OVIPOSITION AND LARVAL ESTABLISHMENT OF SITODIPLOSIS MOSELLANA (GÉHIN) (DIPTERA: CECIDOMYIIDAE) AND ITS PARASITOID, MACROGLENES PENETRANS (KIRBY) (HYMENOPTERA: PLATYGASTERIDAE) ON WHEAT WITH TWO POTENTIAL MECHANICAL RESISTANCE TRAITS

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

By

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ABSTRACT

The orange wheat blossom midge, Sitodiplosis mosellana (Géhin) (Diptera: Cecidomyiidae), is a widespread and invasive pest that has been a serious economic threat to wheat production on the prairies in Canada. One form of antibiosis resistance with a single gene, Sm1, is currently available to reduce wheat midge damage, but other forms of resistance are needed in order to preserve this single resistance gene. Mechanical traits such as awns and pubescent glumes were evaluated alone and in combination over the growing seasons of 2020 and 2021. Pheromone traps were deployed to track emergence and population densities of wheat midge at the sites where experimental wheat was planted. Samples were collected from wheat spikes with different trait combinations from multiple fields in Saskatoon, Saskatchewan to measure 1) the ability of the wheat midge to oviposit on wheat spikes with mechanical resistance traits, 2) identify if mechanical resistance traits reflect a yield increase, decrease, or trade-off, and 3) compare the number of midge-damaged and undamaged seeds of wheat spikes with mechanical resistance traits to those without. These mechanical traits are also studied to see if they influence one of the natural enemies of the wheat midge, *Macroglenes penetrans* (Hymenoptera: Platygasteridae). There was no significant yield increase, decrease, or trade-off present when adding pubescent glumes or awns to wheat lines. Sitodiplosis mosellana Alternative Resistance in Triticum (SMART) lines with the combination of glabrous glumes, awns, and the Sm1 gene resulted in the lowest number of midge-damaged seeds for each sample. Alone or in combination, pubescent glumes did not deter the wheat midge or its parasitoid, *M. penetrans*, from reproducing on wheat spikes with these traits. Wheat spikes with awns did not show a significant decrease of wheat midge oviposition statistically. The timing of heading was recorded for each line and trait combination and different heading dates impacted yield and wheat midge oviposition. *Macroglenes penetrans*. Overall, this research highlights the effects and non-effects of mechanical resistance traits, hairy glumes and awns, on wheat midge.

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DEDICATION

To my Mom, who has always supported my passion and dedication to school and agriculture.

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LIST OF ABBREVIATIONS

CDC	Crop Development Centre
AAFC	Agriculture and Agri-Food Canada
HG	Hairy Glumes
EA	Egg Antibiosis
Sm1	Sitodiplosis mosellana 1
VB	Varietal Blend
OWBM	Orange Wheat Blossom Midge
SMART	Sitodiplosis mosellana Alternative Resistance in Triticum
NILs	Near Isogenic Lines
AC	Agriculture Canada
CGC	Canadian Grain Commission
USD	United States Dollar
CAD	Canadian Dollar
CPSR	Canadian Prairie Spring Red
CWSP	Canada Western Special Purpose
CWSWS	Canada Western Soft White Spring
CNHR	Canada Northern Hard Red
CWES	Canada Western Extra Strong
RCBD	Randomized Complete Block Design
ANOVA	Analysis of Variance
DD	Degree Days
cm	Centimetre
°C	Degrees Celsius
mm	Millimeters
m	Meters
sp.	Species (singular)
USask	University of Saskatchewan
QTL	Quantitative Trait Locus
ZGS	Zadoks Growth Stage
MDK	Midge-Damaged Kernels
VPM	Ventricosa x Persicum x Maine

Chapter 1 Introduction

1.1 Background

Wheat (*Triticum aestivum* L.) is one of the world's most important cereal crops and is cultivated over a wide range of climatic conditions (Varshney et al., 2006). There are currently more acres of farmland used for growing wheat than any other crop in the world. The orange wheat blossom wheat midge (OWBM), *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae), hereafter called wheat midge, is a widespread and invasive pest of wheat, accidentally introduced to North America in the 1800s (Elliott et al., 2009). The first report of wheat midge in North America was from Quebec in 1828 (Fitch, 1865). The pest was recorded over time in various locations throughout the continent including North Dakota, Minnesota, Montana, Alberta, British Columbia and Saskatchewan. (Felt, 1912; Mukerji et al. 1988). Damage from wheat midge is economically devastating to wheat production. For example, an outbreak in Saskatchewan in 1983, resulted in 30% yield reduction, with an economic loss of \$30 million CAD to wheat producers (Kamran et al., 2013; Olfert et al., 1985; Olfert et al., 2003). Since this first major outbreak in Canada, the wheat midge has continued to spread through North America, and is also a major wheat pest in Asia, and Europe and has become a major pest worldwide (Knodel & Ganehiarachchi, 2016).

Antibiotic resistance is a form of resistance that affects the pests' ability to survive and reproduce. Antibiotic resistance protects the plant from insect pests when they attack or begin consuming the plant resulting in mortality of the pest or the inability for the pest to reproduce or develop (Van Emden, 2017). Antibiotic resistance often acts to reduce pest populations (Pedigo & Rice, 2014). There is one current form of antibiotic resistance available to protect wheat from the wheat midge (Lamb et al., 2002). This form of antibiosis resistance exists as the result of a single gene, called *Sm*1. The *Sm*1 gene is located on the short arm of chromosome 2BS and has proven to be highly effective in controlling larval wheat midge (Thomas et al., 2005). *Sm*1 activates a natural response in wheat gene expression that prevents wheat midge larval development. When first-instar midge larvae initially feed on the developing seed of wheat plants with *Sm*1, ferulic and p-coumaric acids are released and the larvae stop feeding and starve to death (Lamb et al., 2002; Ding et al., 2000; Elliott et al., 2011). However, there are some

midge that can develop on wheat with the Sm1 gene and these are called "virulent" wheat midge. Virulent wheat midge can complete larval development on plants with the Sm1 resistance gene and were detected in Manitoba at population levels below 1% (Smith et al, 2004). With the resistance from Sm1 based on a single gene, if these virulent midges become dominant in populations, then this single resistance gene in wheat will be lost. Using another additional form of resistance in combination with the antibiosis resistance provided by Sm1 will help to preserve the Sm1 gene and reduce wheat midge damage and wheat midge populations.

Antixenosis resistance is another form of plant resistance to arthropod pests. This form of resistance involves traits that make the plant less preferable by the pest and deters the settling, feeding or oviposition or other use of the plant by an arthropod pest (Velusamy & Heinrichs, 1986). Antixenosis operates by disrupting normal insect behaviour, whereas antibiosis adversely affects a pest's physiological processes (Kalaiseker et al., 2017). Antixenotic resistance can exist in different plant traits that can range from the colour of the plant, palatability, waxiness, morphology, and hairiness (pubescence). For example, leaf pubescence was effective in deterring the cereal leaf beetle, Oulema melanopus (L.), from ovipositing on common wheat, Triticum aestivum (Gallun et al., 1966; Roberts et al., 1979). Due to the success of this trait against the cereal leaf beetle, pubescent leaves were later employed against the Hessian fly, Mayetiola destructor (Say) (Roberts, et al., 1979). Roberts et al., (1979) discovered that Hessian fly oviposition on wheat with pubescent leaves was significantly less than on glabrous leaves. Only one current antixenotic resistance trait against the wheat midge exists and is called Oviposition Deterrence (OD). Wheat spikes with the OD trait are less preferred by the female wheat midge although they do not have any mechanical traits that can be expected to impact the oviposition behaviour of wheat midge (trichomes, spikes, hairs) (Lamb et al., 2002). OD is a plant characteristic that reduces the number of wheat midge eggs by deterring wheat midge females from ovipositing on wheat cultivars (Lamb et al., 2002). There is more than one gene involved in the inheritance of OD with no evidence of linkage between the antibiosis gene Sm1 (Gharalari et al., 2009). To develop insect-resistant plants with durable resistance against the wheat midge, a combination of antibiosis resistance and antixenosis resistance is required, as it will broaden the basis and increase the levels of resistance to this pest (Golla et al., 2018).

Mechanical traits, pubescent glumes and awns may act as a form of antixenotic resistance and deter oviposition and larval development by the wheat midge. These mechanical traits were

studied in combination with each other and with the Sm1 gene to determine if they contribute to a higher level of resistance than Sm1 alone to preserve the Sm1 gene. These different combinations of pubescent or glabrous glumes, awned or awnless spikes, with the Sm1 gene present or absent have been bred into several different spring wheat lines called the "Sitodiplosis mosellana Alternative Resistance in Triticum" (SMART) lines. These SMART wheat lines were developed by Robert Graf of Agriculture and Agri-Food Canada (AAFC) in Lethbridge, Alberta. Dr. Graf created hundreds of doubled haploid lines with differential expression of all three traits: glumes (pubescent/glabrous), awns (awned/awnless), and Sm1 status (gene present/absent). These SMART wheat lines were developed in Lethbridge, Alberta and characterized in New Zealand prior to testing under Western Canadian field conditions. Dr. Pierre Hucl planted the SMART lines in 2018 and selected only lines with adequate agronomic traits to continue planting in subsequent years. A two-rep agronomic trial of doubled-haploid lines with hairy glume expression, based on greenhouse and field characterization in New Zealand, in 2018/2019 was grown under irrigation near Lethbridge in 2019. The SMART lines included those that were awned or awnless, Sm1 carriers or non-carriers, and those that were positive and negative for VPM (Sr38/Lr37/Yr17). The VPM gene confers resistance to stem rust (Sr38), leaf rust (Lr37) and stripe rust (Yr17) as well as eyespot disease. The VPM acronym for the gene is derived from the species names of the *Triticum* species that were crossed; Ventricosa x Persicum x Marne. This resistance is not common in western Canadian spring wheat germplasm but was not examined in this thesis. The parentage of the SMART lines is CDC Stanley/Jones-Fife//CDC Hughes and the HG trait was introduced into spring wheat through the Jones-Fife winter wheat cross.

Antibiosis and antixenosis resistance on host plants play an essential role in reducing insect pests but have the potential to adversely influence beneficial insects such as predators and parasitoids in agroecosystems (Peterson et al., 2016). Mechanical traits have the ability to deter pests, but these traits can have negative impacts on the natural enemies search efficiency (Peterson et al., 2016). Consequently, parasitoids of the wheat midge could be at a disadvantage if antixenosis resistance traits are added to spring wheat. Hairy glumes and awns have been shown to reduce the ability of some parasitoids to oviposit and reproduce (Kuo, 1986). For example, in the parasitoid, *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae), parasitism rate was reduced when attacking greenhouse whiteflies *Trialeurodes vaporaririrum* Westwood

(Hemiperta: Aleyrodidae), on cucumbers with dense trichomes (Hulspas-Jordaan & van Lenteren, 1978). Additionally, the population density of parasitoids can fluctuate when the population of pests decreases because of these host plant resistance traits (Cai et al., 2009). Specialist parasitoid populations suffer decrease when the availability of suitable hosts is reduced (Agrawal, 2000). The interaction of host plants, parasitoids, and pests needs to be monitored to ensure ecological safety while optimizing integrated pest management techniques (Cai et al., 2009).

This research evaluates the potential of two mechanical traits, hairy glumes and awns to aid in preserving the *Sm*1 gene on spring wheat lines. Additionally, this research also explores the impact of these mechanical traits in spring wheat on the main parasitoid of the wheat midge, *Macroglenes penetrans* (Kirby) (Hymenoptera: Platygasteridae).

1.2 Objectives and Hypothesis

This research investigates the impacts of antixenosis and antibiosis resistant traits in SMART spring wheat lines with and without mechanical resistance traits and the *Sm*1 gene. Specifically, the objective of this research is to identify if different mechanical resistance traits, such as hairy glumes and awns can reduce wheat midge oviposition while increasing yield and seed production in the Saskatoon region of Saskatchewan. This thesis incorporates four main hypotheses:

- Hypothesis 1: Wheat with hairy glumes and awns will have lower yields and fewer seeds per wheat spike because of the trade-off for an added resistance trait.
- Hypothesis 2: Wheat midge will lay fewer eggs on wheat with hairy glumes and awns, resulting in fewer larvae present, resulting in fewer midge-damaged kernels (MDK).
- Hypothesis 3: Wheat spikes with earlier and later heading dates and timing of anthesis will have a reduced number of wheat midge eggs laid and therefore fewer third instar wheat midge larvae present.
- Hypothesis 4: The parasitoid, *M. penetrans*, will not be impacted by the mechanical resistance traits of hairy glumes and awns.

To test the hypotheses, the following experiments were conducted:

- Experiment 1: The effects of antixenotic and antibiotic resistance traits were tested alone and in combination to identify their potential effects on yield and potential to provide mechanical resistance against the wheat midge (Hypotheses 1&2). The timing of heading and anthesis were recorded for all SMART wheat lines with different trait combinations to identify another form of potential resistance (Hypothesis 3).
- Experiment 2: The number of wheat midge larvae parasitized by *M. penetrans* were quantified on a subset of the SMART wheat lines to determine if the resistance and mechanical trait combinations impacted the parasitoids' ability to reproduce and parasitize the wheat midge (Hypotheses 4).

Chapter 2 Literature Review

2.1 Biology of Sitodiplosis mosellana

Adult wheat midge are small insects, measuring approximately 1-3 mm long. Males and females are sexually dimorphic with respect to their antennae (Knodel & Ganehiarachchi, 2016). Males are distinguishable from females through fine hairs covering their antennae and longer antennae compared to females (Elliot et al., 2011). In addition, females possess an ovipositor while males feature "claspers" on their final abdominal segments. Adult and larval stages of wheat midge are recognizable from other species in the family Cecidomyiidae due to their bright orange colour (Figure 2.1) (Olfert et al., 1985).

In Canada, wheat midge are univoltine with four distinct life cycle stages: egg, larval, pupal and adult. In the Canadian prairies, eggs are laid on wheat florets by females in late June to early July (Olfert et al., 1985). Females are active in the evening, laying eggs on wheat florets at dusk when weather conditions are calm and temperatures are above 10-11°C. Eggs are laid singly or in clusters underneath the glumes or along grooves on the surface of wheat florets (Elliott et al, 2011). Prior to laying eggs, the adult female will remain on the wheat spike and use her antennae and ovipositor to probe wheat spikelets before oviposition. She will also tap and drag her ovipositor along the surface of the spikelet sensing for chemicals. Once the eggs are deposited, they hatch in approximately 4-7 days and the first instar larvae begin to feed on the surface of seeds as they develop for 2-3 weeks (Ding & Lamb, 1999; Pivnick & Labbe, 1993). After the 2-3 weeks of larval feeding, mature larvae remain in their cast 2nd instar skin waiting for rain or another high humidity event to drop to the soil. If conditions are dry, the larvae remain in the wheat spikes and will be threshed out during harvest. These mature larvae spin a larval cocoon to over-winter and diapause within the top 5 cm to 10 cm of soil (Stougaard, 2014). Dormancy periods of five years are not uncommon, and some reports indicate wheat midge have periods of dormancy that can last up to 13 years (Stougaard, 2014). Once the soil temperatures rise, diapause is broken and the third instar larvae will leave the cocoons and move closer to the soil surface (Doane & Olfert, 2008). This action initiates post-diapause development, which takes at least three weeks. Once conditions are favourable, usually the result of an increase in

moisture due to heavy rain, both sexes of adult wheat midge will emerge (Barnes, 1952). The female wheat midge will then release a pheromone signal, identified as 2,7-nonanediyl dibutyrate, in the late afternoon after eclosion as a signal to attract males (Gries et al., 2000; Pivnick & Labbe, 1993, 1992). After mating both sexes will disperse and the female will lay her eggs on wheat spikes and the lifecycle repeats (Olfert et al., 1985; Chavelle et al., 2017).



Figure 2. 1. Female wheat midge on an awnless wheat spike with hairy glumes in late June, 2020 in Saskatoon, Saskatchewan.

In the Canadian Prairies, a minimum of 25 mm of precipitation prior to the end of May is required for development of the wheat midge from overwintering cocoon to adult and lower levels of precipitation will reduce the emergence of adults (Elliot et al., 2009). In Montana,

United States of America, 450 degree-days (DD) above 4.5°C from March 1st must occur prior to larvae emerging from cocoons and before 10% emergence (Knodel & Ganehiarachchi, 2016). In Canada, in the Western Prairies, 306 DD above 9°C from March 1st was needed for 50% emergence in a laboratory experiment (Wise & Lamb, 2004). In Saskatchewan, Canada, 693 DD above 5°C from March 1st resulted in a 10% emergence of adults (Elliot et al., 2009). Wheat midge can exist in low populations for several years before becoming a significant problem. If the environmental conditions are suitable, with over 25 mm of accumulative rainfall, populations of wheat midge can rapidly reach epidemic proportions.

The wheat midge is an oligophagous insect and in Canada, spring wheat is the primary host, but winter wheat is susceptible to this pest in the United States, Europe and Asia where the growing conditions are longer. Durum wheat (*Triticum durum* Desf.), rye (*Secale cereal* L.), barley (*Hordeum vulgare* L.) and all other species in the genus *Triticum* are also host plants for wheat midge (Knodel & Ganehiarachchi, 2016). Some other grassy weeds, such as slender meadow foxtail (*Alopecurus myosuroides* Huds.) and quackgrass (*Elymus repens* L.) have been reported to have eggs of wheat midge. However, the larval development is not as successful on these grassy weeds as wheat and these weeds have been questioned as actual host plants (Knodel & Ganehiarachchi, 2016). The severity of the damage resulting from wheat midge infestation in spring wheat depends on the synchrony between emergence of egg-laying females and the heading stage of wheat. Wheat is most susceptible to wheat midge damage when eggs are laid during heading, at Zadoks' growth stages 51-59 (Table 2.1) (Elliot, 1996; Zadoks et al., 1974).

Principal Stage	Secondary Stage &	Description
Seedling growth	 10 - First leaf through coleoptile 11 – The first leaf emerged 	• 12-19 - Two to nine leaves emerged
Tillering	 20 - Main stem only 21 - Main stem and one tiller	• 22-29 - Main stem and two to nine tillers
Stem elongation	 30 - Pseudostem 31-36 - First to sixth node detectable 	 37 - Flag leaf just visible 39 - Flag leaf ligule just visible
Booting	 41 - Flag leaf sheath extending 43 - Boots just visible and swollen 45 - Boots swollen 	 47 - Flag leaf sheath opening 49 - First awns visible

 Table 2. 1. Growth stages of wheat according to the Zadoks' staging scales (Zadoks et al., 1974).

Ear emergence from boot	 51 - Tip of ear just visible 53 - Ear quarter emerged 55 - Ear half emerged 	 57 - Ear three quarters emerged 59 - Ear emergence complete
Anthesis	61 - Beginning of anthesis65 - Anthesis halfway	• 69 - Anthesis complete
Milk development	71 - Kernel water ripe (no starch)73 - Early milk	75 - Medium milk77 - Late milk
Dough development	83 - Early dough85 - Soft dough	• 87 - Hard dough
Ripening	 91 - Grain difficult to divide 92 - Grain not dented by thumbnail 93 - Grain loosening in daytime 94 - Straw dead and collapsing 95 - Seed dormant 	 96 - Viable seed giving 50% germination 97 - Seed not dormant 98 - Secondary dormancy induced 99 - Secondary dormancy lost

2.2 Geographic Distribution of Sitodiplosis mosellana

Geographical regions including Asia, Europe and North America experienced rapid population increases of wheat midge from the 1980s to the 2000s. The wheat midge is Palaearctic in origin and is noted to be extremely common in the 42nd and 62nd parallels (Affolter, 1990; Olfert et al., 2009). Climate is the principal factor for regulating the distribution and population abundance of wheat midge (Doane & Olfert, 2008).

2.2.1 Asia

2.2.1.1 China

Wheat midge was first detected in the early 1310's in China. Since then, outbreaks have occurred many times but more economic damage and yield loss in wheat have occurred in recent years. The 1950s and the 1980s had the two most significant outbreaks of wheat midge reported, resulting in approximately one billion kg of wheat lost per year due to wheat midge (Ni & Ding, 1994; Miao et al., 2013). The wheat midge populations continued to increase over time and were distributed throughout the country due to appropriate weather conditions. Outbreaks continued into the early 21st century and in 2005-2007, Henan and Hebei, which are the two main wheat-

growing areas in northern China, experienced severe damage with the loss of more than two million hectares of wheat (Duan et al., 2013). This pest has spread rapidly throughout Asia resulting in negative impacts on wheat production and remains a pest in some regions of China today.

2.2.1.2 Japan

In Japan, the wheat midge was first recorded in Tochigi Prefecture around 1935. Damage was severe and the midge population spread into the Kyoto Prefecture resulting in yield losses until 1952 (Katayama et al., 1987). After this spread in 1952, wheat production was reduced to decrease the wheat midge population by removing its preferred host plant. In 1978, farmers were encouraged to grow wheat heavily to reorganize rice field rotations. Manageable damage was noted in Ki and Shiga Prefectures. However, in May of 1981, a wheat field in the Kyoto Prefecture, Tamba area, saw an economically devastating outbreak of wheat midge for the first time in approximately 30 years (Katayama et al., 1987).

2.2.2 Europe

The first record of wheat midge in Europe is from a 1741 report of a larva in wheat in England (Knodel & Ganehiarachchi, 2016). Serious outbreaks in the United Kingdom resulted in crop losses of more than 500,000 ha of wheat exceeding £30 million in 1993 and £60 million in 2004 (Chavelle et al., 2015). Since the first discovery in England (1741) the geographical distribution of the wheat midge over the past 30 years expanded through Europe and into Germany, France, and Belgium which all experienced outbreaks in addition to reduced harvest yield and grain quality. Wheat midge commonly coexists with another gall midge in Europe that attacks wheat spikes, called the lemon blossom midge, *Contarinia tritici* (Kirby) (Harris et al., 2003).

2.2.3 North America

2.2.3.1 United States

The wheat midge is thought to have been accidentally introduced to North America in the early 1800s from Europe. It has become one of the most damaging pests of the Northern Great Plains and Canadian wheat production areas. Wheat midge was first recorded in the United States in Vermont in 1820 and was declared a major pest by 1828 (Fitch, 1856). The pest was recorded later in various locations throughout the United States including North Dakota, Minnesota and Montana. (Felt, 1921; Mukerji et al., 1988). During the 1990s, wheat midge was responsible for damaging more than 725,000 acres of wheat per year, valued at approximately US\$30 million in North Dakota alone (Knodel & Ganehiarachchi, 2016). In the 1990s, outbreaks remained low in Montana until 2006, when an outbreak in the northwest resulted in losses over US\$1.5 million in the Flathead County area alone (Stougaard et al., 2014).

2.2.3.2 Canada

Wheat midge was first discovered in Canada as early as the 1800s with the first report in Quebec in 1828. This pest was later found in every province resulting in devastating wheat losses. The first major outbreak of wheat midge in Western Canada was in 1983 affecting millions of hectares (Olfert et al., 2003). In Saskatchewan and Alberta, yields were reduced by an average of 30%, with an economic loss of CAD\$30 million in 1983. This pest spread throughout most of Manitoba and Saskatchewan resulting in significant losses by the 1990s (Doane et al., 2013). Wheat midge occurred in all wheat-growing areas with 95% of spring wheat fields infected with larvae during 1993-1997 (Lamb et al., 1999). Losses from this pest exceeded USD\$38.65 million in Manitoba and USD\$77.30 million in Saskatchewan in 1995 (Lamb et al., 1999; Thompson & Reddy, 2016). Wheat midge populations increased, and outbreaks continued through the 1990s and 2000s.

2.3 Sitodiplosis mosellana Integrated Pest Management

Multiple management practices have been developed and are available to minimize the effect of the wheat midge (Elliot et al., 2011). Management strategies include cultural, chemical, biological and genetic controls. Cultural control is a practice to modify the growing environment such as early planting, crop rotation, increasing seeding rates and tillage (Hill, 1990). Chemical controls, such as insecticides, are available for use but at a premium cost with limited protection

(Furlan and Kreutzweiser, 2015). Classical biological control and inundative biological control introduce a natural enemy or predator of the pest, such as predaceous beetles or parasitic wasps (Affolter, 1990; Basedow, 1973; Reeher, 1945). The genetic approach to controlling wheat midge has proven to be the most promising. Two different types of resistance are currently available to control wheat midge: an antixenotic resistance trait called oviposition deterrence (OD) that was found to reduce the density of wheat midge eggs on wheat and an antibiotic resistance which is based on a single gene called Sm1 (Lamb et al., 2000). Control strategies are discussed in further detail in the following sections.

2.3.1 Cultural Control

Cultural control was the first management approach used in the control of wheat midge. The seeding date is vital in determining the timing of wheat anthesis. When the seeding date is adjusted to earlier or later, this is relative so heading occurs before emergence of wheat midge from diapause. Wheat spikes are most susceptible at the heading stage (Zadoks growth stages 51-59) (Zadoks et al., 1974; Elliott et al., 1991) and much damage can occur when wheat midge oviposition coincides with heading. When anthers are visible, oviposition and larval survival decline, resulting in less damage to kernels. Changing the seeding date, however, is not always practical, and it is of limited use in Canada and Europe due to the short growing season for cereals in northern latitudes. Crop rotation plays an important cultural control role in the reduction of wheat midge by not providing females with a suitable host plant in the field where they emerge, forcing female midge to disperse. The wheat midge has a wide host range (over 18 species in the genus *Triticum*) resulting in neighbouring fields playing an important role in providing a host for the pest (Knodel & Ganehiarachchi, 2016). However, wheat midge can diapause for up to 13 years and will remain in the soil independent of the crop rotation provided, limiting cultural control when used alone. Tillage was attempted as a cultural control method with a hypothesis that burying cocoons deeper in the soil could reduce populations. However, burying cocooned larvae to different depths still resulted in the occurrence of infestations (Barnes, 1956).

2.3.2 Chemical Control

Chemical control is a fundamental tool for controlling and managing insect pests. Insecticides with the active ingredient chlorpyrifos (Lorsban, Nufos, Pyrifos, Pyrinex and Citadel) were used for control of wheat midge until recently when they were deregistered. Dimethoate (Cygon and Lagon) is currently registered to control of wheat midge in Canada (Saskatchewan Crop Production Guide, 2022). The economic threshold for wheat midge is an important tool for deciding whether to apply an insecticide. The loss of kernels lowers the yield and damaged kernels will reduce the grade of harvested wheat (Government of Saskatchewan, 2020). Standards have been established by the Canadian Grain Commission (CGC) that limit wheat midge damage to a No.1 and No.2. Respectively, the No.1 is limited to 2% and the No.2 is limited to 8% before grade is affected. Insecticidal application is recommended at a threshold of one adult midge per four to five wheat spikes to maintain yield and one adult midge per ten wheat spikes to maintain grade (Knodel & Ganehiarachchi, 2008). Chemical control is often the simplest route, but wheat midge is not easily controlled due to the window for the application timing and short heading stage of wheat. When insecticides with the active ingredient chlorpyrifos was applied at the proper timing and at the correct rate it would kill the wheat midge, but it will also result in the death of other natural enemies such as parasitoids and carabid beetles (Elliott & Mann, 1996; Elliot & Mann, 1997).

2.3.3 Biological Control

2.3.3.1 Generalist Predators

Natural enemies play an essential role in controlling insect pests. Predatory insects can consume large numbers of insects, including the wheat midge, in their immature forms (egg, larvae, or nymphs) or adults forms, and sometimes both forms (Gredler, 2001). Wheat midge third instar larvae drop to the soil to overwinter in cocoons, and at this point in their lifecycle they are most vulnerable to predation (Basedow, 1973). Floate et al., (1990) identified fourteen different species in four different genera of carabid beetles, *Agonum, Amara, Bembidon,* and *Pterostichus,* that fed on wheat midge larvae in laboratory conditions when no other prey was present. *Bembidion* species (*Bembidion quadrimaculatum* L., *Bembidion obscurellum* Motschulsky, *Bembidion timidum* LeConte, *Bembidion rapidum* LeConte, *Bembidion nudipenne* Lindroth, *Bembidion nitidum* Kirby, and *Bembidion rupicola* Kirby) fed on the highest number of wheat

midge larvae out of all four genera. In this laboratory study, *B. quadrimaculatum* and *B. obscurellum* consumed 9 to 15 wheat midge larvae per beetle per day (Floate et al., 1990). Floate et al., (1990) additionally performed a field study that quantified the daily predation rate of the same four genera as the laboratory study (*Agonum, Amara, Bembidon,* and *Pterostichus*) with results ranging from <1 to 86 wheat midge larvae consumed per square meter. In a later study in the United Kingdom, an exclusion technique demonstrated that polyphagous predators did not significantly impact the wheat midge population prior to oviposition but did reduce the number of wheat midge returning to the soil (Holland and Thomas, 2000). Basedow (1973) and Barnes (1955) determined that spiders may act as predators of egg, larval and adult stages of wheat midge.

2.3.3.2 Parasitoids

Parasitoids are insects that complete their lifecycle by living on or in a host insect that later dies. Although, usually small, the parasitoids feed on the host and kill it in the process (Gredler, 2001). Macroglenes penetrans (Kirby) (Hymenoptera: Platygasteridae) is a hymenopterous egglarval parasitoid that attacks wheat midge. This small, 1-2 mm insect was first recorded in Canada in 1984 and is currently present in Europe, North America and Asia (Figure 2.2) (Noyes, 2014; Doane et al., 1989; Olfert et al., 1985). The estimated value of this parasitoid in Saskatchewan alone, due to the reduction in insecticide costs was \$248.3 million from 1991 to 2000 (Olfert et al., 2008). A recent study by Dufton et al., (2021) calculated an *M. penetrans* parasitism rate of wheat midge larvae ranging from 36-71%, depending on the location. The success of *M. penetrans* is because of the synchrony of this parasitoid wasp with the oviposition period of wheat midge. The emergence of *M. penetrans* is between 3 to 9 days after wheat midge emergence when temperatures reach 20-22°C (Olfert et al., 2003). The female parasitoid wasp lays her eggs inside the wheat midge eggs or into early-stage larvae. Larvae of M. penetrans feed internally on larvae of wheat midge resulting in the eventual death of the overwintered wheat midge. These parasitoid wasps, *M. penetrans*, then overwinter within the third-instar wheat midge larvae and complete their development after the wheat midge pupates. As a result, crop damage is not reduced in the current growing season, but the wheat midge populations are reduced in the following year (Pivnick & Labbe, 1993).



Figure 2. 2. Female *Macroglenes penetrans* on spring wheat spike in late June, 2020 in Saskatoon, Saskatchewan

2.3.4 Forecasting Maps

The Prairie Pest Monitoring Network, a coordinated insect monitoring and surveillance program developed in 1997, incorporates data from land surveys to create wheat midge forecasting maps (Figure 2.3) (Prairie Pest Monitoring Network 2022). This network includes researchers from Agriculture and Agri-Food Canada (AAFC), Manitoba Agriculture, Food and Rural Initiatives, Saskatchewan Ministry of Agriculture, Alberta Agriculture and Forestry, and university researchers. These forecasting maps are used as a tool to monitor wheat midge emergence on the Canadian Prairies in Alberta, British Columbia, and Saskatchewan each year (Figure 2.3) (Prairie

Pest Monitoring Network 2022). The maps incorporate data collected from all three provinces and are created using a bioclimatic model (Olfert et al., 2016). The bioclimatic model includes temperature, diapause, light, moisture, heat stress, cold stress, wet stress, and dry stress as parameters for which locations are estimated to have higher abundances of the pest abundances (Olfert et al., 2016).



Figure 2. 3. Forecast maps for the wheat midge in Saskatchewan for A) 2019, B) 2020, C) 2021, to D) 2022 (Agriculture and Agri-Food Canada). White = not surveyed, light green = no infestation, green = $< 600 \text{ midge/m}^2$, yellow= 600-1200 midge/m², orange= 1200-1800 midge/m², red = 1800 midge/m².

2.4 Breeding for Resistance

Most practitioners of integrated pest management consider the "gold standard" method of insect pest control to be the use of insect resistant and/or tolerant varieties. Such varieties including the suffix "VB" for varietal blends contain a ratio of 90% seeds resistant to the wheat midge and express the *Sm*1 gene to 10% wheat-midge susceptible genes.

Insect pest resistance can be of two types: antibiosis and antixenosis. Antibiosis resistance protects the plant from insect pests when they first attack or begin consuming the plant, resulting in the pest's mortality or an inability to reproduce (Van Emden, 2017). Whereas antixenosis resistance protects the plant by making the plant less preferable and deters the settling, feeding or oviposition of the pest (Velusamy & Heinrichs, 1986). A combination of antibiosis and antixenosis-resistance in plants provides a more durable resistance (Golla et al., 2018).

2.4.1 Antibiotic Resistance to Wheat Midge

Antibiosis resistance against the wheat midge was first discovered in 1996 by Barker and McKenzie on soft red winter wheat. Following efforts to transfer this resistance into spring wheat, the resistance gene was named Sm1 (McKenzie et al., 2002). This form of antibiosis resistance exists due to a single gene, called Sm1. The Sm1 gene is located on the short arm of chromosome 2BS and has proven to be highly effective in controlling larval wheat midge (Thomas et al., 2005). Sm1 activates a natural response in wheat gene expression that prevents wheat midge larval development. When first-instar midge larvae initially feed on the developing seed of wheat plants with Sm1, ferulic and p-coumaric acids are released and the larvae stop feeding and starve to death (Lamb et al., 2002; Ding et al., 2000; Elliott et al., 2011). Ferulic acid is implicated as the key to the cessation of larval feeding and levels of this acid rise naturally in post-anthesis kernels, and both Sm1 induced and natural levels of ferulic acid decrease by harvest (Elliott et al., 2011).

To keep the wheat midge population from reproducing virulent offspring, overcoming the Sm1 gene, and returning to pest levels (Smith et al., 2004), wheat with the *Sm*1 gene is mixed in a 90:10 ratio with susceptible wheat varieties (a varietal blend, VB), creating a refuge. Having the varietal blend with 10% susceptibility allows for virulent and avirulent individuals to be

produced and mate. The gene for overcoming the *Sm*1 resistance is recessive. The result of a virulent and avirulent wheat midge mating is an offspring with one recessive and one dominant gene that leaves it susceptible to *Sm*1 resistance. Such varieties including the suffix "VB" for varietal blends contain a ratio of 90% seeds resistant to the wheat midge and express the *Sm*1 gene to 10% wheat-midge susceptible genes. The first three wheat cultivars having the *Sm*1 gene were commercially available in the spring of 2010 as varietal blends (VB) (Unity VB, Goodeve VB, and Glencross VB). Fieldstar VB became available in 2011 with three others (Shaw VB, Utmost VB and Conquer VB) becoming available in 2012 (Vera et al., 2013). Varietal blends are offered to growers to improve yield and grade. These varietal blends are offered in Canada Western Red Spring (CWRS), Canada Prairie Spring Red (CPSR), Canada Western Special Purpose (CWSP), Canada Western Soft White Spring (CWSWS), Canada Northern Hard Red (CNHR), Canada Western Extra Strong (CWES) and Durum (Table 2.2).

Wheat Class	Variety	Commercially Available
Canada Western Special Purpose	AAC Awesome VB	2019
Canada Western Special Purpose	KWS [®] Charing VB	2018
Canada Western Special Purpose	KWS® Sparrow VB	2018
Canadian Northern Hard Red	CDC Cordon CL Plus VB	2020
Canadian Northern Hard Red	AC [®] Conquer VB	2012
Canadian Northern Hard Red	AC [®] Unity VB	2010
Canadian Prairie Spring Red	AAC Foray VB	2018
Canadian Prairie Spring Red	AC [®] Enchant VB	2014
Canadian Western Extra Strong	AC [®] Glencross VB	2010
Canadian Western Red Spring	AAC Russell VB	2022
Canadian Western Red Spring	SY Brawn VB	2021
Canadian Western Red Spring	AAC Starbuck VB	2021
Canadian Western Red Spring	AAC Wheatland VB	2021
Canadian Western Red Spring	CDC Succession CL Plus VP	2020
Canadian Western Red Spring	SY Chert VB	2020
Canadian Western Red Spring	AAC Warman VB	2020
Canadian Western Red Spring	AAC LeRoy VB	2020
Canadian Western Red Spring	AAC Alida VB	2020
Canadian Western Red Spring	CDC Adamant VB	2019
Canadian Western Red Spring	CDC Landmark VB	2018
Canadian Western Red Spring	CDC Hughes VB	2018

Table 2. 2. List of wheat midge tolerant varieties currently available for purchase in Canada and the year they became commercially available (Modified from Shelby Dufton; Midge Tolerant Stewardship Team, 2022).

Canadian Western Red Spring	AAC Jatharia VB	2017
Canadian Western Red Spring	CDC Titanium VB	2016
Canadian Western Red Spring	AAC Cameron VB	2016
Canadian Western Red Spring	AC® Vesper VB	2013
Canadian Western Red Spring	CDC Utmost VB	2012
Canadian Western Red Spring	AC® Shaw VB	2012
Canadian Western Red Spring	AC® Fieldstar VB	2011
Canadian Western Red Spring	AC® Goodeve VB	2010
Canadian Western Soft White Spring	AAC Paramount VB	2019
Canadian Western Soft White Spring	AAC Indus VB	2018
Canadian Western Soft White Spring	AC® Sadash VB	2017
Durum	AAC Weyburn VB	2022
Durum	AAC Succeed VB	2021
Durum	CDC Carbide VB	2017
Durum	AAC Marchwell VB	2016

2.4.2 Antixenotic Resistance to Wheat Midge

Only one current antixenotic resistance trait against wheat midge exists, Oviposition Deterrence (OD). OD is a plant characteristic that reduces the number of wheat midge eggs by deterring wheat midge females from ovipositing on wheat cultivars and was studied in the early 2000s (Lamb et al., 2001, 2002). In a laboratory study, Lamb et al., (2002) found OD on spring wheat lines resulted in one line, L10, having a significantly reduced number of larvae per spike. Prior to this, Lamb et al., (2001) identified OD on one durum, *Triticum durum* Desf., wheat cultivar. The OD wheat spikes are less preferred by the female wheat midge but do not present any mechanical traits (trichomes, spikes, hairs) that might impact the oviposition behaviour of wheat midge (Lamb et al., 2002). The mechanism for the resistance is not well understood but rather than mechanical traits it is hypothesized that surface chemicals and volatiles released from the wheat spikes are detected by the female wheat midge when selecting for an oviposition site (Gharalari et al. 2009). There is no evidence of a linkage between the antibiosis Sm1 gene and the OD genes (Gharalari et al., 2009).

In addition to OD, a significant difference in wheat midge oviposition preference throughout the spike has also been observed (Smith and Lamb, 2001). The host spike's middle and upper sections are preferred for wheat midge oviposition (Smith and Lamb, 2001).

2.4.2.1 Glume Pubescence

Pubescent traits can mediate different plant-herbivore interactions, including attracting and deterring insects to host plants (Lill, et al., 2006; Southwood, 1986). Pubescent traits have the potential to act as a form of antixenotic resistance by deterring the insect from ovipositing on the plant (Callahan, 1957; Webster et al., 1975; Roberts et al., 1979; Robinson et al., 1980; Gannon et al., 1994; Haddad and Hicks, 2000; Malakar and Tingey, 2000), surviving and growing on the plant (Poos and Smith, 1931; Schillinger and Gallun 1968; Lambert et al., 1992; Malakar and Tingey, 2000; Gilbert, 1971; Eisner et al., 1998; Haddad and Hicks, 2000), or even moving on the plant (Webster et al., 1975; Eisner et al., 1998; Zvereva et al., 1998). However, sometimes these traits do not act as a form of resistance, but instead they can benefit the insect (Southwood, 1986). Longer legged probing and sucking insects (e.g. Hemiptera) are less deterred by trichomes, whereas insects with more contact on surfaces (Lepidoptera) are more deterred (Levin, 1973; Southwood, 1986; Hare & Elle, 2002). Lill et al., 2006 discovered that pubescence on oak leaves (Quercus spp.) did not prevent oviposition from generalist slug caterpillars (Lepidoptera: Limacodidae) but the pubescence did result in poor larval performance. Slug caterpillars' populations decreased with increasing pubescence on leaves and can be linked to feeding preferences for glabrous leaves (Lill et al., 2006). Leaf pubescence on common wheat, Triticum aestivum was effective in deterring the cereal leaf beetle, Oulema melanopus (L.), from ovipositing (Gallun et al., 1966; Roberts et al., 2079). Due to the success of this trait against the cereal leaf beetle, pubescent leaves on wheat were studied against the Hessian fly, Mayetiola destructor (Say) (Roberts, et al., 1979). Roberts et al., (1979) discovered that Hessian fly oviposition on wheat with pubescent leaves was significantly less than on glabrous leaves. Additionally, the success of eggs hatching, and larval migration of the Hessian fly were reduced when pubescent leaves were present.

While some insect species oviposit on leaves, the wheat midge oviposits on the inner surface of glumes or anthers of host plants (Barnes, 1956). The female wheat midge adults probe the surface of a wheat spikelet with their antenna and ovipositor before laying eggs (Pivnick & Labbé, 1992). Pubescent glumes may impact the probing of the female wheat midge prior to oviposition on wheat spikes. Pubescent glumes could act as a form of antixenosis resistance to deter the adult female wheat midge from laying eggs on the spikelet (Figure 2.4). In addition to

deterring oviposition, pubescent glumes may also prevent feeding by larvae and slowing or stopping larval development.



Figure 2. 4. SMART line 171 with hairy level 2, very hairy, created by Robert Graf of Agriculture and Agri-Food Canada (AAFC), Lethbridge AB.

2.4.2.2 Awns

Awns were bred on wheat lines in the past for their ability to increase yields (Grundbacker, 1963; Goulden & Neatby, 1929; Grantham, 1919; Atkins & Norris, 1955; Patterson et al., 1962; Olugbemi et al., 1976). Studies show an increased yield when awns were present on wheat spikes because they are an important site for photosynthesis, resulting in more starch produced for the kernels compared to the awnless lines (Grundbacker, 1963; McKenzie, 1972). However, other studies did not obtain the same results and a consensus was formed that awned wheat will only increase yield in warm semiarid climates (McKenzie, 1971; Grundbacker, 1963; Miller et al., 1944; Vervelde, 1953). A more recent study has shown that awns are correlated with larger grain

size and yield in less favourable environments but correlated with reduced grain number in more favourable environments (Rebetzke et al., 2016). This study also identified that awns did not affect the number of spikelets. Regardless of their potential to increase or decrease wheat yields, awns have also been used for plant resistance to insects (Havlickova, 2008). Havlickova (2008) found winter wheat cultivars were more resistant against the English grain aphid, *Sitobion avenae* (Fabricius) when long awns were present. Blake et al., (2011) and Zhang et al., (2020) identified QTL's (*QSm.mst-1A*) in spring wheat varieties for awns that were associated with reduced midge damage. Thambugala et al., (2020) later identified that awns on wheat deterred wheat midge oviposition. It was revealed that wheat with the awned genotype provided additional oviposition deterrence to the wheat midge (Thambugala et al., 2020).

Chapter 3

Evaluating the impact of pubescent glumes and awns on spring wheat yield and *Sitodiplosis mosellana* oviposition

3.1 Introduction

One of the most significant factors impacting yield and seed quality in wheat production is damage from insects (Smith, 2000). The worldwide average for yield losses from insect pests are 20-37%, which translates to approximately \$70 billion per year globally (Bradshaw et al., 2016; Pimentel et al., 1997; Kamran et al., 2013). The orange blossom wheat midge (*Sitodiplosis mosellana*) is one of the many major pests in wheat crops. Midge-damaged hard red spring wheat has a very high protein content, dark flour colour, increased flour ash, reduced flour yield, weak sticky dough properties, low baking absorption and poor bread quality (Miller & Halton, 1961; Dexter et al. 1987). In 1983, the damage from wheat midge caused a 30% wheat yield reduction with an economic loss of CAN\$30 million dollars in northeast Saskatchewan (Kamran et al., 2013; Olfert et al., 1985). Areas where midge-damaged kernels is extensive, the SDS sedimentation test can be used to screen the effect of the midge-damaged kernels on gluten properties (Axford et al. 1978; Dexter et al. 1987).

The wheat midge can be found in all western Canadian provinces, with 95% of spring wheat fields in Manitoba showing some infestation between 1993 and 1997 (Lamb et al., 1999). In 2014, wheat midge populations reached densities of over 1800 midge per square metre in the northeastern portion of Alberta (Dufton et al., 2021). Since wheat midge was first discovered in Canada, insecticides have been the main control method (Jacquemin et al., 2014; El-Wakeil et al., 2013), along with resistant wheat varieties (Jacquemin et al., 2014; Ellis et al., 2009). Insecticide applications are recommended when an average of 4-5 wheat spikes have at least one adult wheat midge present during heading (ZGS 51-59) (Elliot et al., 2009; Elliot et al., 2007).

Wheat lines have been developed with antibiosis and antixenosis resistance to prevent insect damage (Clark et al., 1972; Kogan and Ortman, 1978). Antibiosis resistance is when the plant causes mortality of the insect or inhibits the growth or development of the insect. Antixenosis is when the plant repels the insect or makes the insect unattracted to the host plant (Oki et al., 2012). Currently, there is only one trait for antibiotic resistance against the wheat
midge available. The form of resistance for the antibiotic trait is based on a single gene, called Sm1 (Berzonsky et al., 2002; Mckenzie et al., 2002; Ding et al., 2000, Lamb et al., 2000). The Sm1 gene works by reducing wheat midge larval feeding on the seed. Sm1 has proven to be very effective and is bred into different varieties of wheat (Smith et al., 2004). However, when a single antibiotic resistance gene is used to protect a crop from insects, the resistance often breaks down rapidly (Smith et al., 2004). This breakdown occurs because the frequency of virulent insects increases in pest populations (Smith et al., 2004). For example, Mayetiola destructor (Hessian fly) is another cereal insect pest that causes substantial yield losses to spring wheat. Three antibiotic resistance genes (H3, H6, and H5) were deployed in homozygous cultivars and within nine years after the release, the gene H3, broke down completely due to virulent hessian fly populations exceeding threshold (Smith et al., 2004; Foster et al., 1991). Like the H3 gene, the Sm1 genes providing resistance against the wheat midge are similarly likely to break down unless protected, and this is why the "refuge" system was employed. A refuge of susceptible wheat planted as a varietal blend keeps avirulent wheat midge alive, breeding out virulent genes in the population and has helped prevent the Sm1 gene from breaking down in resistance (Smith et al., 2004; Gould, 1986; Rausher, 2001).

Oviposition Deterrence (OD) is a plant characteristic that reduces the number of wheat midge eggs laid on the plant and is first discovered in wheat in the late 1990s (Lamb et al., 2001). This form of resistance is not available on the market, but OD is an antixenotic resistance trait that deters wheat midge females from ovipositing on wheat cultivars (Lamb et al. 2002). In a laboratory study, Lamb et al., (2002) found that oviposition deterrence on spring wheat lines resulted in one line, L10, having a significantly reduced number of larvae per spike. Before this, Lamb et al., (2001) identified oviposition deterrence on one durum, *Triticum durum* Desf., wheat cultivar. These wheat spikes are less preferred by the female wheat midge but do not present any mechanical traits that impact the oviposition behaviour of wheat midge (Lamb et al., 2002). Gharalari et al., 2009 identified that surface chemicals and volatiles were released by wheat spikes that deterred the female wheat midge when selecting for oviposition sites (Gharalari et al., 2009, 2011). In addition to OD studied in the early 2000s, Blake et al., (2011) and Zhang et al., (2020) identified QTL's (*QSm.mst-1A*) in spring wheat varieties for awns that were associated with reduced midge damage. Thambugala et al., (2020) later determined that wheat with the awned genotype does provide additional oviposition deterrence to the wheat midge.

In addition to using varietal blends (90:10 ratio of resistant wheat and susceptible wheat), another mechanism to prevent the breaking down of a resistance trait, is to combine several resistance traits (Crowder and Carriere, 2009). Combining an antibiosis trait, such as the Sm1 gene, with an antixenotic trait, such as the oviposition deterrence trait, will help increase the longevity of both forms of resistance (Smith and Lamb, 2001). Alternatively, combining these traits could result in a trade-off between spike size and the number of seeds produced (Dwivedi et al., 2021; Ferrante et al., 2007). The potential trade-off between yield and quality is a concern for plant breeders, seed companies and producers when developing and growing new resistant wheat varieties (Smith, 2000). High yielding wheat lines have been developed with small spikes and high spike density (ie. 'Kauz') and the alternative with large spikes and low spike density (ie.'Babax'). The SMART wheat lines developed by Robert Graf of Agriculture and Agri-Food Canada (AAFC) Lethbridge, Alberta had differential expressions of glume pubescence rated as glabrous, slightly pubescent, or strongly pubescent which were introduced by crossing with Jones Fife. The glume pubescence in combination with awned and awnless heads, and presence/absence of the Sm1 gene. The parentage of the SMART wheat lines is CDC Stanley/Jones Fife//CDC Hughes and includes a gene cluster known as VPM which confers rust resistance to CWRS wheat. The hairy-glumed traits comes from the winter wheat variety Jones Fife. The CDC Teal Near Isogenic lines (NIL) out of Pierre Hucl's wheat breeding program at the University of Saskatchewan's Crop Development Center (CDC) either have pubescent glumes or glabrous glumes and represent a good system to compare only the effect of pubescent glumes on wheat midge oviposition and subsequent damage. The hairy glumed (HG) trait on the CDC Teal HG lines was classed as HG1 by AAFC (Wist Lab) researchers based on wheat spikelets' visual appearance. Glume pubescence is a distinct characteristic that can be seen on individual wheat spikes. The Wist Lab, has classified the SMART line glumes as glabrous (scored as 0), slightly pubescent (scored as 1), or strongly pubescent (scored as 2) (Figure 3.1, Figure 3.2). Strongly pubescent and slightly pubescent glumes (hairy glumes) may be used as form of antixenotic resistance against the wheat midge, where pubescent, or 'hairy' glumes may act as a physical barrier to prevent oviposition by female wheat midge and/or may interfere with their chemical analysis of the wheat spike surface layer which is likely a prerequisite to oviposition.

Finding a way to preserve the resistance Sm1 gene is crucial for wheat producers worldwide. It has led to substantial research efforts to identity additional sources of resistance and deterrence against the wheat midge. This research study investigated if adding awns to spring wheat lines in combination with a pubescent (hairy) glume trait will deter wheat midge oviposition, reducing the number of midge-damaged kernels. Combining different resistance mechanisms (Sm1 gene, hairy glumes, and awns) this study provided information that will contribute to delaying the breakdown of the Sm1 gene. Different levels of midge-damaged kernels (damaged and unharvestable, damaged and harvestable, and undamaged) were studied based on the different resistant trait combinations. The kernel production (number of kernels produced per spike) and yield (kg/ha) were studied to identify any differences with all the trait combinations in SMART spring wheat lines.



Figure 3. 1. Two SMART lines with different glume trichome densities. Slightly pubescent glumes, HG level 1 (left) vs glabrous glumes, HG level 0 (right).



Figure 3. 2. Two SMART lines with different glume trichome densities. Strongly pubescent glumes, HG level 2 (left) vs slightly pubescent glumes, HG level 1 (right).

3.2 Methods

3.2.1 Site Description

To assess the effect of the mechanical resistance traits (hairy glumes and awns) impact on yield and seed production, experimental field plots were examined. This study was conducted over two years (2020 and 2021) at four different sites in Saskatoon, Saskatchewan (The University of Saskatchewan's Brown Research Farm (dryland), The University of Saskatchewan's Preston Research Farm (irrigated), The University of Saskatchewan's Campus Research Farm (irrigated), and at the AAFC Saskatoon Research Centre's Lowe Road Farm (irrigated, when necessary) (Table 3.1).

Research farms and at the AAFC Saskatoon Research Centre's Lowe Road farm).				
Site Name	Long/Lat	Irrigated (Y/N)	Planting Type	
Brown Research Farm	52.16352°N	Ν	Hills	
	106.52951°W			
Preston Research Farm	52.12546°N	Y	Hills	
	106.61853°W			
Campus Research Farm	52.13197°N	Y	Hills	
	106.61537°W			
AAFC Research Farm	52.15183°N	Y	Rows	
	106 57390°W			

Table 3. 1. Site details for the four different sites with SMART wheat lines grown in Saskatoon, Saskatchewan for the year 2020 (University of Saskatchewan's Brown, Preston, and Campus Research farms and at the AAFC Saskatoon Research Centre's Lowe Road farm).

Crop rotations for the previous years differed slightly from site to site (Table 3.2). Hourly wind averages for May, June, July, and August were 17.65 km/h in 2020 and 16.67 km/h in 2021 in Saskatoon, SK (Environment and Climate Change Canada https://saskatoon.weatherstats.ca/charts/wind_speed-monthly.html). Based on the Saskatoon, Saskatchewan Historical Data RCS weather station, average temperatures for May, June, July, and August in the year 2021 was 16.83°C (24.62°C was the average high, and 9.03°C was the

average low). In 2020, the average temperature was 15.85°C for those four months (23.03°C was

high, and 8.68°C was low). The amount of precipitation, and added irrigation were recorded for each site and are presented in Table 3.3.

Year 1	Brown	Preston	Campus	AAFC
2020	SMART hills	SMART hills	SMART hills	SMART rows
2019	Fallow	Fallow	Fallow	Spring wheat
2018	Canola	Durum wheat	Pulse plots	Spring wheat
Year 2	Brown	Preston	Campus	AAFC
2021	SMART hills	SMART hills	SMART hills	SMART rows
2021 2020	SMART hills Fallow	SMART hills Fallow	SMART hills Fallow	SMART rows SMART rows

Table 3. 2. Crop rotations for the four sites in Saskatoon, SK for 2018, 2019, 2020 and 2021 (University of Saskatchewan's Brown, Preston, and Campus Research farms) at the AAFC Saskatoon Research Centre's Lowe Road farm).

Table 3. 3. Average precipitation and irrigation data for the four different sites in Saskatoon, SK for the months of May, June, July and August in 2020 and 2021 (University of Saskatchewan's Brown, Preston, and Campus Research farms and at the AAFC Saskatoon Research Centre's Lowe Road farm)

Year	Site	Precipitation	Irrigation Total	Precipitation +
	Name	Total		Irrigation Totals
2020	Brown	209.5 mm	Dryland	209.5 mm
	Research			
	Farm			
	Preston	238.0 mm	40.0 mm	278.0 mm
	Research			
	Farm			
	Campus	238.0 mm	50.8 mm	288.8 mm
	Research			
	Farm			
	AAFC	168.1 mm	0 mm	168.1 mm
	Research			
	Farm			
2021	Brown	116.5 mm	Dryland	116.5 mm
	Research			
	Farm			
	Preston	139.0 mm	110.0 mm	249.0 mm
	Research			
	Farm			
	Campus	139.0 mm	127.0 mm	266.0 mm
	Research			
	Farm			
	AAFC	101.1 mm	50.8 mm	151.9 mm
	Research			
	Farm			

At each site, ten different SMART wheat lines were grown per combined trait (hairy, awnless, Sm1+; hairy, awnless, non- Sm1; hairy, awned, Sm1+; hairy, awned, non- Sm1; glabrous, awnless, Sm1+; glabrous, awnless, non- Sm1; glabrous, awned, Sm1+; glabrous, awnless, awned, non- Sm1) totalling eighty lines (Table 3.4). In addition to these eighty SMART lines, AC Lillian, CDC Hughes, AC Waskada, and CDC Stanley spring wheat varieties were grown as control lines. These four control lines were chosen based on the performance of high yields with

a combination of wheat midge susceptibility and resistance. Control lines had awns present and absent and AC Waskada, although having slightly hairy (lower) glumes, was considered glabrous for this study. Each SMART line was planted at each of the four locations and had one of the eight trait combinations hairy, awnless, Sm1+; hairy, awnless, Non-Sm1; hairy, awned, Sm1+; hairy, awned, Non-Sm1; glabrous, awnless, Sm1+; glabrous, awnless, Non-Sm1; glabrous, awned, Sm1+; glabrous, awned, Sm1+; glabrous, awned, Non-Sm1.

Line No.	Glumes	Awns	Sm1Status
147, 153, 157, 158, 166, 168, 169, 170, 173, 191	Hairy	Awnless	Non- <i>Sm</i> 1
171, 175, 182, 185, 186, 187, 188, 193, 194, 225	Hairy	Awnless	Sm1+
255, 257, 260, 261, 268, 271, 274, 281, 286, 296	Hairy	Awned	Non- <i>Sm</i> 1
258, 262, 267, 276, 278, 280, 283, 295, 297, 299	Hairy	Awned	Sm1+
198, 201, 203, 210, 219, 221, 228, 230, 232, 243	Glabrous	Awnless	Non-Sm1
202, 205, 208, 212, 215, 218, 234, 239, 247, 248	Glabrous	Awnless	Sm1+
301, 303, 316, 319, 326, 329, 330, 338, 352, 365	Glabrous	Awned	Non- <i>Sm</i> 1
302, 307, 310, 318, 325, 333, 334, 339, 345, 350	Glabrous	Awned	Sm1+
CDC Hughes	Glabrous	Awned	Sm1+
CDC Stanley	Glabrous	Awnless	Non-Sm1
AC Lillian	Glabrous	Awnless	Non-Sm1
AC Waskada	Glabrous	Awned	Non-Sm1

Table 3. 4. Line numbers grown with different trait combinations (hairy or glabrous glumes, awns present or absent (awnless), and Sm1+ or Non-Sm1 combinations.

The hairy glume trait was classified and scored as glabrous (no trichomes) (scored as 0), slightly pubescent (scored as 1), or strongly pubescent (scored as 2). Scoring for the slightly pubescent (1) and strongly pubescent (2) was based on the visual appearance of the trichomes present on the outer glumes (Figure 3.2; Figure 3.3; Table 3.5). With respect to the hairiness level of each SMART line, there was no significant difference in any of the results when analysing if different hairy levels made a difference. Therefore, SMART lines with hairy level 1 and 2 were grouped as "hairy" for the remainder of the study. The SMART lines and their glume level of hairiness are presented in Table 3.5 but are not discussed in further detail throughout this study.

Table 3. 5. Wheat lines were planted at four locations in Saskatoon, Saskatchewan (University of Saskatchewan's Brown, Preston, and Campus Research farms, and at the AAFC Saskatoon Research Centre's Lowe Road farm for the years 2020 and 2021. Lines are grouped into levels of hairiness ranging from very hairy (2), slightly hairy (1), and glabrous (0) as identified by the WistLab at AAFC.

Line No.	Level of Hairiness
147, 153, 157, 158, 168, 170, 171, 185, 187, 188,	2
194, 255, 257, 261, 262, 267, 274, 278, 281, 286	
166 169 173 175 182 186 191 193 258 260	1
100, 109, 175, 175, 162, 180, 191, 195, 258, 200, 268, 271, 276, 104, 280, 282, 205, 206, 207, 200	1
208, 271, 270, 194, 280, 285, 295, 290, 297, 299	
198, 201, 203, 210, 219, 221, 228, 230, 232, 243,	0
202, 205, 208, 212, 215, 218, 234, 239, 247, 248,	
AC Lillian, CDC Stanley, CDC Hughes, AC	
Waskada	

The field plot at the AAFC Saskatoon Research Centre's Farm was organized in a randomised complete block design (RCBD) with all eighty SMART lines and the four control lines replicated four times, totalling 336 rows (84x4). The plot was planted 28 rows wide and 12 rows long. Individual rows measured 4.57 m (15 ft) long 0.30 m (1 ft) wide (Figure 3.3 & Figure 3.4). The wheat seeds at this site were planted at an approximate depth of 2.5 cm with a Hege plot seeder.

2021 SMART Lines



Figure 3. 3. Layout of the field plot experiment for the SMART wheat lines at the AAFC Saskatoon Research Centre's Lowe Road farm in 2021. The orange arrows show the direction of seeding. It was seeded from North to South and the numbers on each plot identify the line number planted.

2020 SMART Lines



Figure 3. 4. Layout of the field plot experiment for the SMART wheat lines at the AAFC Saskatoon Research Centre's Lowe Road farm in 2020. The orange arrows show the direction of seeding. It was seeded from North to South and the numbers on each plot identify the line number planted.

The University of Saskatchewan's Brown, Preston and Campus Research Farms plots were organized as a RCBD with four replicates at each site, but plots were planted in hills instead of rows. These three sites were seeded with a hill seeder with a 30 cm seeding space between hills. The planting dates at all four sites varied slightly from 2020 to 2021 and are presented in Table 3.6. Wheat spikes were monitored from the beginning to the end of July for wheat midge eggs on the outside of glumes and the rachis. Data collection began after second instars were present on wheat spikes. All four sites were equipped with one pheromone trap (Trece Incorporated Pherocon VI Trap) in order to monitor the number of adult wheat midge males that were present at each site for the 2020 and 2021 growing seasons.

Table 3. 6. Seeding and harvest dates at four locations in Saskatoon, Saskatchewan (University of Saskatchewan's Brown, Preston, and Campus Research farms and at the AAFC Saskatoon Research Centre's Lowe Road farm) for the years 2020 and 2021.

Year	Site	Seeding Date	Susceptible Date	Harvest Date
			(Z51-60)	
2020	Preston	June 2 nd	July 23 rd	September 14 th
	Campus	May 27 th	July 17 th	September 14 th
	Brown	May 27 th	July 17 th	September 17 th
	AAFC	May 25 th	July 15 th	September 18 th
2021	Preston	May 28 th	July 18 th	September 23 rd
	Campus	May 11 th	July 1 st	September 19 th
	Brown	May 31 st	July 21st	September 25 th
	AAFC	May 31 st	July 21st	October 12 th

Pheromone traps were placed in all four site locations (University of Saskatchewan's Brown, Preston, and Campus Research Farms and at the AAFC Saskatoon Research Centre's Lowe Rd. Farm) to monitor wheat midge populations over time (Figure 3.5). In 2020, populations were measured from July 7th to August 4th and in 2021, populations were measured from July 9th.



Figure 3. 5. Pheromone traps used to monitor wheat midge populations at all four locations (University of Saskatchewan's Brown, Preston, Campus Research Farms and AAFC Saskatoon Research Centre's Lowe Rd. Farm in 2020 and 2021.

3.2.2. Experimental Design and Methodology

The timing of heading was recorded for the individual lines at the AAFC Saskatoon Research Centre farm and categorized as early, medium, and late on July 21st, 22nd, and 23rd of 2021 (Figure 3.6). This timing was reflected within a 3-day envelope of growth from the seeding date to 51-53 days of growth for the single site (AAFC Saskatoon Research Centre's Lowe Rd. Farm). SMART lines that were past the most susceptible stage of heading and onto the anthesis stage (ZGS 61-65) were designated as early timing. These early timing SMART lines were either at the beginning of anthesis (few anthers at middle of ear) or half-way through anthesis (anthers occurring halfway to tip and base of ear). The medium timing SMART lines were at the most susceptible stage (ZGS 51-59) with ear emergence from the boot beginning to just completing. The late timing SMART lines were scored on the appearance of the sheath and flag leaf and had no ear emergence from the boot (ZGS 45-49).



Figure 3. 6. Different growth stages of wheat. A. Early (ZGS 61-65), B. Medium (ZGS 51-59) and C. Late (ZGS 45-49) wheat lines.

Due to different planting methods (row versus hills), the AAFC site data was analyzed separately from the three sites at the University of Saskatchewan (Brown, Campus and Preston). Ten wheat spikes of each SMART line were collected (four replicates) to count the number of seeds and the number of third instar wheat midge larvae present per spike from only the AAFC site on July 23rd, 2020, and July 29th, 2021. These collection points were chosen haphazard in each line from their 4.57 m rows and wheat spikes were clipped approximately 10cm below the bottom of the spike and placed into paper bags. These ten spikes were later dissected individually

and sequentially, starting at the bottom of the spike, counting the third instars and the number of seeds per spike. The number of third instars was recorded per spike, per line, and per rep. The third instar larvae were all alive at this point in the season and were then placed in 6.35 cm cups filled ³/₄ full of soil collected from the field to be used in the parasitoid study (Chapter 4). The number of seeds produced for all ten spikes was counted and combined for a total of ten spikes. The remaining wheat lines at the AAFC site were harvested on September 18th, 2020 and October 12th, 2021 with a Wintersteiger Quantum combine using a Harvest Master Grain-gage system to weigh plots and calculate yield. The yield (kg/ha), and plot weight (kg) (row) was recorded for each line and rep.

The University of Saskatchewan's hilled sites (Brown, Campus, and Preston) had ten spikes collected from each SMART line (four reps) on September 3rd, 2020 and on September 9th, 2021. All ten spikes were threshed together and the number of seeds were counted and recorded. The seeds from these three sites were then placed into small envelopes and seeds were classified into three groups of either damaged and unharvestable, damaged and harvestable, and undamaged. Damaged and unharvestable seeds were classified if they had wheat midge larvae present, feeding on the seed enough that it had shrivelled and would not be harvestable, or a wheat midge skin cast left behind by the larva. These damaged and unharvestable seeds are shrivelled by wheat midge larval feeding and are lost as chaff when harvesting, resulting in lost yield. Damaged but harvestable seeds are large enough to harvest, but the seed coat will have visible scarring from the larval feeding which can reduce the grade of the wheat sample (Figure 3.7).



Figure 3. 7. Susceptible (without the *Sm*1 gene) wheat kernels that have wheat midge damage. A shows a damaged and harvestable seed. B shows a third instar larval cast (present on the left side of the seed). C and D are considered damaged and unharvestable.

3.2.3. Statistical Analysis

Linear mixed-effects models were used to test for significant differences in total yield (kg/ha), the number of seeds from each line, and the number of midge-damaged kernels (MDK) with the various trait combinations. Traits (presence or absence of awns, glumes, and *Sm*1) were treated as fixed effects. Year and line number were treated as random effects. Analyses were performed in RStudio (version 3.6.1; R Core Team 2019) using the package "lme4" and "glmmTMB" (version 1.1-27.1) to run the models, and "emmeans" to obtain least-squares means for the mixed models with significant interactions. The linear mixed-effects models were chosen based on the AIC value and are presented in Table 3.7. Generalized linear models with Poisson discrete probability distributions were used to test for the random effects and pheromone trapping data. All data were examined for normality and heteroscedasticity visually using q-q plots, residual plots, and histograms.

Table 3. 7. Mixed models with fixed and random effects results for yield (kg/ha), total number of seeds in wheat spikes, number of third instar wheat midge, and total number of damaged seeds.

Model Response	Model Type	Fixed Effects	F	p-value	Random Effects
Yield (kg/ha)	Linear Mixed- Effects (lmer)	Glumes	1.0856	0.3008	Year
		Awns	0.8329	0.3640	Line Number
		<i>Sm</i> 1	0.7897	0.3769	
Total number of seeds per 10 wheat spikes (AAFC site only)	Linear Mixed- Effects (lmer)	Glumes	0.0550	0.8152	Year
		Awns	0.1248	0.7248	Line Number
		<i>Sm</i> 1	2.5251	0.1160	
Total number of seeds per 10 wheat spikes	Linear Mixed- Effects (lmer)	Glumes	0.0342	0.8533	Rep/Site
		Awns	0.0626	0.8025	Year
		<i>Sm</i> 1	0.2856	0.5933	
Model Response	Model Type	Fixed Effects	Chi Sq	p-value	Random Effects
Total number of third instars on wheat spikes	Generalized Linear Mixed Models (glmm)	Glumes	4.1072	0.0427	Year
		Awns	3.5348	0.0601	Line Number
		<i>Sm</i> 1	64.5165	<0.0001	
Total number of damaged seeds	Generalized Linear Mixed Models (glmm)	Glumes	0.6698	0.4131	Year Site
		Awns	0.5393	0.4627	Line Number
		<i>Sm</i> 1	30.9243	<0.0001	

3.3 Results

3.3.1 Yield

The 80 SMART wheat lines with the eight different trait combinations were harvested at the AAFC Saskatoon Research Centre's Farm on September 18th, 2020 and October 12th, 2021. The linear mixed-effects model showed no significant differences in the median yield (kg/ha) in lines with hairy or glabrous glumes ($F_{1, 76.03}$ = 1.0856, p= 0.3008), awns or awnless spikes ($F_{1, 84.34}$ =0.8329, p=0.3640), and the presence or absence of the *Sm1* gene ($F_{1,78.65}$ =0.7897, p=0.3769). Traits were tested alone and in combination and had no significant impact on the yield (kg/ha) ($F_{1, 79.91}$ = 2.3528, p= 0.1251). Year was a significant factor that influenced the yield (kg/ha), regardless of trait ($F_{1,656}$ =462.486, p=<0.0001; Figure 3.8). The seed yield (g) of control lines (AC Lillian, CDC Hughes, CDC Stanley, and AC Waskada) did not differ significantly from SMART lines ($F_{1, 670}$ = 2.038, p= 0.154). There was no significant interaction between any of the fixed effects (Glumes:Awns= $F_{1, 76.65}$ = 0.2776, p= 0.5998; Glumes: *Sm*1= $F_{1, 76.65}$ = 0.1437, p= 0.7056; Awns: *Sm*1= $F_{1, 76.03}$ = 1.4694, p= 0.2292; Glumes:Awns: *Sm*1= $F_{1, 76.03}$ = 1.1686, p= 0.2831).



Figure 3. 8. Yield (kg/ha) for the AAFC Research Centre's Lowe Road farm in Saskatoon, Saskatchewan in 2020 and 2021. The boxes represent the interquartile range and the horizontal line inside the boxes represents the median for each timing. The space above the median is the

 75^{th} percentile and the space below the median is the 25^{th} percentile. The whiskers show the maximum and minimums.

The year 2021 they had a higher average temperature with less rainfall and lower average wind speeds in comparison to the year 2020. Therefore, a second model was run for the year 2020 individually. The results from the model for 2020 showed no significant differences in the yield (kg/ha) among SMART lines with hairy or glabrous glumes ($F_{1, 76}$ = 0.2242, p= 0.6372) or awned or awnless heads ($F_{1, 76}$ = 0.2335, p= 0.6303) lines. However, there was a significant increase in yield for SMART lines that had *Sm*1 present ($F_{1, 76}$ = 7.0368, p= 0.0097; Figure 3.9). There was no significant interaction between any of the fixed effects (Glumes:Awns= $F_{1, 76}$ = 1.6691, p= 0.2003; Glumes: *Sm*1= $F_{1, 76}$ = 0.8259, p= 0.3663; Awns: *Sm*1= $F_{1, 76}$ = 0.9215, p= 0.3401; Glumes:Awns: *Sm*1= $F_{1, 76}$ = 1.1881, p= 0.2792).



Figure 3. 9. Effects of eight different trait combinations on the yield (kg/ha) produced for the AAFC Saskatoon Research Centre's Lowe Road farm in Saskatoon, Saskatchewan in 2020. The boxes represent the interquartile range and the horizontal line inside the boxes represents the median for each timing. The space above the median is the 75th percentile and the space below the median is the 25th percentile. The whiskers show the maximum and minimum.

3.3.2 Pheromone traps

Wheat is most susceptible during the Zadok's GS 51-59 and susceptibility dates are mentioned in Table 3.6 for each individual field. Results from the pheromone traps deployed from July 7th to August 4th in 2020 and from June 21st to July 29th in 2021 for all four site locations (University of Saskatchewan's Brown, Preston, and Campus Research Farms and at the AAFC Saskatoon Research Centre's Lowe Rd. Farm) are presented in Figure 3.10; Figure 3.11; Table 3.8. Only wheat midge males are attracted to pheromone traps and counts are based off the males alone.



Figure 3. 10. Pheromone based wheat midge trapping data at four different site locations (University of Saskatchewan's Brown, Preston, and Campus Research farms and at the AAFC Saskatoon Research Centre's Lowe Road farm) found in Saskatoon, Saskatchewan in 2020.



Figure 3. 11. Pheromone based wheat midge trapping data at four different site locations (University of Saskatchewan's Brown, Preston, and Campus Research farms and at the AAFC Saskatoon Research Centre's Lowe Road farm) found in Saskatoon, Saskatchewan in 2021.

Table 3. 8. Pheromone based wheat midge trapping data at four different site locations (University of Saskatchewan's Brown, Preston, and Campus Research farms and at the AAFC Saskatoon Research Centre's Lowe Road farm) found in Saskatoon, Saskatchewan in 2020 and 2021.

Total Number of Male Wheat Midge adults			
	2020	2021	
Brown	562	225	
Campus	658	154	
Preston	1275	57	
Lowe	850	647	

The year 2020 had a significantly higher number of wheat midge caught on pheromone traps than 2021 (χ^2 =1211.90, df=1, p=<0.0001). In 2020, a total of 3,345 adult wheat midge were trapped at the four sites, while in 2021 a total of only 1,083 wheat midge were trapped. The four sites differed significantly, the Preston and AAFC Lowe sites had a significantly higher number of wheat midge than the Brown and Campus sites (χ^2 =356.22, df=3, p=<0.0001). Preston

had a total of 1,332 wheat midge trapped, AAFC Lowe had a total of 1497, Brown had a total of 787, and Campus had a total of 812 wheat midge trapped over the two years of monitoring.

3.3.3 Wheat midge third-instar larval counts

In total, 2,163 third-instar wheat midge larvae were found on the SMART lines at the AAFC Saskatoon Research Centre's Lowe Rd. Farm. SMART lines with the trait *Sm*1 present had significantly fewer third instar larvae (χ^2 =59.5013, df=1, p=<0.0001). Only 12 of the 2,163 third instars were found on SMART wheat with the *Sm*1 gene. These smart lines included: SMART 171 with three larvae present, SMART 185 with one larva present, and SMART 225 with eight larvae present. No virulent midge were present on control line Hughes, which has the *Sm*1 gene present. SMART lines with hairy glumes had more third-instar larvae (χ^2 =4.1072; df=1, p=0.04270) compared to the glabrous lines. While wheat with the hairy-glumed trait had more third-instar larvae, the level of hairiness on the glumes ranging from slightly hairy (1) to very hairy (2) did not result in any significance on the number of third instars present per line (F_{2,669}= 1.0907, p= 0.3366). SMART lines with the awned trait had a slight, but non-significant, decrease in the number of third-instar larvae (χ^2 =3.5348, df=1, p=0.06009; Figure 3.12) compared to the awnless lines. The number of third-instar larvae present on control lines (AC Lillian, CDC Hughes, CDC Stanley, and AC Waskada) did not differ significantly from the SMART lines (F_{1,670}= 1.4186, p= 0.2341).

Due to the zero inflation in the above model caused by few larvae on the lines with the *Sm*1 gene, an additional model was examined with the SMART lines that did not have the *Sm*1 gene present. This model did not demonstrate any significant differences in midge numbers among wheat lines that were awnless or awned (χ^2 =1.9569; df=1, p=0.1618), with hairy glumes or without (χ^2 =2.2688, df=1, p=0.1320) or the interactions between Awns:Glumes (χ^2 =0.6419; df=1, p=0.4230).



Figure 3. 12. Effect of eight different trait combinations on the total number of wheat midge third instar larvae found on wheat spikes at the AAFC Saskatoon Research Centre's Lowe Road farm in Saskatoon, Saskatchewan in 2020 and 2021. The boxes represent the interquartile range and the horizontal line inside the boxes represents the median for each timing. The space above the median is the 75th percentile and the space below the median is the 25th percentile. The whiskers show the maximum and minimums.

In addition to the *Sm*1 gene reducing the total number of wheat midge-third instar larvae, the year ($F_{1,670}$ = 92.439, p= <0.0001), and timing of heading also had significant effects on the number of third instars per trait combination ($F_{2, 659}$ =3.9044, p=0.02276). SMART wheat lines that had medium timing (ZGS 51-59) had the fewest total third-instar larvae per SMART line. While the early (ZGS 61-65) and late (ZGS 45-49) timing had significantly more third instar wheat midge (Figure 3.13).



Timing

Figure 3. 13. Effect of timing of heading on the total number of wheat midge third instars per ten wheat spikes at the AAFC Saskatoon Research Centre's Lowe Road farm in Saskatoon, Saskatchewan in 2020 and 2021. The boxes represent the interquartile range and the horizontal line inside the boxes represents the median for each timing. The space above the median is the 75th percentile and the space below the median is the 25th percentile. The whiskers show the maximum and minimums.

3.3.4 Seed production and seed damage

In total, 150,269 seeds were counted at the three University sites (Brown, Campus and Preston Research Farms) on the different SMART lines from four reps over the years 2020 and 2021.

There was no significant difference on the total number of seeds produced per ten wheat spikes for any trait: hairy or glabrous glumes ($F_{1,556}$ = 0.0342, p= 0.8533), awned or awnless wheat ($F_{1,556}$ =0.0626, p=0.8025), or presence of the *Sm*1 gene ($F_{1,556}$ =0.2856, p=0.5933) (Figure 3.14). In the year 2020, the SMART wheat spikes had a higher seed count ($F_{1,560}$ =41.7709, p=<0.0001) per 10 spikes compared to the year 2021. The seed count did not differ among the four sites ($F_{2,552}$ =0.4254, p=0.6537) or among the four control lines ($F_{1, 670}$ =0.3424, p=0.5586). No significant interactions between any of the traits and the number of seeds produced per 10 wheat spikes was identified.



Figure 3. 14. The number of seeds per ten wheat spikes with eight different trait combinations at the three different site locations (University of Saskatchewan's Brown, Preston, and Campus Research farms) in Saskatoon, Saskatchewan in 2020 and 2021. The boxes represent the interquartile range and the horizontal line inside the boxes represents the median for each timing. The space above the median is the 75th percentile and the space below the median is the 25th percentile. The whiskers show the maximum and minimum.

In addition to the different years affecting the number of seeds per 10 wheat spikes, the timing of heading and anthesis (early, medium, and late) was significant ($F_{2,669}$ = 3.6503, p= 0.0265). Six of the 80 lines were in the early heading category (ZGS 61-65), five of the 80 lines were in the late heading category (ZGS 45-49), and the remaining lines were in the medium category (ZGS 51-59). Wheat lines that had an earlier anthesis had a lower seed count and wheat with the late anthesis had a significantly higher seed count ($F_{2, 659}$ =3.2788; p=0.03829; Figure 3.15). Control lines (AC Lillian, CDC Hughes, CDC Stanley, and AC Waskada) all had medium timing (ZGS 51-59) so no statistical analysis was performed on significance of timing between control and SMART lines. No significant interactions between the traits or timing were found in the yield (kg/ha) or number of seeds produced per spike analysis (Glumes:Awns= $F_{1, 79.84}$ = 0.0986, p= 0.7543; Glumes: $Sm1=F_{1, 79.85}$ = 1.2014, p= 0.2763; Awns: $Sm1=F_{1, 76.04}$ = 0.4532, p= 0.5029; Glumes:Awns: $Sm1=F_{1, 76.04}$ = 0.2466, p= 0.6209).



Figure 3. 15. Effect of timing of heading on the number of seeds per wheat spike at the three different site locations (University of Saskatchewan's Brown, Preston, and Campus Research farms) in 2020 and 2021. The boxes represent the interquartile range and the horizontal line inside the boxes represents the median for each timing. The space above the median is the 75^{th} percentile and the space below the median is the 25^{th} percentile. The whiskers show the maximum and minimums.

The same 150,269 seeds that were counted in the seed production analyses were categorized as damaged (damaged and harvestable, damaged and unharvestable) or undamaged for each line number and each trait combination. A total of 3,836 seeds were categorized as damaged out of the 150,269 for the years (2.60%). The trait combinations with the *Sm*1 gene (χ^2 =30.943, df=1, p=<0.0001) had significantly fewer damaged seeds than the lines without the *Sm*1 gene (Figure 3.16; Figure 3.17). Glumes (χ^2 =0.6698, df=1, p=0.4131) and awns (χ^2 =0.5393, df=1, p=0.4627) had no significant effect on the number of damaged seeds. However, a significant interaction among the three factors Awns: Glumes: *Sm*1 was present (χ^2 =10.2677, df=1, p=0.001354; Figure 3.18). This interaction was driven by the trait combination, hairy glumes, awnless, and non- *Sm*1 having a significantly higher number of damaged seeds than the lines (AC Lillian, CDC Hughes, CDC Stanley, and AC Waskada) had a significantly lower number of damaged seeds compared to the SMART lines (F_{1,670}=12.158, p=0.0005)



Figure 3. 16. The number of damaged seeds per ten wheat spikes with eight different trait combinations at the three different site locations (University of Saskatchewan's Brown, Preston, and Campus Research farms) in Saskatoon, Saskatchewan in 2020 and 2021. The boxes represent the interquartile range and the horizontal line inside the boxes represents the median for each timing. The space above the median is the 75th percentile and the space below the median is the 25th percentile. The whiskers show the maximum and minimums.



Figure 3. 17. Total number of damaged seeds found at the three research sites (University of Saskatchewan's Brown, Preston, and Campus Research Farms) in 2020 and 2021. Trait combinations included glume type (G=glabrous, H=hairy), awned (AW) or awnless (AL), and the *Sm*1 gene present (+) or absent (-).



Figure 3. 18. Relationship between *Sm*1 status and number of damaged seeds, in awned and awnless and pubescent or glabrous lines. Data are pooled for all University of Saskatchewan sites in all years.

There was a significant difference between the number of damaged seeds in 2020 versus 2021 ($F_{1, 574}$ =113.26, p=<0.0001). In 2020, a total of 2,974 damaged seeds were present on SMART lines, while in 2021 a total of 862 damaged seeds were present on SMART lines.

3.4 Discussion

The overall goal of this study was to determine whether the mechanical traits, hairy glumes and awns, could provide increased or alternative resistance against the wheat midge on spring wheat. Presently, Sm1 is the only antibiotic resistant trait against the wheat midge and is heavily relied on. OD, an antixenotic resistant trait reduces the number of wheat midge eggs and was tested in the early 2000s but not used on a commercial level (Lamb et al., 2001). Thambugala et al., (2020) identified an awned genotype that provided additional oviposition deterrence against the wheat midge. This study examined the impact of mechanical traits, awns and hairy glumes, in combination with and without the Sm1 gene. This study provided some key observations. First, adding the antibiosis resistant trait, Sm1, along with adding other mechanical resistant traits such as hairy glumes and awns to the wheat lines, did not reduce or increase yield (kg/ha) or the number of seeds produced per spike. However, removing the Sm1 gene from wheat lines did reduce yield in the year 2020 (kg/ha). Some studies have demonstrated a trade-off between resistance and yield; however, this can be overcome. For example, Golan et al (2019) identified that one GNI-AI allele from wild emmer 'Zavitan' and one 1B allele from bread wheat 'Weebil' decreased trade-offs in yield (kg/ha) and seed number in wheat breeding. Negative regulators in rice (GSN1) have been identified to overexpress and increase seed number but reduce yield (kg/ha) or alternatively, reduce expression and receive a heavier yield (kg/ha) with fewer seeds (Dwivedi et al., 2021; Guo et al, 2018b). There was no sign of a trade-off in this study and no indication that breeding these additional resistant traits into spring wheat lines compromised yield or seed production.

The second key observation is that the temperature and precipitation had a significant impact on the yield and seed production in the SMART wheat lines. According to the Saskatoon, Saskatchewan Historical Data RCS weather station results, in the year 2021, there was an increase in temperature compared to 2020. In Saskatoon, Saskatchewan, over the growing months of May, June, July and August the sites in 2020 had an average temperature of 15.85°C for those four months (23.03°C as a high, and 8.68°C as a low). In 2021, there was an average temperature of 16.83°C (24.62°C as a high, and 9.03°C as a low) over the four months. In 2021, from June 26th to August 2nd temperatures were at an all-time high with 31 of the 38 days reaching temperatures at or above 27°C (Saskatoon, Saskatchewan Historical Data RCS weather

station). Out of those 31 days, 18 days were at or above 30°C. In addition to these extreme temperatures in 2021, there was also a decrease in precipitation for all four sites (University of Saskatchewan's Brown, Preston, and Campus Research Farms and at the AAFC Saskatoon Research Centre's Lowe Rd. Farm). A lack of precipitation and higher temperatures have been known to significantly reduce spring wheat and other cereal yields and seed production (Dwivedi et al., 2021). The results from this study supported that the lack of precipitation and higher temperatures in 2021 resulted in significantly lower yields for Saskatoon, Saskatchewan for all trait combinations.

This study also highlighted that the growth stage timing of heading and anthesis in SMART wheat lines played a crucial role in seed production. Other studies have shown that lines that mature earlier usually produce less seeds than lines that mature over a more extended period of time (Dwivedi et al., 2021). The timing of anthesis (early, medium late) impacted the number of seeds produced by the SMART spring wheat lines. The SMART lines that had earlier heading dates (ZGS 61-65) had a significantly lower number of seeds than SMART wheat lines with the late heading dates (ZGS 45-49). This observation could arise from biotic and abiotic factors such as lack of precipitation, low fertility, temperature, and/or growing degree days. Early heading and flowering of wheat is usually an indication of temperature stress (Hu et al., 2005). These wheat lines with earlier heading dates may have endured more temperature and moisture stress when developing compared to the later heading SMART lines. These earlier heading lines presented signs of stress with less time given to the plant to fill the seed resulting in less seeds produced and lower yield (kg/ha).

Interestingly, wheat midge oviposition was not deterred by hairy glumes. This mechanical trait did not reduce the number of wheat midge larvae or damaged seeds on any of the SMART lines. Instead, in some cases, SMART wheat with hairy glumes had a higher number of damaged seeds in comparison to SMART wheat lines with glabrous glumes. This is possibly due to the hairy glumes doing the opposite of its proposed purpose and making it more appealing for wheat midge oviposition instead of deterring the wheat midge. Differences in hairy glume levels were determined, from slightly hairy to very hairy, based on the appearance of hairiness conferred by trichomes but there was no significant difference in the results when comparing slightly hairy to very hairy glumes in this study. Overall, the hairy glumes did not deter the wheat midge from ovipositing on wheat spikes.

In the study by Thambugala et al. (2020), QTL *QSm.mrc-1A*, the awned genotype was linked to a slight reduction in MDK. Our results identified a small reduction of third instar larvae when SMART lines had awns present.. We hypothesized that SMART lines with hairy glumes, awns, and the *Sm*1 trait would work in combination and have the lowest number of third instars and damaged seeds per wheat spike. However, a three-way interaction identified a combination of glabrous glumes, awns, and the *Sm*1 trait was the most successful in reducing the number of damaged seeds. Lines with the combination of hairy glumes, awnless heads, and Non-*Sm*1 SMART lines had the most damaged seeds.

The Sm1 gene is the only antibiotic resistance trait currently available in wheat on the market (Lamb et al., 2002; Thomas et al., 2005). In our study, the Sm1 gene was the most significant contributor of resistance, increasing yield (kg/ha), reducing the number of third instar wheat midge larvae, and reducing the number of damaged wheat seeds. In a previous study by Wise et al. (2015), several dead second instar wheat midge larvae were found on lines with the Sm1gene in the field. Another study reported no survival of third instar wheat midge larvae on lines with the Sm1 gene (Smith et al., 2007). However, this study found a total of 12 virulent and healthy third instar wheat midge on resistant lines (Sm1 present) over the two field years. Three SMART lines, (171, 185, and 225) were the only lines to have wheat midge larvae present on them. SMART 171 had three third instar larvae present, SMART 185 had one third instar larvae present, and SMART 225 had eight third instar larvae present. These virulent midge could be present as result of the SMART lines failing to express the Sm1 gene, or this difference related to previous studies could also suggest potential breakdown of the Sm1 gene's resistance in the wheat midge population in Saskatoon, Saskatchewan. In addition to the number of third instars on SMART lines, significant differences in the number of damaged seeds were observed among lines with the Sm1 gene present and absent. Seeds were analyzed in two different categories, either damaged or undamaged. Damaged seeds had third instar larval casts current, were significantly shrunken in size, distorted in shape, and had more damage on them in comparison to "tubby" seeds from first instars feeding, as first described by Barker and McKenzie (1996). Like the results from Wise et al. (2015) the SMART lines with Sm1 had a lower number of damaged seeds than those without the resistant gene.

Synchronization of wheat midge oviposition and timing of heading on wheat is key for the wheat midge to survive (Elliot & Mann, 1996). Wheat spikes are most susceptible to wheat

midge oviposition at Zadoks growth stages 51-59 (Zadoks et al., 1974; Elliott et al., 1991). However, in our study, wheat lines with the ZGS 51-59 after 51-53 days post seeding had the lowest number of third-instar larvae present on wheat spikes. Wheat that was heading earlier after 51-53 days post seeding (ZGS 61-65) and heading later (ZGS 45-49) had higher numbers of third instar larvae on the wheat spikes. Wheat midge usually emerge in late June and early July in western Canada in response to warm temperatures and moist conditions (Elliot et al., 2009; Elliot, 1988). The pheromone trapping results identified 2020 as having a higher number of wheat midge in comparison to the year 2021. In this scenario, it is assumed that the emergence of wheat midge was affected due to the lack of precipitation and higher temperatures in the year 2021. Additionally, 2020 had a higher number of damaged seeds compared to 2021. There are two main observations that would explain these results. First, the lack of precipitation in the year 2021 would have reduced the number of wheat midge emerging that year. Secondly, the seeding date and reduced moisture from increased temperatures would have affected the synchronization of wheat midge emergence with the timing of heading.

In 2020, USask's (University of Saskatchewan) Preston and AAFC Lowe Rd. sites had the highest number of wheat midge adults captured on the pheromone traps. However, USask's Brown and Campus sites had the highest number of damaged seeds in 2020 which contradicts the pheromone data. This is likely a result of the timing susceptibility of the SMART wheat and the wheat midge emergence. Spring wheat is most susceptible during the heading stage (ZGS 51-59). The majority of the SMART lines at the AAFC Lowe Rd. site were most susceptible during the dates of July 15th-18th, 2020 and the USask's Preston site SMART wheat was most susceptible during the dates of July 23rd-26th, 2020. The pheromone trapping data results show a peak in adult wheat midge at the AAFC Lowe Rd. site on July 21st (521 adults). This peak is past the susceptibility of the SMART lines at the AAFC Lowe Rd. site. The USask's Preston site had 1275 wheat midge captured but the majority (1235) were caught from July 7th-July 21st. The SMART wheat at the Preston site were able to escape most of the wheat midge damage because most of the lines were not susceptible until several days later because of the later seeding date. The USask's Brown and Campus sites had the highest number of wheat midge-damaged kernels. This is likely due to the SMART lines being susceptible from July 17th-20th and the pheromone trapping data identifying 109 wheat midge at Brown on July 14th and 241 wheat midge at Campus on July 14th.

In 2021, pheromone trapping data identified a decrease in wheat midge populations at all four sites. The AAFC Lowe Rd site had two peaks in wheat midge on June 28th, 2021 (251 adults) and July 22nd, 2021 (130 adults). The SMART lines were most susceptible on July 21st so the first peak of wheat midge (June 28th) would not have resulted in any MDK. Whereas the second flush would have resulted in MDK to most of the SMART lines. This same year, the AAFC Lowe Rd site had the highest number of wheat midge adult males (647) compared to the other sites (Brown (225), Campus (154), Preston (57). If the SMART lines at the AAFC Lowe Rd site were all early maturing or planted earlier, they would have been susceptible to the first and larger flush of wheat midge. However, the majority of the SMART lines were able to escape the wheat midge because of the planting date. The USask's Brown site had a peak on July 12th, 2021 (149 adults) but most of the SMART lines were susceptible from July 19th-21st. This flush of wheat midge would have resulted in some MDK but not as many as the previous year, reducing the number of MDK by more than half in 2021 at the Browns site.

Although not identified in this study, some plants can modify their phenology to delay their growth or escape insect pests by transitioning into an early reproductive stage (Mitchell et al., 2016). A lower number of wheat midge larvae and midge-damaged kernels are present when wheat spikes are exposed after anthesis was completed (Elliot & Mann, 1996). Kernel damage was the lowest during the stages of ZGS 65-70 and field inspections during or after this time for wheat midge are unnecessary. The highest frequency of wheat midge larvae counted, and wheat damaged kernels was when spikes were exposed to oviposition during ZGS 51-59 (Elliot & Mann, 1996). Ding & Lamb (1999) also discovered that wheat midge females laid eggs on wheat heads throughout the heading stage to anthesis. However, after anthesis the oviposition substantially decreased. These results could explain the results of the pheromone trapping data in regard to the number of midge-damaged kernels. Spring wheat damage from wheat midge can be minimized by selecting early or later maturing varieties or by seeding earlier or later so the plant can escape the synchronization of wheat midge emergence with anthesis. Overall, wheat lines with the *Sm*1 gene had the lowest number of third instar larvae and the lowest number of damaged seeds.

This present study aimed to sample all four site locations in Saskatoon, Saskatchewan for all field experiments. Unfortunately, due to constraints brought on by the pandemic restrictions we could not access all fields each year at the appropriate times. Additionally, the AAFC
Research Farm was planted in rows instead of hills and had to be analysed differently for yield (kg/ha). A second antibiotic resistant trait was studied at the AAFC Lowe Road farm in 2020 and 2021 called egg-antibiosis (EA). This trait was theorized to cause mortality to wheat midge eggs and prevent eggs from hatching based upon data from bioassays conducted on one of the parent wheat lines. However, the EA trait alone did not prevent the hatching of wheat midge eggs the first year (2020). In the second year, a flock of geese invaded the AAFC Field site and consumed the EA lines destroying all data available. Laboratory bioassays were attempted but emergence from the wheat midge colony started at AAFC in Saskatoon, Saskatchewan was unsuccessful. Over thirty different attempts were made to have wheat midge emerge for laboratory bioassays, and all were unsuccessful. Therefore, bioassays targeting specific lines at susceptible times could not be performed.

Despite these obstacles, the sampling collected from the fields provided this study with observations show potential for plant breeders and geneticists to add antibiotic and antixenotic resistant traits to spring wheat lines with minimal trade-offs to yield and seed production. The lack of significant effects (statistical or biological) could have arisen from the minimal sites studied in addition to the temperature and precipitation differences over the specific years studied. The pandemic played a large role in restricting access and restricting data collection. However, the results from this study determined that the lack of precipitation and extreme heat in 2021 is likely to cause disruptions for wheat midge emergence and should be investigated further. Future studies could explore how the heading date (early, medium, and late) of spring wheat can influence oviposition of wheat midge to further improve pest management strategies in Western Canada.

3.5 Conclusion

This study demonstrated that temperature and precipitation variation from year to year in Saskatoon, Saskatchewan is a factor driving spring wheat seed production and yield. Mechanical traits, hairy glumes and awns did not reduce or improve the yield when wheat midge populations were present. There were no trade-offs to yield, or the number of seeds produced when adding mechanical traits. Precipitation and temperature were the main factors that differed from year to year and further investigation into trade-offs because of resistance traits in the Canadian Prairies

could prove very beneficial. Future research is needed to further elucidate the different factors driving yield variation and seed production across the Canadian Prairies.

This study confirmed that the Sm1 gene, a novel antibiotic resistance trait, was dominant over mechanical traits when reducing the number of wheat midge present on wheat spikes. The Sm1 gene significantly reduced both the number of third instar wheat midge larvae found on wheat spikes as well as the number of damaged seeds found per trait combination in both years, while increasing yield (kg/ha). Even though virulent wheat midge larvae were present on wheat with the Sm1 gene, the Sm1 gene is still the most notable form of resistance present now. The breakdown of this resistance gene still needs to be monitored with different scouting techniques and continued yearly forecasting maps. Additional antibiotic resistance genes need to be created and studied to preserve the longevity of the Sm1 gene to help protect wheat producers in Canada.

Chapter 4 Identify if *Macroglenes penetrans* is impacted by hairy glumes or awns on spring wheat

4.1 Introduction

The orange blossom wheat midge, Sitodiplosis mosellana, is a common pest of wheat in Western Canada. To avoid damage from the wheat midge, integrated pest management approaches combining synthetic insecticides and resistant cultivars are typically applied (Jacquemin et al., 2014; El-Wakeil et al., 2013). However, biological control with natural enemies such as polyphagous predators (Carabidae, Staphylinidae and spiders) (Chavelle et al., 2015; Holland & Thomas, 2000) and parasitoids (Chavelle et al., 2015; Barnes, 1956) known to attack wheat midge is another potential integrated pest management component. As many as 27 insect species parasitize Sitodiplosis mosellana and/or Contarinia tritici (Kirby) (Chavelle et al., 2015; Affolter, 1990). Contarinia tritici, commonly known as the yellow wheat blossom midge, also has larvae that overwinters in the soil. Like the orange wheat blossom midge (Chavelle et al., 2018). Most common among the hymenopteran parasitoids of S. mosellana and C. tritici is Macroglenes penetrans (Kirby) (Hymenoptera: Chalcidoidea, Pteromalidae). Macroglenes *penetrans* is a particularly common parasitoid and is a significant population control on overwintering wheat midge. Olfert et al., (2003, 2009) monitored parasitism levels of M. penetrans in Canada to evaluate the future biological control of wheat midge and found the mean rate of parasitism by M. penetrans averaged 22-45% from 1991-2000, and 47-71% in 2001 (Olfert et al., 2003; Olfert et al., 2009).

The success of *M. penetrans* as a natural control agent of the wheat midge depends on the synchronized emergence of the two insects. Forecasting models for wheat midge have been used in North America (Chavelle et al., 2015; Elliot et al., 2009; Knodel & Ganehiarachchi, 2008; Wise & Lamb, 2004) and Europe (Chavelle et al., 2015; 1982; Kurppa, 1989; Oakley et al., 1998) to predict wheat midge emergence. Temperature and moisture conditions regulate wheat midge emergence, and the development of *M. penetrans* is affected by the same factors as its host (Chavelle et al., 2015). Adding an antibiotic resistant trait such as the *Sm*1 gene, with additional mechanical resistant traits such as awns and hairy glumes could result in a negative impact on wheat midge, reducing their populations (Lamb et al., 2001). However, the

combination of these resistant traits reducing wheat midge populations could also result in a negative impact on beneficial insect predators, such as *M. penetrans* (Lamb et al., 2002). Opposite in nature, Cai et al., (2009) identified host plant resistance on wheat heads influenced parasitism of the aphid species *Sitobion avenae* (F). However, to protect parasitoids populations and other beneficial predators of the wheat midge, a refuge of susceptible wheat is planted with resistant wheat to provide plants upon which the host midge can complete development. The motivation for this study is to gain a better understanding of these added mechanical resistance traits, hairy glumes and awns, on the parasitoid, *M. penetrans*.

In this study, the ability of *M. penetrans,* to parasitize the wheat midge on spring wheat with awns and hairy glumes was measured from third-instar wheat midge larvae overwintered in soil from two sites. The hypothesis is that the parasitoid, *M. penetrans,* will not be impacted by the mechanical resistance traits, hairy glumes and awns.

4.2 Methods and Materials

To study parasitism rates by *M. penetrans*, on July 23rd, 2020, and July 29th, 2021, spring wheat spikes of the SMART wheat lines were harvested, dissected, and counted from two spring wheat fields in Saskatoon, Saskatchewan. The *Sm*1 gene was not studied in this section because the SMART lines with the gene would act by minimizing the number third-instar larvae present. Five SMART lines for the four trait combinations (hairy glumes, awned; hairy glumes, awnelss; glabrous glumes, awned; and glabrous glumes, awnless) were planted at the two sites, totalling 20 different SMART lines (Table 4.1). No control lines were used for this study. Sites were chosen based on the seeding date and the ability to access them during the COVID-19 pandemic. Both sites (AAFC Saskatoon Research Centre's Lowe Rd. Farm and the University of Saskatchewan's Brown Research Farm) were planted on the same date in 2021 (May 31st) and only two days apart in 2020 (May 25th and May 27th). Each site had pheromone traps to track the emergence of the adult wheat midge. Yellow sticky traps were used at one site (AAFC Saskatoon Research Centre's Lowe Rd. Farm (52.16352°N, 106.52951°W) was planted as a RCBD in hills and the AAFC Saskatoon Research Centre's Lowe Rd. Farm (52.15183°N,

106.57390°W) was planted as an RCBD in 4.57 m (15ft) rows. Wheat spikes were selected at random from both sites and cut 10cm below the bottom of the spike.

Line No.	Glumes	Awns	Site
158, 166, 169, 170, 191	Hairy	Awnless	AAFC Lowe U of S Brown
201, 203, 210, 232, 243	Glabrous	Awnless	AAFC Lowe U of S Brown
255, 257, 271, 281, 296	Hairy	Awned	AAFC Lowe U of S Brown
316, 319, 329, 353, 365	Glabrous	Awned	AAFC Lowe U of S Brown

Table 4. 1. Line numbers and trait combinations for parasitism dissection data at two different locations in Saskatoon, Saskatchewan (University of Saskatchewan's Brown Research farm and at the AAFC Saskatoon Research Centre's Lowe Road farm) for the years 2020 and 2021.

Ten spikes per line (200 wheat spikes) were dissected individually and sequentially, starting at the bottom of the spike, counting the third instar wheat midge larvae found per spike. The number of third instars were recorded per spike, per line, and per site. The third instar larvae were still alive and were then placed in envelopes and transferred into containers for overwintering. The number of third instar larvae varied from 6-30 per container. The overwintering of the third instar wheat midge was described by Hinks and Doane (1988). Third instar wheat midge from each line and for each location were counted and placed into plastic cups (6.35 cm tall x 6.35 cm wide) that were filled $2/3^{rd}$ with soil from the AAFC Lowe Road Farm. Containers were topped with an additional 1.5 cm of soil to cover the larvae. Soil was then moistened with distilled, room temperature water and left at room temperature with ambient humidity the soil remoistened at the 2-week mark and the containers were sealed with an airtight lid and maintained at $22^{\circ}C +/-1^{\circ}C$ for a 6-week conditioning period (Figure 4.1). At the end of

this period, the third-instar wheat midge larvae were left to overwinter in a refrigerator at 2°C+/-1°C for approximately 6 months. After the 6 months' time, containers were removed from the freezer and left to acclimatize to room temperature for 72 hours. The soil was then examined for cocoons under a stereoscopic microscope (Nikon SMZ-2B). Cocoons that were found were dissected with pins to identify if they were parasitized or non-parasitized. Cocoons that were solid and alive were counted as non-parasitized. Cocoons that were popped easily were counted as parasitized.



Figure 4. 1. Third instar wheat midge diapause container set up. Containers $2/3^{rd}$ (6.35 cm tall x6.35 cm wide) full of soil had wheat midge larvae placed on top of soil and sealed with a lid.

4.3 Results

After performing this experiment, the vast majority of the wheat midge cocoons were unparasitized resulting in true zeros. Statistical analyses were attempted using several modeling methods including zero inflated models. Ultimately data results were limited based on many zeros and a very low rate of parasitism combined with the inefficiency of the zero inflated models, and consequently no statistical analysis are presented.

In total, 576 of the 918 (62.7%) third instar wheat midge larvae successful pupated and were recovered in the plastic cups during the laboratory dissection. Out of the 576, 15 pupae (0.03%) were parasitized and killed by *Macroglenes penetrans* over the years 2020 and 2021.

The parasitism rate by *M. penetrans* varied from 0.019-0.039% over the two years. In 2020, there was a total of 7 parasitized cocoons and 372 successful overwintering cocoons and recovered cocoons (0.019% parasitism rate). In 2021, 8 parasitized cocoons were found from the 204 recovered cocoons (0.039%).

The mechanical traits, hairy glumes and awns, appear not to affect the parasitism of *M*. *penetrans*. All of the different trait combinations of SMART lines had a minimum of 3 parasitized cocoons over the two years and two site locations. SMART lines with the hairy awned traits, glabrous awned traits, and glabrous awnless traits all had 4 parasitized cocoons. The SMART line with the hairy awnless traits had 3 parasitized cocoons (Figure 4.2).



Figure 4. 2. Total number of parasitized cocoons found on SMART wheat lines with different trait combinations at two different locations (University of Saskatchewan's Brown Research farm and AAFC Saskatoon Research Centre's Lowe Road farm) in 2020 and 2021 in Saskatoon, SK.

Yellow sticky cards were used at the AAFC Research Farm to identify parasitoid populations at the AAFC Research Farm in 2021 and caught over 3000 *M. penetrans* over six days. Pheromone traps were used to identify the presence of wheat midge. The University of Saskatchewan's Research Farm (Brown) pheromone traps caught a total of 562 wheat midge in 2020 and 225 wheat midge in 2021 totalling 787 wheat midge over the months of June, July, and August. The AAFC Saskatoon Research Farm (Lowe) had a total of 850 wheat midge in 2020 and 647 wheat midge in 2021 totalling 1,497 wheat midge caught. The AAFC Saskatoon Research Farm (Lowe) had 11 total parasitized cocoons with a total of 308 recovered cocoons (0.04%). In contrast, the University of Saskatchewan's Research site (Brown) only had 4 total parasitized cocoons with a total of 268 recovered cocoons (0.01%). While the data were not conducive to statistical analysis, parasitism rate between the years did not appear to differ.

4.4 Discussion

The parasitism rate of the hymenopterous parasitoid, *M. penetrans*, was studied on wheat lines with glabrous and hairy glumes with the presence or absence of awns. In this present study, as well as previous studies, the parasitism rates varied from site and year. Chavelle et al., (2015) studied *M. penetrans* as well as two other parasitoids *Euxestonotus error* (Fitch) and *Platygaster tuberosula* (Kieffer) at two different field locations in 2015. In this 2015 study, parasitism rate for all three parasitoid species ranged from 19.5%- 48.0% between sites, with *M. penetrans* responsible for 37.5%- 65.6% of those rates. Additional studies show parasitism rates by *M. penetrans* varying anywhere from 22-100% (Chavelle et al., 2019; Olfert et al., 2003; Affolter, 1990). However, this present study on *M. penetrans* indicated a significantly lower parasitism rate totalling 0.03% over both sites and both years (2020 and 2021). Yellow sticky traps in 2021 confirmed the presence of *M. penetrans*. Despite this, the number of parasitized cocoons was still significantly lower than in previous studies. However, due to the low parasitism numbers no conclusions can be made.

The low parasitism rate seen in this study could be a result of the overwintering process undertaken during this laboratory study (Colinet and Boivin, 2011). The sudden temperature changes of $22^{\circ}C$ +/- $1^{\circ}C$ to $2^{\circ}C$ +/- $1^{\circ}C$ may not have impacted the wheat midge diapause, but it may have influenced mortality of *M. penetrans*. Each parasitoid species has a unique

overwintering process that cold storage could affect (Colinet and Boivin, 2011). Future studies using laboratory bioassays (see Chavelle et al., 2015; Olfert et al., 2003) to measure live parasitoid emergence and parasitoid oviposition preference could result in more accurate and higher parasitism rates. A laboratory bioassay with more observational components (parasitoid oviposition preference, number of parasitoids present, etc.) would assist in measuring whether *M. penetrans* parasitism rate is impacted by mechanical traits, hairy glumes and awns.

This study was unable to identify any significant differences in SMART wheat lines with mechanical traits because the numbers of parasitized cocoons were too small to make assumptions. The impact of hairy glumes and awns as mechanical traits should be further investigated. Future investigation on the effects these mechanical traits may have against *M*. *penetrans* and other hymenopterous parasitoids could provide new insights into improving wheat midge pest management strategies. Additionally, further examination of *M. penetrans* synchronization of emergence and parasitism rate response with higher precipitation would assist in understanding the parasitoid's biology.

4.5 Conclusion

This study did not identify variation of *M. penetrans* parasitism rate in field sites in the Saskatoon, Saskatchewan area due to the low parasitism rates between sites overall. Differences in the previous agronomic practices (tillage) and neighbouring fields could be the influence on *M. penetrans* populations in field sites. The results from this study's parasitism rates of *M. penetrans* were significantly lower and not consistent with other studies. Therefore, it cannot be confirmed if the rate of parasitism by *M. penetrans* was affected by the mechanical traits, hairy glumes and awns, on wheat spikes. Other factors (*ie.*, timing of heading, rainfall, temperature, overwintering) should be explored in follow up studies to identify practices that would increase *M. penetrans* populations across Western Canada. Future research with additional field site locations and more observations in laboratory bioassays will identify more accurately if the mechanical traits, hairy glumes and awns can increase or decrease *M. penetrans* parasitism rates across Western Canada.

General Discussion

The orange wheat blossom midge continues to be a threatening pest for agriculture producers (Knodel & Ganehiarachchi, 2016). This insect pest is found everywhere wheat production occurs between the 42^{nd} and 62^{nd} parallels (Affolter, 1990). Although there is one current form of antibiotic resistance available to protect wheat from the wheat midge, it is based on a single gene (Lamb et al., 2002). This single gene, *Sm*1, as a form of resistance is in danger of break down due to the presence of virulent wheat midge in the overall population. These virulent midge are capable of completing larval development on plants with the resistance gene (Smith et al, 2004). Varietal blends are currently used at a 90:10 ratio of resistant to susceptible wheat to create an interspersed refuge to protect the *Sm*1 gene (Vera et al, 2013). However, using both an antibiosis resistance in combination with an antixenosis form of resistance could help to protect and prolong breakdown of the gene. While there are midge tolerant varieties, one registered insecticide, natural predators (parasitoids) and cultural controls available to control this insect pest it is important to keep looking for alternative methods of wheat midge control.

Mechanical traits such as pubescent (hairy) glumes and awns have been used to deter other insect pests and prolong the lifespan of resistance traits (Robert et al., 1979). Previous studies have identified that breeding different traits results in trade-offs for yield and seed number in wheat breeding (Golan et al., 2019). To investigate the effect of awns and pubescent glumes on wheat midge populations, this study sampled different SMART wheat lines in Saskatoon, Saskatchewan. These lines had been created at the AAFC Lethbridge Research Centre to determine the effect of hairy glumes, awns and their combination on added resistance to wheat midge. I hypothesized that breeding mechanical traits into wheat lines would result in a trade-off, reducing the overall yield. Over two growing years, the yield remained consistent for all SMART lines, regardless of their mechanical traits. However, the year differed significantly based on precipitation and temperature. Higher temperatures with reduced precipitation during April and August negatively affected wheat crops in Canada and this was identified in this study (Morgounov et al., 2018).

Roberts et al., (1979) identified the Hessian fly oviposition was significantly lower on wheat with pubescent leaves versus wheat with glabrous leaves. Thambugala et al., (2020) identified an awned genotype that was linked to reduced midge-damaged kernels. With this

information from previous studies, I hypothesize that the lines with hairy glumes and awns would deter wheat midge females from ovipositing, reducing the number of wheat midge larvae and midge-damaged kernels on wheat heads with these traits. My findings revealed that wheat midge females were not deterred from laying eggs by the hairy glumes and the larvae could find and feed on wheat kernels despite the hairy glumes. However, when the three traits; glabrous glumes, awns, and the Sm1 gene were present, this specific combination of traits reduced the number of midge-damaged kernels on wheat heads. The results from this study identify that hairy glumes do not deter the wheat midge from ovipositing on wheat spikes. Additionally, further research investigating the awned trait would be beneficial for preserving the Sm1 gene.

Timing of wheat heading and anthesis is a factor that contributes to the increase or decrease of wheat midge larvae and midge-damaged kernels. When oviposition occurs during the early stages of anthesis (ZGS 51-59) the highest proportion of larvae and midge-damaged kernels occur (Elliot & Mann, 1996). When wheat is exposed to oviposition after anthesis is completed, the number of larvae and midge-damaged kernels decreased (Elliot & Mann, 1996; Ding & Lamb, 1999). With this information from previous studies, this study identified that wheat spikes could to escape wheat midge oviposition depending on the seeding date and maturity of different SMART lines. If the seeding date was earlier or later than the emergence of the wheat midge females, the wheat spikes were not at the most susceptible stage and escaped oviposition, resulting in a reduction of larvae and midge-damaged kernels.

The wheat midge parasitoid, *Macroglenes penetrans*, and it's parasitism rates, have been at anywhere from 29.5-100% in the years 1991-2019 (Olfert et al., 2003, 2009; Chavelle et al., 2015, 2019). The same method of overwintering the third instar midge larva to determine parasitism rates was used in this study (Hinks & Doane, 1988). However, this study's parasitism rates were very low, less than 1%, during both years of the study. Emergence, longevity, fecundity and sex ratio, and many other important fitness traits may have impacted the overwintering process, therefore impacting the parasitoid emergence (Colinet & Boivin, 2011). Therefore, no conclusion was made on whether the hairy glumes and awns affect this parasitoids' ability to parasitize wheat midge. Further studies need to be performed to assess this.

Overall, this study explored how hairy glumes and awns affected wheat midge and its parasitoid (*Macroglenes penetrans*) over two growing seasons. The following key conclusions were made:

- There was no sign of a trade-off to indicate breeding additional resistant traits, hairy glumes and awns, into spring wheat lines decreasing yield or seed production.
- 2. Wheat midge females are still able to oviposit on wheat spikes with or without different mechanical traits (hairy glumes and awns).
- 3. Wheat midge larvae can move and feed on wheat kernels with mechanical traits (hairy glumes and awns) present.
- 4. Earlier and later seeding dates are an integrated pest management strategy that shows potential to decrease the number of midge-damaged kernels.

The study's finding provide information on wheat midge's ability to oviposit on wheat spikes with two mechanical resistance traits. Further studies on *Macroglenes penetrans* are required to ensure that mechanical resistant traits on wheat spikes will not deter the parasitoid from parasitizing wheat midge eggs and larvae. This study can add to the information available to wheat producers and plant breeders to protect the Sm1 gene and manage wheat midge populations.

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