment	**Model**		**Variance distribution**
Sticky card comparison – female capture	Glmer (season total females) ~ card type + (1|Site)	Random intercept	Poisson – log link
Sticky card comparison – male capture	Lmer (season total males) ~ card type + (1|Site)	Random intercept	normal
Sticky card comparison – parasitoid capture	Lmer (season total parasitoids) ~ card type + (1|Site)	Random intercept	normal
Sticky card comparison, cm^2 – female capture	Glmer (season total females) ~ card type+ offset(log(cm^2)) + (1|Site)	Random intercept	Poisson – log link

Table 3.8 Summaries of linear models used to compare viable cocoons per m^2 to total number of midges captured on emergence traps (n=20) and total number of midges captured on pheromone traps (n=8) at 9 field sites.

Experiment	**Model**	**Variance distribution**
Cocoon comparisons – emergence traps	Lm (cocoons per m^2) ~ total midges in emergence traps	Normal
Cocoon comparisons – pheromone traps	Lm (cocoons per m^2) ~ total midges in pheromone traps	normal

Chapter 4: Discussion

The overall goals of this study were to understand wheat midge biology in the most northern distribution of its invasive range and to develop appropriate monitoring tools for its management in this region. Wheat midge is an invasive pest of Palearctic origin and has a widespread distribution including China, Japan, and North America (Basedow and Schütte, 1971; Borkent, 1989; Helenius and Kurppa, 1989; Miao et al., 2013). In the Peace River region of Alberta (55.19°N, -119.40°W), wheat midge has been a sporadic but damaging pest since 2011. We examined life history traits of wheat midge that could vary with range expansion such as overwintering success, parasitism rates, emergence patterns, phenology, adult longevity, and periods of adult activity. We compared the effectiveness of available monitoring tools (different commercially available sex pheromone lures and traps, different sizes of yellow sticky cards, soil core samples) for wheat midge in this region. Additionally, we examined whether these tools could be used to establish nominal action thresholds in the Peace River region.

All biological characteristics studied were consistent with previous literature except for adult emergence and phenology of wheat midge. The emergence of adult wheat midge in the Peace River region did not follow any of the previously developed phenological models. Wheat midge in this likely have different thermal requirements than what has been reported in Europe. In Canada, wheat midge is predominantly a pest on spring-seeded wheat rather than winter-seeded wheat. Patterns of adult wheat midge emergence vary widely from region to region and depend on both temperature accumulation and precipitation (Basedow, 1972; Cheng et al., 2017; Elliott et al., 2009; Jacquemin et al., 2014; Kurppa, 1989). This regional variation is associated with differences in host phenology. Wheat midge larvae collected from different hosts emerge at different times when held under the same conditions (Basedow, 1972). It is also possible that genetics or maternal effects, rather than phenotypic plasticity, drive different emergence patterns.

In the Peace River region, adult emergence followed a bimodal pattern at some field sites. Long emergence periods in Europe are attributed to prolonged diapause (Wise and Lamb, 2004), but long emergence periods have not been reported before in Canada. Basedow (1972) suggested that late emerging midges may maintain populations on secondary wheat tillers or on couchgrass, *Elymus repens*. The mechanism behind this phenomenon is unclear but may be the result of prolonged diapause based on different thermal requirements, or genetic or phenotypic differences within the same midge cohort. Late season emergence may be a form of “bet hedging”, as wheat phenology depends on human farming practices as well as abiotic conditions. Bimodal peaks in male midge capture in sex-pheromone baited traps occurred at the same field sites where bimodal emergence was recorded. This emergence pattern exposes wheat fields to two periods of peak adult activity. The second peak of midges emerged after the period when the main wheat stems were susceptible, but secondary stems were still susceptible.

Pheromone-baited traps were identified as the most sensitive monitoring tools, but the number of male midges captured did not predict larval densities or subsequent damage. Larger delta traps baited with flex lures were the most sensitive of the trap-lure combinations tested. There were no significant relationships between pheromone traps and larval densities, seed yield, thousand seed weight, or wheat midge damage. An action threshold using male wheat midge capture in pheromone-baited traps has been developed in the UK (Bruce et al., 2007), but studies in Saskatchewan showed no relationship between adult males captured in pheromone-baited traps and larval density (Mircioiu, 2004). The pheromone traps are baited with the female-produced sex pheromone and only males are attracted. Male wheat midges mate at the site of emergence (Smith et al., 2007), so a single pheromone trap may only sample males from a small area. Dispersing females may actively travel 1 km in addition to passively travelling on the wind for much farther distances (Miao et al., 2013). Pheromone-baited traps are useful for indicating periods of wheat midge activity to determine when to conduct in-field scouting. If indication of activity is the goal, research should optimize pheromone trap monitoring to increase sensitivity and reliability.

Currently, the only sampling tool available to obtain an action threshold for wheat midge control is in-field scouting that is difficult to conduct and labour intensive. Scouting for wheat midge at twilight is recommended. In the Peace River region, wheat midge activity peaks between 10:00 pm and 11:00 pm in July but activity continues throughout the night. In southern Saskatchewan, wheat midge oviposition occurs between 8:00 pm and 9:45 pm (Pivnick and Labbé, 1993). Not only is it difficult to convince producers to scout fields at 10:00 pm, but there is also a risk of misidentification of the target species. Producers may confuse wheat midges with Lauxanid (Diptera: Lauxaniidae) flies, or different orange Cecidomyiidae flies (Figure 4.1).

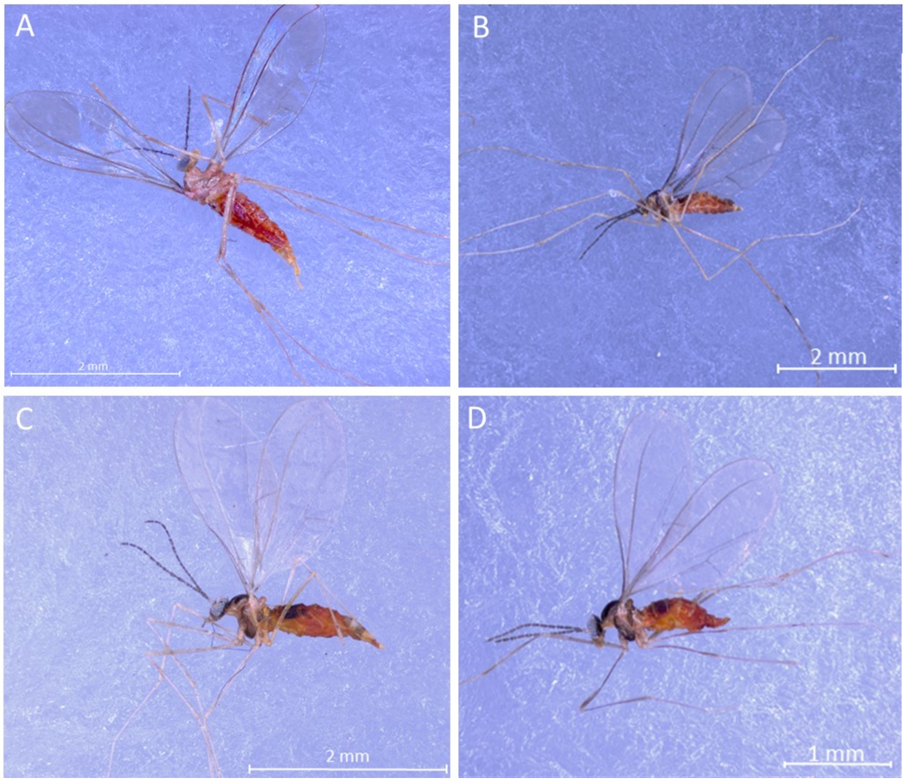


Figure 4.1 Photos of Cecidomyiid flies captured with a malaise trap. A. Wheat midge female. B.-D. Unidentified Cecidomyiidae females.

An ideal sampling tactic for wheat midge would monitor females active in wheat fields. Yellow sticky cards positioned in wheat fields passively capture dispersing midge, and the trap capture is female biased. In this study, we found that a larger number of small cards is a more sensitive monitoring tool than use of fewer large cards. A long, narrow trap transect may be optimal since it can span areas of larval aggregation (Doane et al., 2000) from which multiple clusters of dispersing adult females emerge. This approach could prevent population estimation errors caused by sampling only within or between clusters of aggregated insects (Alexander et al., 2005).

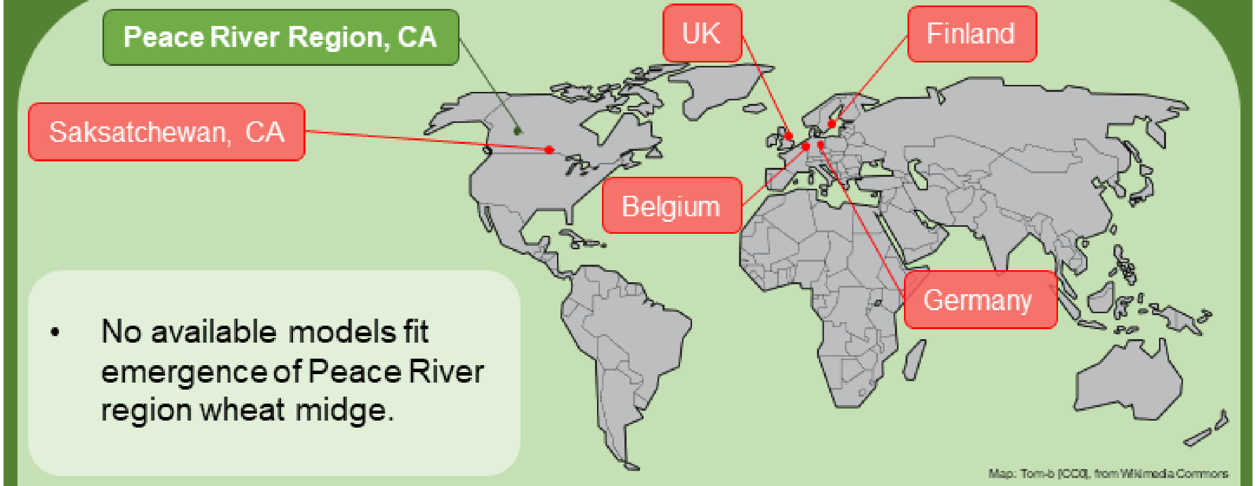
Unfortunately, nominal population thresholds for wheat midge density could not be developed using the monitoring tools tested. Capture of male midges in pheromone-baited traps did not predict larval densities. We were unable to develop thresholds for yellow sticky cards due to the low number of field sites; however, this tool appears to have the most potential for development of nominal thresholds for wheat midge. Further studies should determine whether yellow sticky cards can accurately predict midge population density and crop damage. Previous studies indicate that capture of adult midges on sticky cards is indicative of larval densities in older wheat varieties in Manitoba (Lamb et al., 2002), yet producers have not adopted this sampling procedure.

Further studies should examine regional patterns of wheat midge emergence, as we were unable to develop degree day models based on the temperature requirements (250-300 GDD base 7 °C) revealed in this study. We did not have enough sample sites to partition data into “model development” and “model testing” groups. Additionally, exact determination of dates of rainfall events and midge emergence events was not possible due only recording emergence over 3-day intervals and rainfall over 1-week intervals. A model based on temperature accumulation after rainfall, such as the Jacquemin model (Jacquemin et al., 2014), may more accurately determine wheat midge emergence in the Peace River region. A different temperature requirement is needed, however, due to the shifted phenology of wheat midge in the Peace River region. Future studies examining 300 GDD base 7 °C are recommended.

The mechanism driving the observed wheat midge phenological shifts remains unclear. Understanding whether wheat midge shifts in phenology are due to genetic mutations, gene by environment interactions, or phenological plasticity would provide information on how phenological shifts maintain host synchrony. It is important to predict how wheat midge and its hosts will react to climate change. Insect species that are phenotypically flexible, polyphagous, have a large geographic range or high dispersal ability are less likely to be impacted by climate change-associated phenological asynchronies (Bale et al., 2002). Insects that use the same developmental cues as host plants are also less likely to experience phenological asynchronies, regardless of environmental variation (van Asch and Visser, 2007). Understanding mechanisms behind wheat midge phenology would also improve insect pest management systems. We could predict the proportion of wheat midge in the soil with extended diapause regardless of conditions or predict the proportion of the population that emerge early in the season. If genetic polymorphisms can be linked to phenological phenotype then we could better understand the proportion of midges that undergo extended diapause in the field.

This study describes wheat midge biology and ways to monitor populations in the northernmost region of its range (Figures 4.2 & 4.3). In the Peace River region, wheat midge has adapted to match the phenology of fast maturing spring wheat cultivars. In this study, an emergence model that begins temperature accumulation for pupal development after an inductive rainfall event minimized annual and between-site variability. Determination of the correct temperature accumulation for the second period of this model needs to be determined and 300 GDD base 7 °C is recommended based on the wheat midge emergence temperature requirements observed in this study. A regionally-optimized model will suggest appropriate monitoring periods, during which activity should be monitored with pheromone-baited traps. Currently available lures need more stringent manufacturing standards to ensure consistent release from lures within batches. When wheat heads emerge, and midge activity is indicated by capture in pheromone-baited traps, in-field scouting should be done between 10:00 pm and 11:00 pm in the Peace River region. Further research should be conducted to determine if soil core samples are more effective in other regions, or in other years. Yellow sticky cards should be better developed as a monitoring tool to determine if adult midge capture can predict larval densities and make a useful economic threshold. Understanding the changes in phenology, combined with optimized monitoring tools for activity monitoring and damage prediction will strengthen the integrated pest management program for wheat midge.

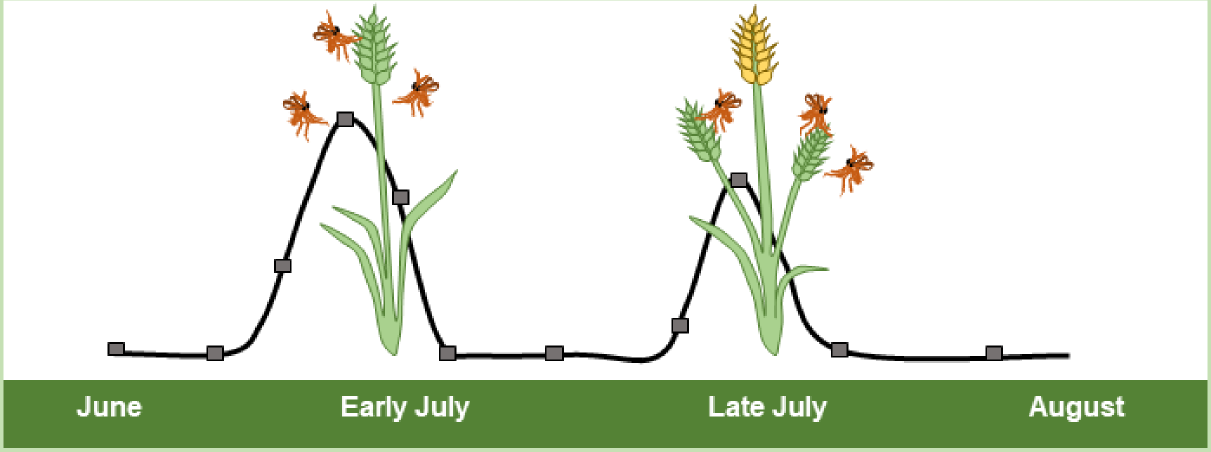
Wheat midge emergence models:



Map: Tom-b [CC0], from Wikimedia Commons

- No available models fit emergence of Peace River region wheat midge.

Model	°C > PRR ?	°C < PRR ?	moisture?	>1 step?
Saskatchewan, CA	✓		✓	
Belgium		✓	✓	✓
UK		✓	✓	✓
Germany		✓	✓	✓
Finland		✓		

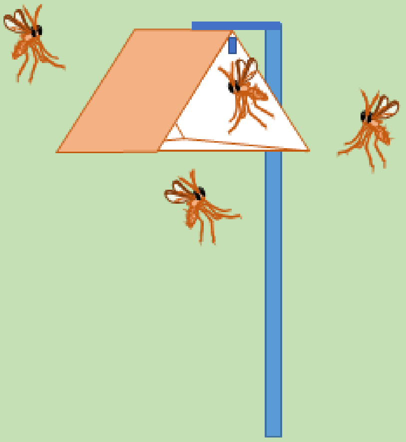


- Two periods of wheat midge emergence & flight.
  - increases risk of overlap with susceptible wheat growth stages
  - allows midge to develop in secondary tillers.

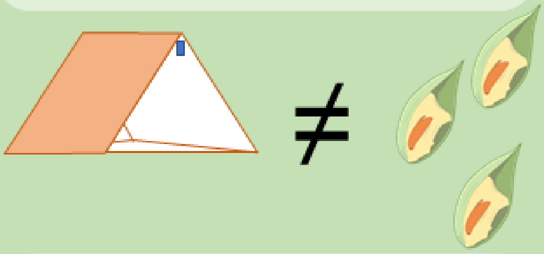
Figure 4.2 Infographic (page 1 of 2) depicting major findings of thesis.

Wheat midge monitoring tools:

Pheromone traps

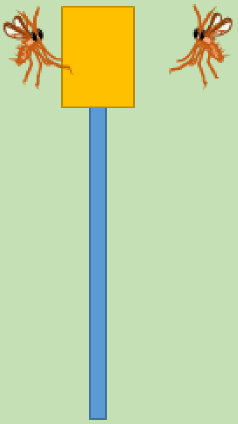


- Sensitive for capture of **male** wheat midge.
- Size, not colour, matters.

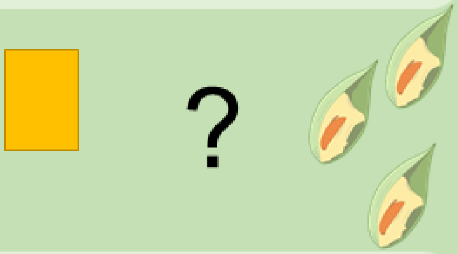


- Not able to predict larval densities
- Good for detecting wheat midge activity & presence

Yellow sticky traps



- Less sensitive, but captures **female** wheat midge when in wheat fields.
- Small, plain cards were best.



- Needs more research – may be able to predict larval densities

Future steps:

- Develop lures with more consistent release rates

- Examine potential for economic thresholds in northern Alberta

Figure 4.3 Infographic (page 2 of 2) Depicting major findings of M.Sc. thesis.

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