DISSERTATION

IMPROVING INTEGRATED PEST MANAGEMENT OF WHEAT STEM SAWFLY (HYMENOPTERA: CEPHIDAE)

Submitted by

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ABSTRACT

IMPROVING INTEGRATED PEST MANAGEMENT OF WHEAT STEM SAWFLY (HYMENOPTERA: CEPHIDAE)

The wheat stem sawfly (*Cephus cinctus* Norton) has been a major pest of cultivated wheat (*Triticum aestivum* L.) for over 100 years. It is difficult to estimate the damage of this insect. Still, conservative calculations estimate the grain-yield loss to exceed 30%, and the economic losses exceed $350 million annually in affected regions in the United States of America. This estimate does not include Colorado, so we expect the current figure to be much higher. Most economic loss is caused when the larva reaches the final instar when it creates a hibernaculum (stub) by cutting the stem above ground level just before harvest, which causes the wheat stem to fall. Fallen stems are difficult to harvest and are easily blown away. The life cycle of this pest makes it difficult to control, and current management strategies are not always effective. My dissertation aims to improve current integrated pest management strategies to control the wheat stem sawfly better. Chapter 1 briefly introduces the three aspects of integrated pest management that I studied: plant resistance, biological control, and cultural control using trap crops.

Breeding for host-plant resistance is of particular importance for the management of wheat stem sawfly and is often considered the most effective. In Chapter 2, I review the current literature on plant resistance to the wheat stem sawfly. First, I examine host plant resistance using solid pith expression. Solid pith expression is the most common mechanism of resistance to the wheat stem sawfly. However, expression and control are inconsistent and often impacted by environmental variables. Another drawback to solid stemmed varieties is how biological

control can be affected by solid stem expression. Next, moving away from the solid stem, I explore the literature on resistance due to host-preference and new resistance mechanisms derived from wheat landraces. I conclude this chapter by suggesting improvements to breeding for wheat stem sawfly resistance, such as screening for resistance and a deeper analysis of host plant metabolism.

Given the variability of current resistant genotypes, I developed a new screening method for resistance described in Chapter 3. I report a novel screening method where plants are grown in the greenhouse in conetainers under optimal conditions and then subjected to a natural infestation in the field. Using this method, I was able to examine host preference as well as host suitability. For this experiment, I chose seven winter wheat genotypes with different levels of pith expression to measure host preference and suitability and compared results to field trials. Results of the conetainer study showed similar sawfly infestation amounts as those observed in the field study, which confirms the robustness of the conetainer assay. In addition, the smallest larvae and lowest infestation amounts were found in a solid-stemmed variety in 2019 and a semi-solid stemmed variety in 2020. This screening method will allow wheat breeders and entomologists to evaluate host plants for various resistance traits gather information on host preference and suitability.

The wheat stem sawfly can be successfully controlled by biological control. However, in Colorado, we have not observed high populations of the common biological control agents of the wheat stem sawfly. Chapter 4 examined the prevalence of two *Bracon* parasitoids, *Bracon cephi* Gahan and *B. lissogaster* Muesebeck, and their host the wheat stem sawfly. I assessed the degree of non-crop and crop host plant use and responses to landscape composition. I also found no parasitism by either *Bracon* species in our three-year statewide winter wheat survey. Still, I

found small populations of *Bracon* in non-crop landscapes throughout eastern and western Colorado. I used model selection to examine how local (500 m scale) and landscape (5 km scale) cover of suitable non-crop and crop habitats potentially affect *Bracon* and wheat stem sawfly abundances. My best fit model for wheat stem sawfly suggests that a decrease in non-crop cover at the landscape scale increases wheat stem sawfly infestation in non-cultivated grasses. My best fit model for *Bracon* parasitism suggests an increase in wheat cover at the local level results in the greatest increase in the odds of parasitism by either species of *Bracon*. Herbaceous cover at local and landscape scales were also significant predictors of *Bracon* parasitism. This study suggests that pests and natural enemies respond differently to landscape composition, and these responses should be evaluated before management decisions are made.

In my final chapter, I evaluated the potential of using winter triticale (x *Triticosecale*) as a trap crop for the wheat stem sawfly. Forage triticale is on average taller and has a larger stem diameter than winter wheat. These traits are considered attractive to adult females when choosing hosts for oviposition. I conducted a two-year field study of one winter wheat and one winter triticale genotype combination for its potential as a trap crop. To complement the field study, I grew three genotypes of winter triticale and one winter wheat genotype in cone-tainers and infested them in the field. The cone-tainer and field studies revealed that the chosen winter triticale genotypes were not more attractive than the winter wheat genotypes for adult wheat stem sawflies. The field study also evaluated the average larval position in the stem and found the average position was variable between sampling dates in both crops. Thus, future studies should evaluate the precise timing of field swathing potentially destroying significant portions of larval populations.

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DEDICATION

To all the insects, who never cease to amaze me.

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CHAPTER 1 - Introduction

Introduction

Global wheat (*Triticum aestivum* L.) production in 2020 was at a record high, with over 760 million tonnes produced (FAO, 2020). Despite increasing production and demand, wheat can experience yearly yield losses of more than 9% due to insect and other animal pests (Oerke, 2006), and damage due to insects is only expected to increase. Models examining effects of temperature predict an increase of 10-25% to insect caused yield loss in wheat for every degree of warming (Deutsch et al., 2018). Temperate environments where wheat is often grown are projected to experience the most losses from insects with every degree of warming compared to corn and rice (Deutsch et al., 2018). To ameliorate the situation, more effective management strategies must be developed.

The wheat stem sawfly (Hymenoptera: Cephidae: *Cephus cinctus* Norton) is a destructive pest that causes damage to wheat (*Triticum aestivum* L.) in North America. Recent grain yield loss due to the wheat stem sawfly can exceed $350 million in the northern Great Plains. Damages are expected to increase as the wheat stem sawfly infests more wheat-growing regions in the Great Plains (Olfert et al., 2019; Cockrell et al., 2021). Current management methods such as resistant wheat, biological control, trap crops, and chemical controls have not effectively controlled the wheat stem sawfly (Beres et al., 2011). This introduction and dissertation focus on three aspects of the integrated pest management of the wheat stem sawfly: plant resistance, biological control, and cultural control using trap crops.

Wheat stem sawfly biology

A comprehensive review of wheat stem sawfly biology and management practices is reported by Beres et al., 2011. In short, the wheat stem sawfly is particularly difficult to control since most of its life cycle occurs inside the host stem. The adult sawfly lay eggs within the stem of their host, and as the larvae develop, they consume parenchyma tissue (Ainslie, 1920). If there are multiple larvae in the stem, they will cannibalize each other until one is left. Towards the end of the season, the one surviving larva creates a hibernaculum (stub) at the stem's base, causing the seed head to fall to the ground. Fallen wheat heads are difficult to harvest and prone to being blown away (Ainslie, 1920). It is also important to note the wheat stem sawfly has gradually adapted to new hosts by emerging earlier in the season to infest, in addition to spring wheat, the earlier maturing winter wheat (Morrill and Kushnak, 1996).

Plant resistance

Currently, host-plant resistance is considered the first line of defense for controlling pests and pathogens (Dara, 2019; Tian et al., 2020) and is regarded as a sustainable pest management strategy (Ramankutty et al., 2018). However, resistance to insects has varying levels of success. Resistance mechanisms often fall into three categories; chemical signals, physical barriers, and reduced palatability (Mitchell et al., 2016).

Breeding for host-plant resistance is of particular importance for managing wheat stem sawflies and is often considered an effective strategy (Beres et al., 2011). For or over 70 years, the focus of resistance breeding has been on solid pith expression. However, solid stem genotypes do not completely deter or stop damage despite the adverse effects on wheat stem sawfly. Other resistance has been evaluated, including genotypes that are not preferred by female wheat stem sawflies for oviposition or wheat landraces to find novel sources of resistance (Beres et al., 2011).

A significant limitation for breeders is the difficulty of screening genotypes for resistance. Most screening for wheat stem sawfly resistance in the field occurs at stem maturity, and plots are given a visual score based on the degree of stem lodging (Weaver et al., 2009). While field trials can provide a lot of data, this method cannot provide information on the type of resistance mechanisms. Greenhouse choice tests are conducted to compare wheat stem sawfly host preference; however, such tests take a considerable amount of time to perform and are typically limited to the evaluation of small numbers of breeding lines (Buteler et al., 2009; Weaver et al., 2009; Buteler and Weaver, 2012). Hence, there is a need to develop methods that can combine the high throughput capabilities of field evaluation and the ability to control the growing environment and, in turn, germination success and plant phenology.

In my first chapter, I review the literature on host-plant resistance to wheat stem sawfly in more depth. Then in my second chapter, I explore a novel method of evaluating host-plant resistance

Biological control

Wheat stem sawfly has seven recorded parasitoids, *Bracon cephi* Gahan, *Bracon lissogaster* Muesebeck, *Eupelmella vesicularis* Retzius, *Eupelmus allynii* French, *Eurytoma atripes* Gahan, *Pleurotropis utahensis* Crawford, and *Scambus detritus* Holmg. that are primarily encountered in non-cultivated grasses (Davis et al., 1955). However, *B. cephi* and *B. lissogaster* are the only known parasitoids to parasitize wheat stem sawfly in spring and winter wheat in Canada (Nelson and Farstad, 1953; Cárcamo et al., 2012), Montana (Morrill et al., 1998; Runyon et al., 2002), and Nebraska (Bradshaw and Peterson 2016). *B. lissogaster* males have a black head, thorax and yellowish-orange metasoma (Runyon et al., 2001). Females are yellowish-orange with a black head. *Bracon lissogaster* is gregarious, with often three or four larvae found on a single wheat stem sawfly larva (Somsen and Luginbill, 1956). The other major parasitoid *B. cephi* is yellowish-orange when an adult and considered solitary since only one egg is laid per larva (Nelson and Farstad 1953). Often, *B. cephi* is the main parasitoid encountered (Morrill et al., 1998). Both *Bracon* species are idiobiont ectoparasitoids that are known to parasitize multiple larval instars of the wheat stem sawfly (Nelson and Farstad, 1953; Rand et al., 2011).

Biological control is predicted to be effective when at least 68% of the wheat stem sawfly population within a field is parasitized by these two *Bracon* species (Rand et al. 2017). However, this level of parasitism is only occasionally recorded in wheat (Rand et al., 2011, 2014, 2017). To date, parasitism in Colorado is rarely observed in cultivated wheat. Hence, there was a need to evaluate where and if *B. cephi* and *B. lissogaster* were present in Colorado non-cultivated grasses. If there is parasitism, did the surrounding landscape affect populations of the wheat stem sawfly and its parasitoids? In my fourth chapter, I evaluate how land use can influence future parasitoid and pest populations to answer these questions.

Trap crops

Trap crops are used to attract insect pests away from the crop of interest. Typically, plants are planted in a small stand near the crop of interest and are destroyed or treated with pesticides once pest densities are high (Hokkanen and Jokioinen, 1991). Some common methods of trap cropping include perimeter planting where the trap crop is planted around the border of the main crop; sequential planting where the trap crop is planted earlier than the main crop; multiple planting where several other trap crops are used; and push-pull where the trap crop is highly attractive, and the main crop is not (Hokkanen and Jokioinen, 1991; Shelton and Badenes-Perez, 2006).

Early studies in wheat stem sawfly trap crops found smooth brome (*Bromus inermis)* and fall rye (*Secale cereale* Will)could be effective for management (Criddle, 1922). Smooth brome was found to mature earlier than spring and winter wheat, and wheat stem sawfly have lower larval survival and higher parasitism when developing in smooth brome (Criddle, 1922). Similarly, fall rye was harvested early in the summer, killing larvae before hibernation chambers could be formed.

More recent studies found potential use for winter wheat as a trap crop for wheat stem sawflies in spring wheat cropping systems (Morrill et al., 2001; Beres et al., 2009; Buteler et al., 2010). The early planting of winter wheat in Montana attracted wheat stem sawfly adults from neighboring fallow wheat stubble and served as a sink for eggs. Later, the trap crop could be harvested or swathed to destroy larvae developing within the stems (Morrill et al., 2001). Studies suggest that stem height, stem solidness, and semiochemicals play a role in the attractiveness and retention of winter wheat to wheat stem sawfly (Morrill et al., 2001; Beres et al., 2009; Buteler et al., 2010; Buteler and Weaver, 2012).

Since most wheat grown in Colorado is winter wheat, in my final chapter, I focused on assessing the retention, attractiveness, and host suitability of winter triticale (x *Triticosecale* Wittmack)*,* a cross between wheat and rye (*Secale cereal,* Will.), as a trap crop for the wheat stem sawfly in a winter wheat cropping system. Winter triticale has tall plant stature, good grain and forage yield potential, early maturity, and desirable forage quality (Oettler, 2005; Estrada-Campuzano et al., 2012; Randhawa et al., 2015), making this crop attractive to wheat farmers who also feed cattle (Harper et al., 2017; Coblentz et al., 2018).

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CHAPTER 2 - Solid stems and beyond: challenges and future directions of resistance to wheat stem sawfly[1]

Introduction

Global wheat (*Triticum aestivum* L.) production in 2020 was at a record high, with over 760 million tonnes produced (FAO, 2020). Despite increasing production and demand, wheat can experience yearly yield losses of more than 9% due to insects and animal pests (Oerke, 2006), and damage due to insects is only expected to increase due to global warming (Deutsch et al., 2018). Among the insect pests, the wheat stem sawfly (Hymenoptera: Cephidae: *Cephus cinctus* Norton) is considered the most important in North America (Beres et al., 2011; Buteler et al., 2015). Current management methods such as biological control, trap crops are not always effective (Beres et al., 2011). Grain yield loss due to the wheat stem sawfly can exceed $350 million annually in the northern Great Plains. Damage is expected to increase as the wheat stem sawfly infests more wheat-growing regions in the Great Plains (Olfert et al., 2019; Cockrell et al., 2021). A comprehensive review of wheat stem sawfly biology and management practices is reported by Beres et al., 2011. The wheat stem sawfly is particularly difficult to control since most of its life cycle occurs inside the host stem. The adult sawfly lay eggs within the stem of their host, and as the larvae develop, they consume parenchyma tissue (Ainslie, 1920). Towards the end of the season, the surviving larva creates a hibernaculum (stub) at the stem's base, causing the stem to lodge. Lodged stems result in fallen wheat heads which are difficult to harvest and prone to being blown away (Ainslie, 1920). The wheat stem sawfly has gradually

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adapted to new hosts by emerging earlier in the season to infest, in addition to spring wheat, the earlier maturing winter wheat (Morrill and Kushnak, 1996).

Breeding for host-plant resistance is of particular importance for the management of wheat stem sawfly. Host-plant resistance is often considered the first line of defense for controlling pests and pathogens (Dara, 2019; Tian et al., 2020) and is regarded as a sustainable pest management strategy (Ramankutty et al., 2018). Resistance to insects has been applied to varying levels of success, and mechanisms often fall into three categories; chemical signals, physical barriers, and reduced palatability (Mitchell et al., 2016). Controlling the wheat stem sawfly is exceedingly complex and multifaceted, making it essential to review recent developments in resistance mechanisms to create robust breeding strategies. In this review, we highlight current strategies of resistance such as solid stems, impacts on biological control, non-preference, plant metabolism, and future directions.

Host plant resistance to wheat stem sawfly

Solid stem resistance

For over 70 years, solid pith expression has been a significant focus of resistance breeding for wheat stem sawfly. Undifferentiated parenchyma cells create a solid pith (Berzonsky et al., 2003) which tends to inhibit egg-hatching, serves as a mechanical barrier to wheat stem sawfly larvae movement, and early pith drying results in desiccation of the larvae (Platt et al., 1948; Holmes and Peterson, 1962; Delaney et al., 2010; Buteler et al., 2015). Solid stemmed genotypes can also reduce female weights, sizes, and fecundity, and in some cases, delay adult emergence (Cárcamo et al., 2005, 2011). Additionally, when compared to hollow stemmed susceptible genotypes, solid stemmed genotypes can increase yield by an average of 16% and reduce losses due to cutting by 7% under high wheat stem sawfly pressure (Beres et al., 2007, 2009). Timing of solid stem expression is considered important as early stem solidness during female sawfly flight has been shown to reduce oviposition and increase larval mortality (Varella et al., 2016, 2019b).

The source of the primary solid stem allele on chromosome 3BL can determine the timing and effectiveness of the genotype. Haplotype analysis by Cook et al. (2017) found almost all hexaploid wheat stem sawfly resistant varieties in North America contained the haplotype from a Portuguese landrace, 'S-615' (Platt et al., 1948). The one exception was the resistant spring wheat variety 'Conan'. Evaluation of the alleles on chromosome 3BL found genotypes containing alleles derived from S-615 showed stable solidness over the growing season (Varella et al., 2016). In addition, lines derived from Conan showed early solid stem expression and lower infestations when compared to lines derived from S-615 (Varella et al., 2016; Cook et al., 2019). The mechanism behind the solid stem trait in wheat is due to an increased copy number of *TdDof*, a gene controlling stem solidness in wheat (Nilsen et al. 2020). In the same study, Nilsen et al. (2020) developed the most up-to-date DNA marker which can be found at *usw275* in bread and durum wheat. Minor QTL (Quantitative Trait Locus) have also been identified on chromosomes 2A, 2D, 4A, and 5A, which are known to enhance the solid stem allele on chromosome 3BL (Nilsen et al., 2017).

The effectiveness of solid stem resistance is not always reliable as the degree of expression is variable based on several environmental conditions and management strategies (Subedi et al., 2020). Choosing the correct genotype and sowing density can impact solidness. Sowing densities of 350 seeds m^{-2} or less can maximize pith expression, decreasing at higher densities (Beres et al., 2012; Nilsen et al., 2016). Environmental conditions such as high precipitation (De Pauw and Read, 1982; Beres et al., 2017) and light intensity can also decrease solid pith expression. During internode elongation, low light intensities can inhibit pith tissue cell division, resulting in reduced solidness (Holmes, 1984; Nilsen et al., 2016). Additionally, conflicting evidence suggests stem solidness may be negatively correlated with grain yield (McNeal et al., 1965; Cook et al., 2018, 2019), shows no correlation at all (Lebsock and Koch, 1968; McNeal and Berg, 1979; Hayat et al., 1995; Sherman et al., 2015), or is positively correlated with grain yield under water deficit conditions (Saint Pierre et al., 2010). Solid stemmed varieties can also still experience large amounts of damage due to larval stem cutting under high wheat stem sawfly infestation pressure (Beres et al., 2009). Breeding programs are starting to evaluate alternative mechanisms for resistance that could complement current solid-stemmed genotypes. Interaction of solid stem and biological control

There is conflicting evidence that solid stemmed genotypes may influence the biological control of the wheat stem sawfly. Two native braconid parasitoids, *Bracon cephi* (Gahan) and *B. lissogaster* (Muesebeck), can effectively control wheat stem sawfly populations (Nelson and Farstad, 1953; Rand et al., 2011). However, parasitism can be highly variable in wheat (Rand et al., 2011, 2014, 2017). In some wheat-growing regions, such as Colorado, significant parasitism has yet to be detected (Peirce et al., 2021). Stem solidity can reduce parasitism, but this impact is highly variable between genotypes (Rand et al., 2012) or not observed (Morrill et al., 1994; Wu et al., 2013). According to a population model created by Rand et al. (2020), sufficient parasitism needed to halt wheat stem sawfly population growth was 22% in resistant genotypes and 86% in susceptible genotypes. Results from the population model and reviews by Bottrell et al. (1998) and Cortesero et al.(2000) highlight the importance of combining natural enemies and plant resistance for the best long-term results. Future genotype selection should evaluate biological control and resistance interactions to ensure one strategy is not working against another.

Resistance due to non-preference

One alternative to solid stem resistance is manipulating the wheat stem sawfly's oviposition behavior, also known as host preference. This type of resistance is based on the preference-performance hypothesis where adult females select hosts for oviposition that are most suitable for offspring survival and development. The performance-preference hypothesis is also known as 'optimal oviposition theory' (Jaenike, 1978) or 'mother knows best' (Valladares and Lawton, 1991).

Insects with immobile immature stages are especially affected by female host selection (Thompson, 1988; Craig and Itami, 2008). A meta-analysis conducted by Gripenberg et al. 2010 found the most substantial evidence of the preference-performance hypothesis for insects with an intermediate level of diet specialization and a less mobile immature stage. The wheat stem sawfly has an immobile immature stage (Ainslie, 1920) and has been documented from numerous known hosts (Cockrell et al., 2017), making it an ideal candidate for developing host resistance through non-preference.

Wheat stem sawfly adult preference is often determined by stem height, diameter, and plant maturity (Holmes and Peterson, 1960; Buteler et al., 2009). Alternative mechanisms for preference have been considered since these characteristics are difficult to alter and are closely related to yield. Volatile compounds released by host plants may also determine host preference. The wheat stem sawfly has 28 unique antennal odorant receptor transcripts (Gress et al., 2013), allowing adult sawflies to respond behaviorally to pheromones such as 9-acetyloxynonanal (Cossé et al., 2002) or other semiochemicals. Based on Y-tube studies and caged choice tests, wheat volatiles (Z)-3-hexenyl acetate, and β-ocimene were attractive to adult females (Piesik et al., 2008; Weaver et al., 2009; Buteler and Weaver, 2012). A small plot field experiment concluded that differences in attraction between genotypes might be attributed to QTLs found on wheat chromosomes 1B and 4A (Sherman et al., 2010; Varella et al., 2015). Future studies could also examine the number of eggs found in the stem to rule out early mortality or low adult populations as confounding variables to preference. Additionally, while there is evidence for preference, often whole fields are planted with a single genotype creating a no-choice environment. In this situation, adult females may lay their eggs in hosts regardless of potential preference.

The preference-performance hypothesis is also closely related to the optimal patch theory, which suggests overall fitness decreases with the time spent finding and handling hosts rather than feeding or ovipositing (Charnov, 1976). A study by (Varella et al., 2017b) identified QTLs on wheat chromosomes 2D, 3B, and 4A that influenced wheat stem sawfly host evaluation behaviors. A QTL on chromosome 3B (*Qss-msub-3BL*) and 2D (*Qwss.msub- 2D*) decreased oviposition preference by reducing ovipositor insertions and egg deposition. A QTL on chromosome 4A (*Qwss.msub-4A.1*) reduced host selection from a distance. Females spent more time examining the susceptible parent 'Reeder' than the resistant parent 'Conan', but the number of ovipositor insertions was double for the susceptible parent (Varella et al., 2017b). Initial examination of plant structures suggests increased wax content of platelet-type crystals on leaf cuticles on Conan compared to Reeder, which had tubular crystals (Lavergne et al., 2018). These findings are supported by previous research, which found epicuticular waxes can play a role in enhancing or deterring other insects oviposition, movement, and feeding after a host is found (Eigenbrode and Espelie, 1995). Breeding genotypes that influence wheat stem sawfly adult preference and behavior has the potential to decrease populations, but will likely have the most impact in combination with other resistance mechanisms (Beres et al., 2009; Cárcamo et al., 2016) and areas with low to moderate infestation (Weiss et al., 1990).

Other sources of resistance

Since the effectiveness of solid stem and non-preference traits are variable based on environmental conditions, there is an urgent need to explore alternative sources of resistance. An essential resource for resistance traits may be the genetic diversity found in wild wheat relatives and landraces (Kishii, 2019; Pour-Aboughadareh et al., 2021). Using landrace accessions from regions where wheat stem sawflies are endemic (Varella et al., 2017a) examined wheat landraces for resistance traits. All accessions were rated for solidness, and 82 hollow-stem accessions were identified as resistant to wheat stem sawfly. Expanding on this study, Varella et al. (2019a) developed six recombinant inbred line populations and found QTL on wheat chromosome 4A was closely linked to resistance unrelated to solid stems. Another study on wheat stem sawfly resistance of North American spring wheat germplasm found a high frequency of the favorable allele on chromosome 4A (Varella et al., 2015). Identified alleles found in the landrace populations contributed to similar cutting, infestation, and mortality of wheat stem sawfly larvae compared to the solid, stemmed check. Thus, suggesting novel traits of resistance could be taken from the landraces tested. However, the underlying mechanism involved in the resistance observed was difficult to quantify in the field.

Understanding the metabolic changes in plants during their interaction with pests can provide valuable insight into the biochemical responses associated with resistance. Two types of herbivore-induced defense mechanisms, sequestration of toxic chemicals and mechanical defenses. Upregulation of toxic chemicals such as benzoxazinoids, glucosinolates, and mechanical defenses such as increased density of thorns and spikes can provide induced resistance to herbivore damage (Markovich et al. 2013, Leybourne et al. 2019, Batyrshina et al. 2020). Observed differences in molecular responses in spring wheat following wheat stem sawfly infestation between resistant and susceptible genotypes (Biyiklioglu et al. 2018, Lavergne et al. 2020). Initial analysis of a hollow-stemmed variety 'Hatcher', considered to be variably resistant to wheat stem sawfly, found differences in plant metabolism when compared to susceptible genotypes (Lavergne et al., 2020). The metabolite profile of Hatcher shifted from primary metabolites to an increased expression of known antiherbivore compounds such as protease inhibitors which impact insect digestion (Singh et al., 2020), and phytochemicals such as benzoxazinoids (Wouters et al., 2016). Resistance of the non-solid stemmed genotype, Hatcher, could be attributed to plant metabolism (Lavergne et al., 2020). When examining primary metabolites, microRNAs play a role in controlling an organism's metabolism and physiological processes (Bordoloi and Agarwala, 2021). Another mechanism for resistance may come from noncoding RNAs and microRNAs. Unique miRNA in a solid stemmed resistant genotype Choteau were identified and may play a role in a stress response to wheat stem sawfly by up or down-regulating the plant metabolism in response to wheat stem sawfly infestation (Cagirici et al., 2017; Biyiklioglu et al., 2018).

We summarize the different types of resistance and when to look for them during the growing season of wheat relative to wheat stem sawfly development. Figure 2.1 is based on the Zadoks maturity scale (Zadoks et al., 1974).

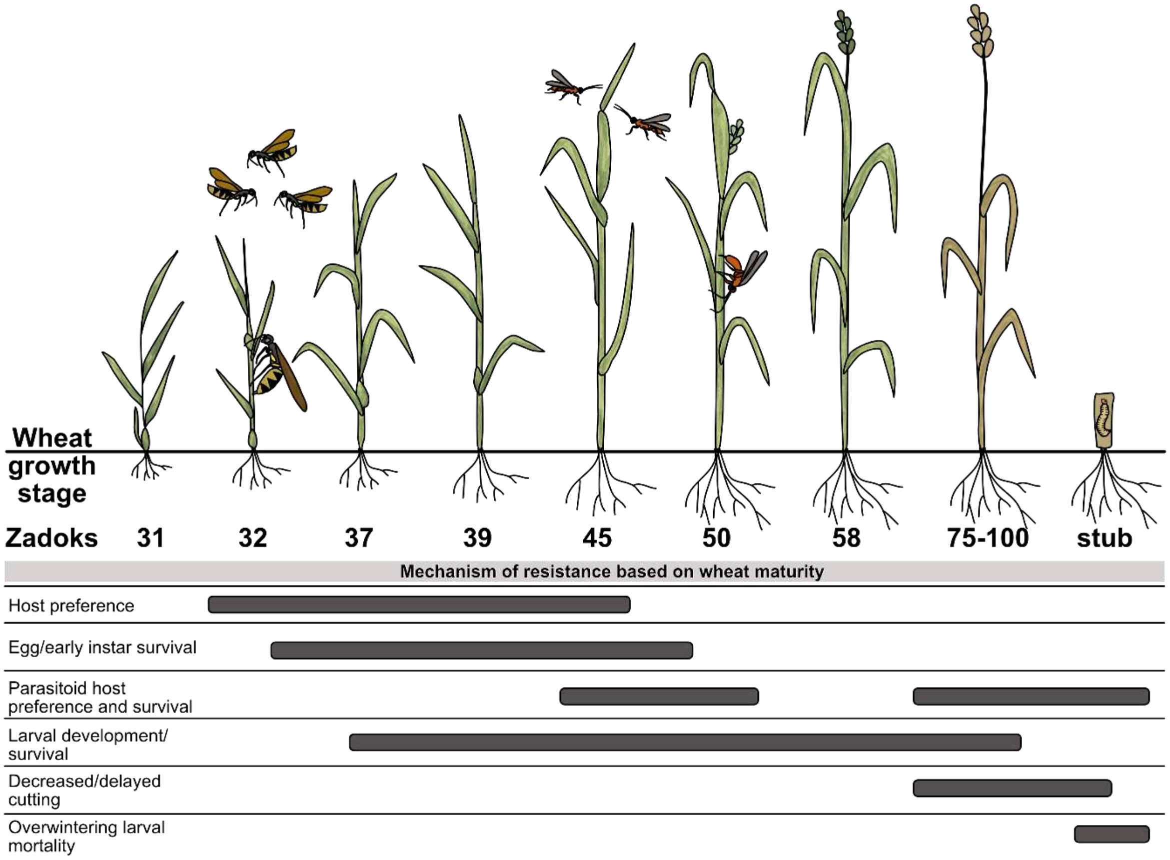

Figure 2.1. Wheat growth stages in the Zadoks scale and when resistance to the wheat stem sawfly would be expressed.

Future research directions

As stated previously, there is evidence that combining biological control and resistant genotypes can decrease wheat stem sawfly population growth and damage when compared to either strategy alone (Rand et al., 2020). Assessing parasitism rates and wheat stem sawfly mortality can help improve future resistant cultivars. Developing resistant varieties that positively impact biological control can only improve wheat stem sawfly management.

Another avenue to decrease damage caused by the wheat stem sawfly would be to explore host tolerance. Tolerance is often difficult to select for but is considered more sustainable since it is difficult for pests to evolve resistance to tolerance traits (Peterson et al., 2017). Examples of traits associated with tolerance include increased photosynthesis despite herbivory, high growth rates, more carbon storage in roots and changes in resource allocation after herbivory (Strauss and Agrawal, 1999). Unfortunately, a major bottleneck for breeding for plant tolerance is the difficulty in identifying traits for phenotyping.

There are also inherent issues concerning the current field screening for wheat stem sawfly resistance. The many confounding variables such as stem maturity, height, stem diameter that impact egg-laying of adults compounded with the time-consuming task of splitting stems open to determine mortality and infestation of larvae make field and greenhouse evaluations difficult. Visual scoring of field plots can be a fast way to assess cutting, often considered a proxy for infestation (Weaver et al., 2009). However, visual inspection of breeding lines to either measure or grade traits of individual crop plants often represents a major logistical constraint, and it may also be associated with considerable inconsistency due to subjectivity caused by human error often resulting in a lack of robustness and repeatability (Araus et al., 2018). Thus, there is a need to develop a high-throughput phenotyping technology using image analysis to screen a more significant number of crop breeding lines accurately and in a timely manner at reduced costs.

Aegilops and *Triticum* genera are examples of known wild wheat species that may harbor useful alleles shown to provide resistance to other insects and abiotic stressors (Kishii, 2019; Pour-Aboughadareh et al., 2021). Using the genetic diversity in landraces and wild wheat species could provide new traits for biochemical responses in wheat, aiming to recover important alleles that can provide resistance to the wheat stem sawfly.

Examining new traits of resistance can provide more robust control to this devastating insect.

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CHAPTER 3 - A novel and efficient screening method to evaluate wheat stem sawfly (Hymenoptera: Cephidae) host selection and suitability

Introduction

The wheat stem sawfly (Hymenoptera: Cephidae: *Cephus cinctus* Norton) is a devastating pest that causes damage to wheat (*Triticum aestivum* L.) and durum wheat (*Triticum durum*), with grain-yield loss exceeding $350 million in the northern Great Plains (Beres et al., 2011). In 2020, wheat stem sawfly damage resulted in an estimated $30 million loss in Colorado (Peairs and Haley, unpublished data). Damages are expected to increase as the wheat stem sawfly's host and geographic range expands (Olfert et al., 2019; Cockrell et al., 2021). Wheat stem sawfly is particularly difficult to control since most of its life cycle occurs inside the host stem. The adult sawfly lays its eggs within their host's stem, and as the larvae develop, they consume parenchyma tissue (Ainslie, 1920). During development, larvae within the stem cannibalize each other until one remains. Towards the end of the season, the one surviving larva creates a hibernaculum (stub) at the stem's base, causing the seed head to fall to the ground. In addition, to yield loss from the larvae consuming the stems, fallen stems result are difficult to harvest and prone to being blown away (Ainslie, 1920).

Current management practices include biological control, trap cropping, mechanical controls by swathing, tillage and rotations, and resistant genotypes (Beres et al., 2011). However, biological control by larval ectoparasitoids *Bracon cephi* and *B. lissogaster* are only considered effective when parasitism exceeds 60 percent, which is rarely observed in the field (Rand et al., 2017). Some trap crops have shown the potential to decrease oviposition in neighboring wheat fields (Morrill et al., 2001; Buteler et al., 2010). However, trap crops are rarely adopted by farmers (Hokkanen and Jokioinen, 1991) and are often not used for cereal crops due to environmental conditions, landscape variation, and cultivation practices associated with grain production (Sharma et al., 2019).

Plant resistance is considered one of the most effective ways to control the wheat stem sawfly (Beres et al., 2011; Buteler et al., 2015). Resistant wheat varieties tend to possess solid stems, which is expressed when undifferentiated parenchyma cells create a solid pith (Berzonsky et al., 2003). The primary source of stem solidness was originally derived from the Portuguese landrace wheat 'S-615' (Lanning et al., 2006). Survivorship of wheat stem sawfly eggs and larvae is lower in solid stem varieties suggesting solid stem expression is a mechanism of resistance (Holmes and Peterson, 1962; Buteler et al., 2015). Additionally, the larvae that develop in resistant plants are often smaller than those in susceptible genotypes (Holmes and Peterson, 1962; Cárcamo et al., 2005). Solid pith expression is considered highly variable within genotypes (Holmes, 1984; Beres et al., 2012; Nilsen et al., 2016). Some solid stem genotypes have been shown to decrease *Bracon* parasitism (Rand et al., 2012; Buteler et al., 2015). Given the issues with solid stem resistance, other resistance mechanisms, such as tolerance to cutting, are currently being investigated (Talbert et al., 2014; Varella et al., 2019). One source of resistance may come from wheat relatives which are considered untapped genetic diversity (Haas et al., 2019; Kishii, 2019). Recent field experiments on wheat land races identified some accessions that exhibit resistance. However, not all lines could be evaluated due to poor germination or mismatch between plant phenology and wheat stem sawfly flights (Varella et al., 2017). Wild wheat species are challenging to grow under field conditions, so ensuring proper germination and growth is critical for screening, which can be done in a greenhouse.

Most screening for wheat stem sawfly resistance in the field takes place at stem maturity, and plots are given a visual score based on the degree of stem cutting (Weaver et al., 2009).

While field trials can quickly provide a lot of data, this method cannot provide information on the type of resistance mechanisms. Greenhouse choice tests may be conducted to compare wheat stem sawfly host preference; however, such tests take a considerable amount of time to perform and are typically limited to the evaluation of small numbers of breeding lines (Buteler et al., 2009; Weaver et al., 2009; Buteler and Weaver, 2012).

In this study, we develop a novel wheat stem sawfly resistance screening method that combines the high throughput capabilities of field evaluation and the ability to control the growing environment and, in turn, germination success and plant phenology. We developed a technique using plants grown in cone-tainers to examine differences in host preference and host suitability of seven winter wheat genotypes. We measured host preference by counting the number of eggs laid in stems by adult sawflies in the field and used larval size and percentage of the stems infested as a measure of host suitability. Larval measurements conducted in controlled conditions can provide invaluable information on how genotypes affect insect development (Stenberg and Muola, 2017).

Methods

Cone-tainer experiment

We germinated seeds of seven winter wheat genotypes (cultivars or experimental lines), described in Table 3.1, on 5 x 10 mm germination blotter paper (Anchor Paper Co. St. Paul, MN) with 5 ml of tap water. Seeds were kept at an average temperature of 24 °C for three days. Germinated seeds were then vernalized by placing them in a cold room held at 4 °C for seven weeks. After vernalization, a single seedling was planted in a cone-tainer (Stuewe & Sons SC10U UV-stabilized cones: 3.81 cm diameter x 20.9 cm depth, 164 ml volume) with a soil mixture of 7 parts soil and 2 parts perlite with a cotton ball at the bottom to prevent soil loss. Due to changes in our soil distributor, we used Metro Mix (Sungro, Agawam, MA) in 2019 and 20B/30V (lambert, Québec, Canada) in 2020.

Table 3.1. Description of CSU wheat genotypes (cultivars or experimental lines) screened using the conetainer method.

ID	Stem type	Wheat stem sawfly susceptibility	Release date	Experimental number	Pedigree
Hatcher	Hollow	Low	2004	CO980607	Yuma/PI372129//TAM-200/3/4*Yuma/4/KS91H184/Vista
Denali	Hollow	High	2011	CO050303-2	CO980829/TAM 111
Avery	Hollow	High	2015	CO11D174	TAM 112/Byrd
Monarch	Hollow	Low	2018	CO13D1383	CO07W722-F5/Snowmass//CO07W722-F5
Steamboat	Hollow	Low	2020	CO15D098R	TAM 114/Antero//Byrd
CO16SF070	Solid	Low	….	….	Antero/Judee//Antero
Fortify SF	Semi-solid	Low	2019	CO15SFD107	Byrd/Bearpaw//Byrd

Plants were grown in a greenhouse with supplemental light [430W HPS (High-Pressure Sodium) fixtures - P.L. Light Systems, bulbs - GE Lucalox lu400 series, 400W]. The greenhouse had a 16L: 8D photoperiod and the day: night temperature of 23:18 °C. Plastic trays were placed under 42 cone-tainers, and all trays were bottom watered as needed. Germination and planting dates were staggered over two weeks in 2019 and three weeks in 2020. We did this as peak sawfly flight is highly variable across years, and we wanted to ensure uniform maturity of plants during exposure to wheat stem sawfly. Genotypes were placed randomly in each tray, and trays were considered blocks placed randomly in the greenhouse (Figure 3.1 A-B). In 2019 we planted 30 plants of each genotype per planting date, and in 2020 we planted 40 plants. Prior to moving the plants to the field, they were fertilized once a week with 300 ml 15-16-17 N-P-K Peters General Purpose Fertilizer (J. R. Peters, Allentown, PA) at 296 ppm in an aqueous solution. Fertilization began a week after planting. To promote primary stem growth, we cut and removed secondary tillers leaving only the primary tiller. In 2019 once plants started to tiller, tillers were cut once a week. In 2020 we cut tillers once, three weeks after planting.

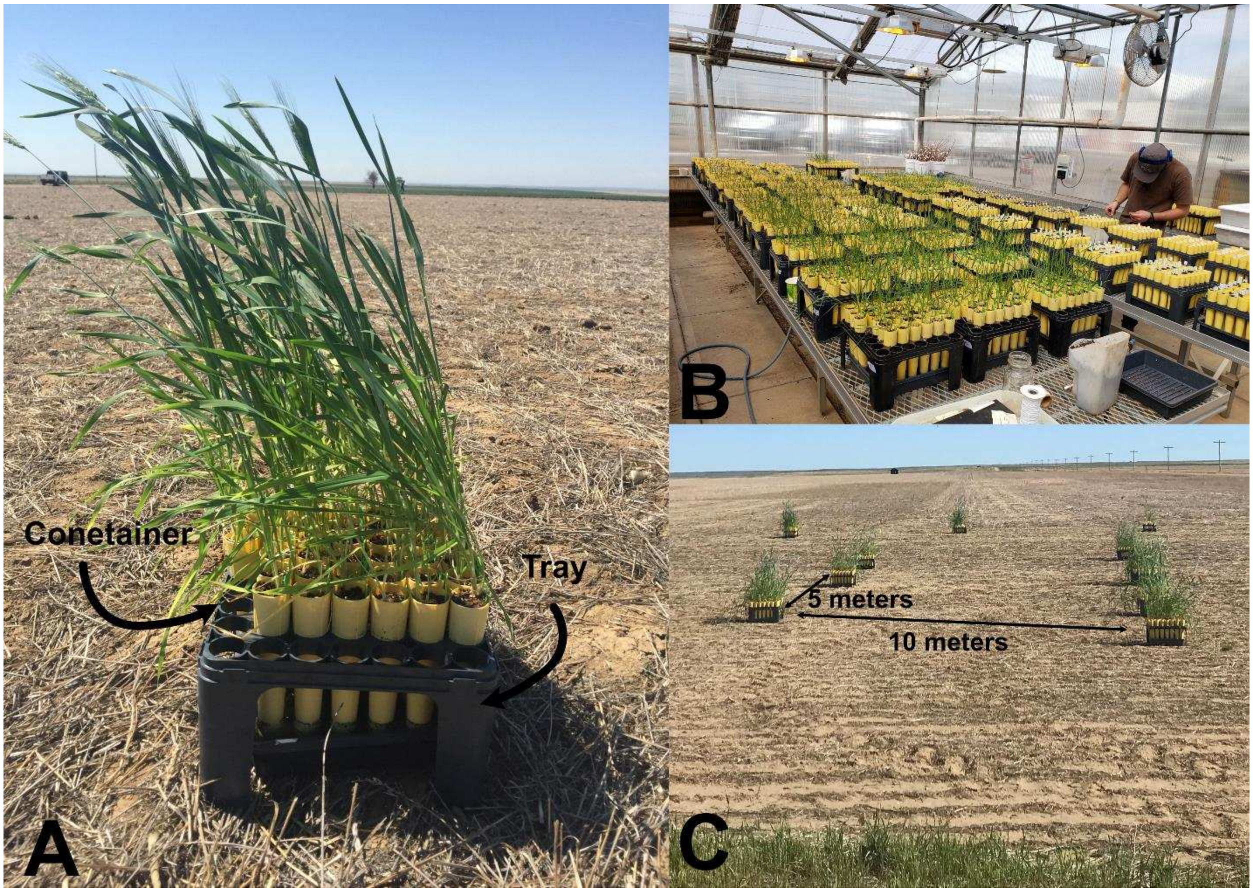

Figure 3.1. A-C: Pictures showing experimental setup. (A) Wheat in conetainers after being placed on a field of wheat stubble. (B) Greenhouse set up with conetainers showing staggered planting dates. (C) Arrangement of conetainer trays in the field.

Sawfly infestation and data collection

We transported wheat plants to the field for infestation once plants reached Zadoks growth stage 32-75 (Zadoks et al., 1974). The wheat stem sawfly requires a stem for larval development;

hence we considered Zadoks 32 (when two to three nodes were visible) to Zadoks 75 (ripening of kernels) as the appropriate stages for oviposition (Criddle, 1923). This range was chosen due to large varietal differences in maturity. We only brought plants to the field on a calm sunny day, conditions favorable for adult sawfly activity. We walked along the edge of a winter wheat field that bordered a fallow field to measure infestation pressure. While doing so, we swept 100 times using 180-degree pendulum sweeps with a standard 38 cm- diameter sweep net. All infestations occurred on a field of infested wheat stubble bordering growing wheat at Wickstrom Farms, near Orchard, CO. Columns of trays were placed 10 meters apart, and within rows trays were set 5 meters apart, as shown in Figure 3.1C.

In 2019, cone-tainer trays of a single planting date were placed in the field for 24 hours on May 24 and 1 hour 30 mins on May 30. In 2020, trays were placed in the field for 2 hours on May 22 and June 5 and 4 hours on May 29. Exposure times were variable which was based on variable adult infestation pressures from adult sweeps. Plants in each round of exposure were from the same planting date. All infestations started at 9 am. Once we brought the trays back to the lab, half of the plants (15 in 2019 and 20 in 2020) were dissected to examine stems for eggs, while the other half remained in the greenhouse to allow larvae to mature. While examining stems for eggs, we recorded stem diameter by measuring the first visible node's width, the stem's maturity on the Zadoks scale, and the number of eggs present. We also evaluated stem solidness by rating each internode on a 1-5 scale, where hollow internodes were considered a 1 and completely solid internodes a 5 and summed the scores from 5-25 (Berzonsky et al., 2003).

Thirty days after infestation, the remaining stems were dissected to assess wheat stem sawfly presence. All stems were examined on the same day. We considered a stem infested if there were frass and/or larvae present in the stem. If larvae were present, they were weighed, and pictures of the head capsule were taken. Using ImageJ (Rueden et al., 2017), the length of the head capsule was measured. Body measurements were recorded as a proxy to compare growth and development among genotypes, as suggested by Kumar and Venkatesan 2019. We noted if stems had been cut to form hibernaculum chambers. In 2019 we only dissected plants with more than one visible node. To collect more precise measurements on stem development in 2020, we recorded the Zadoks growth stage of the stem during dissections and only considered plants at Zadoks growth stages higher than 32 (plants with more than two nodes) for further analysis.

Statistical analysis

Analysis of data was conducted using R (R-Core Team 2019, Version 3.6.2) and R packages lme4 (Bates et al., 2015), ggplot2 (Wickham, 2009), and emmeans (Lenth, 2020). Separate analyses were conducted for 2019 and 2020. Model assumptions were checked using residual diagnostic plots. Also, the leverage of values was plotted against the Pearson residuals to ensure that small sets of observations did not have an undue influence on models. All multiple comparisons were adjusted using the false discovery rate (FDR) with a significance level of 0.05 (Benjamini and Hochberg, 1995).

Before analyzing the sawfly oviposition data, we removed data where the Zadoks growth stage was less than 32, as immature plants are not suitable hosts for wheat stem sawfly. We used a linear mixed model with the infestation date as a random effect and year, Zadoks, genotype, and stem diameter as fixed effects to analyze the number of eggs within a stem. We included year, Zadoks growth stage, and stem diameter in the model as they are all known confounding variables for oviposition. Analysis of stem diameters was conducted using a linear model with genotype and year as independent variables.

Stem infestation data were considered binomial since only one larva can survive per stem (Ainslie, 1920). The proportion of stems infested was calculated as the number of infested stems divided by the total number of stems sampled. These data were analyzed using a generalized linear mixed model (GLMM) with a binomial error distribution and a logit link function. In the GLMM model, infestation date was a random effect, and genotype was a fixed effect. We also analyzed the number of stems where a hibernaculum (stub) was formed. The number of stubs was also analyzed using a generalized linear model with a binomial error distribution and a logit link function with genotype and year as independent variables. Larval head length and weight were analyzed using a linear mixed model with infestation date as a random effect and genotype as a fixed effect.

Results

Infestation pressure: The number of adult sawflies for each infestation date varied across dates. In 2019, 767 sawflies were captured on May 24 and 1,148 on May 30. In 2020, we collected 145 sawflies on May 22, 54 sawflies on May 29, and 4 sawflies on June 5.

Number of eggs per stem. There were no differences among genotypes in the number of eggs found per stem for either year ($X^2 = 6.27$, df = 6, $P = 0.39$) (Figure 3.2). In 2019, a high of 28 eggs were found in one stem of Avery. Across all genotypes, we found an average of 5.09 ± 1.40 (Mean ± SE) eggs per stem in 2019 and 1.30 ± 0.26 in 2020.

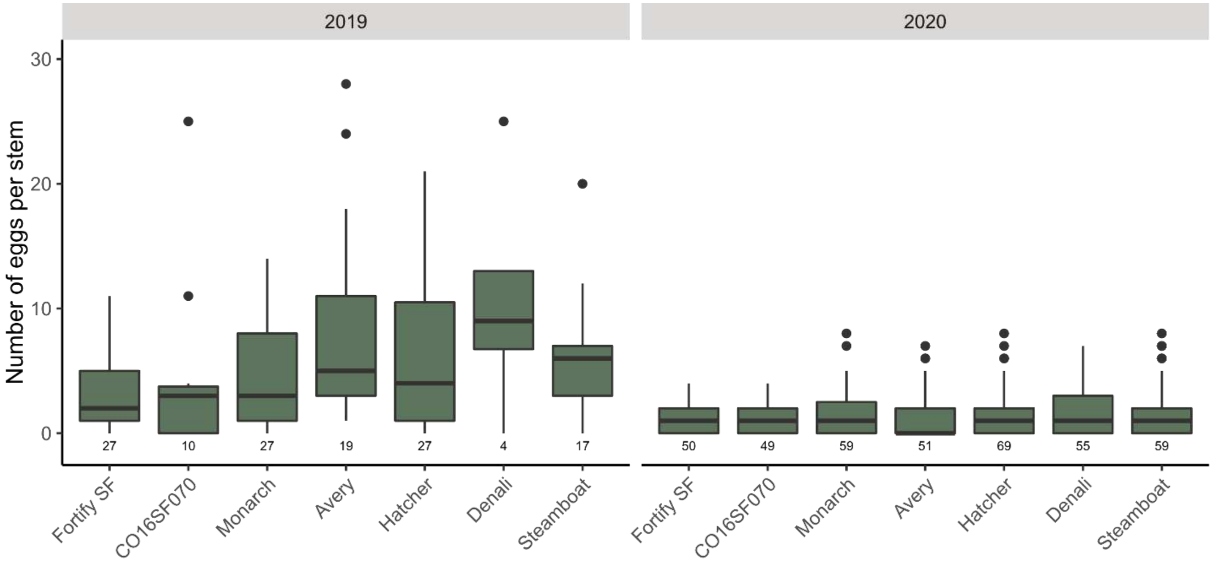

Figure 3.2. Effects of genotype on the number of wheat stem sawfly eggs found in genotypes grown in 2019 and 2020. The total number of stems cut is indicated by the number below each boxplot. The boxes represent the 25th and 75th percentiles; the horizontal line in each box indicates the median. The whiskers signify maximum and minimum values, and circles indicate outliers.

Proportion of infested stems. Proportion of infested stems. The proportion of infested stems differed among genotypes for both years (2019: X^2 = 27.24, df = 6, P = 0.0001; 2020: X^2 = 22.51, df = 6, P = 0.0009). In 2019, pairwise comparisons using binomial tests corrected with a FDR adjustment indicated that the proportion of infested stems of CO16SF070 (0.29 ± 0.06) were lower ($P < 0.05$) than those of Fortify SF (0.70 ± 0.03), Monarch (0.72 ± 0.12), Steamboat (0.74 ± 0.02), or Hatcher (0.80 ± 0.02) (Figure 3.3). In 2019, the proportion of infested stems of Denali (0.33 ± 0.12) was only less than those observed for Hatcher. In 2020, the pairwise comparisons indicated the proportion of Fortify SF's infested stems (0.25 ± 0.03) was lower than all other genotypes. Complete pairwise comparisons and estimated marginal means are provided in Figure 3.4.

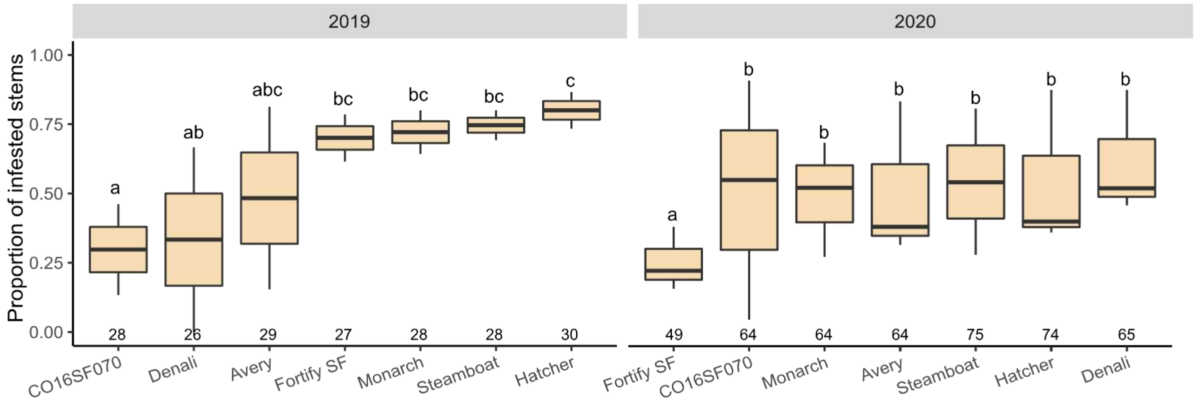

Figure 3.3. Effects of genotype on the proportion of stems infested with wheat stem sawfly. The total number of stems cut is indicated by the number below each boxplot. Letters indicate significant pairwise differences ($P < 0.05$, FDR corrected post hoc test). For more in-depth pairwise comparisons and estimated marginal means, please see Figure 3.4.

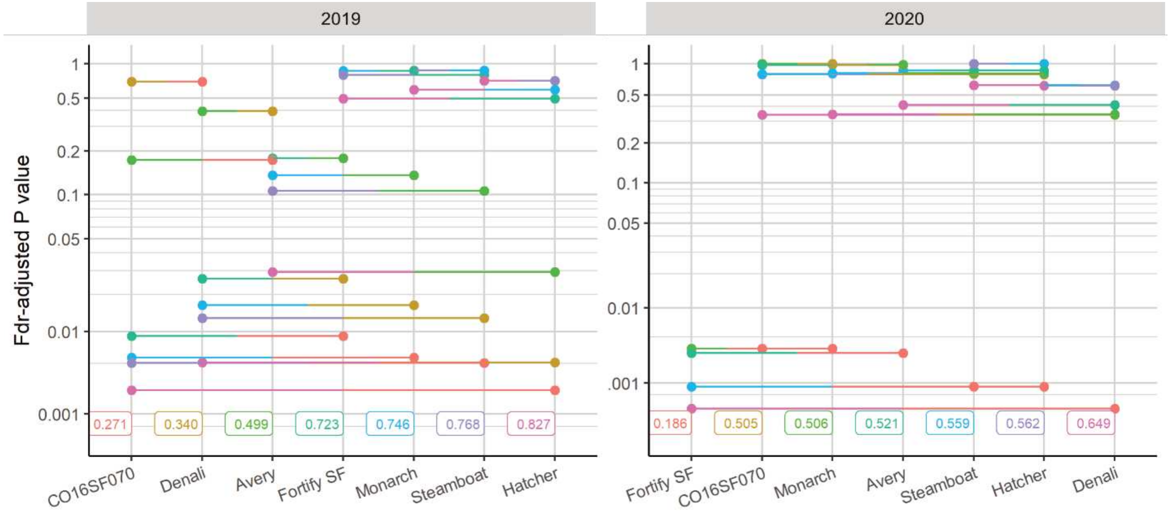

Figure 3.4: Estimated marginal means of the effects of genotype on the proportion of stems infested with wheat stem sawfly. The pairwise p-value plot shows FDR adjusted p-value comparisons. Each comparison is associated with a horizontal line segment that joins the scale positions of the two estimated marginal means being compared and whose vertical position is determined by the P-value of that comparison.

Larval weights and length of head capsules. In 2019, Fortify SF (length: 0.72 ± 0.05 mm, weight: 7.05 ± 1.7 mg) produced larva with shorter ($P < 0.05$) head lengths and weights than Monarch (length: 0.87 ± 0.03 mm, weight: 16.91 ± 2.99 mg), Avery (length: 0.89 ± 0.04 mm, weight: 16.60 ± 1.89 mg) and Denali (length: 0.89 ± 0.02 mm, weight: 15.91 ± 1.49 mg) (Figure 3.5 and 3.6). In 2020, Fortify SF was excluded from the larval head length and weight analyses, as only one living larva was found. Pairwise comparisons indicate larvae found in Hatcher (0.65 ± 0.02 mm) had shorter head lengths than Avery (0.73 ± 0.02 mm), Steamboat (0.73 ± 0.02 mm), or Denali (0.74 ± 0.02 mm). Larval head lengths found in Monarch (0.67 ± 0.02 mm) were shorter than those found in Denali (Figure 3.5). There were no differences in larval weight among genotypes in 2020 (Figure 3.6). Complete pairwise comparisons and estimated marginal means are provided in Figure 3.7 and 3.8.

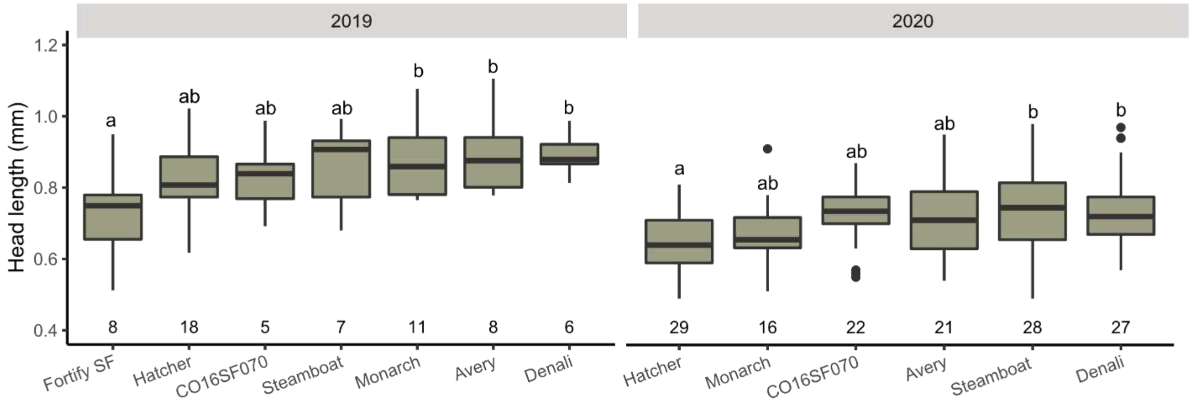

Figure 3.5. Effect of genotype on wheat stem sawfly larval head capsule length. The total number of stems cut is indicated by the number below each boxplot. Letters indicate significant pairwise differences (*P* < 0.05, FDR corrected post hoc test). In 2020, Fortify SF was excluded from the larval head length and weight analyses, as only one living larva was found. For more in-depth pairwise comparisons and estimated marginal means, please see Figure 3.7.

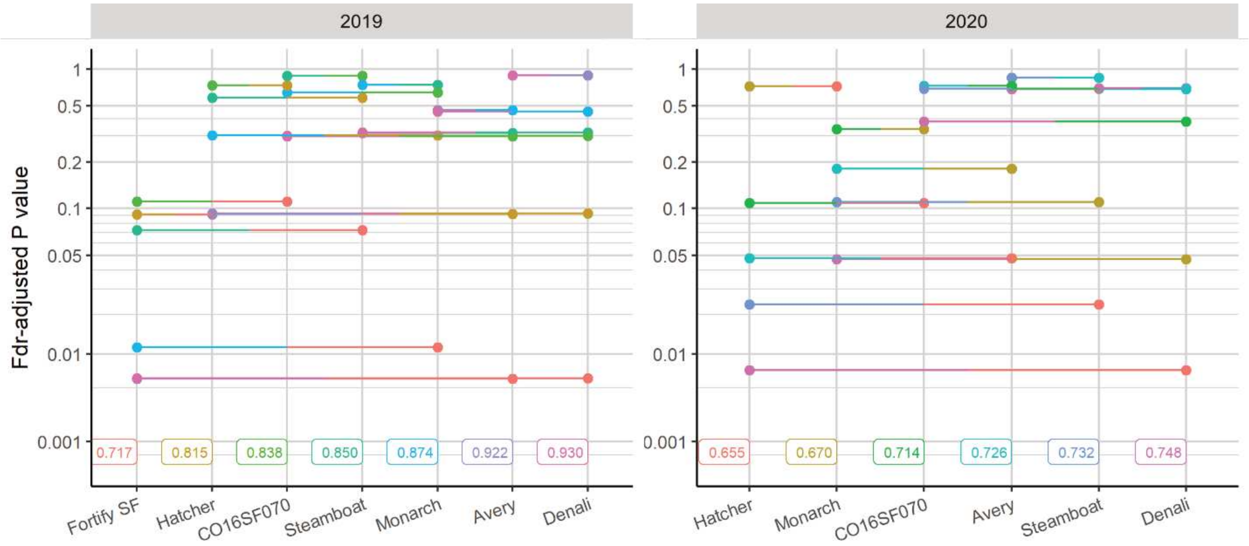

Figure 3.6: Estimated marginal means of the effects of genotype on wheat stem sawfly larval head capsule length (mm). The pairwise p-value plot shows FDR adjusted p-value comparisons. Each comparison is associated with a horizontal line segment that joins the scale positions of the two estimated marginal means being compared and whose vertical position is determined by the P-value of that comparison.

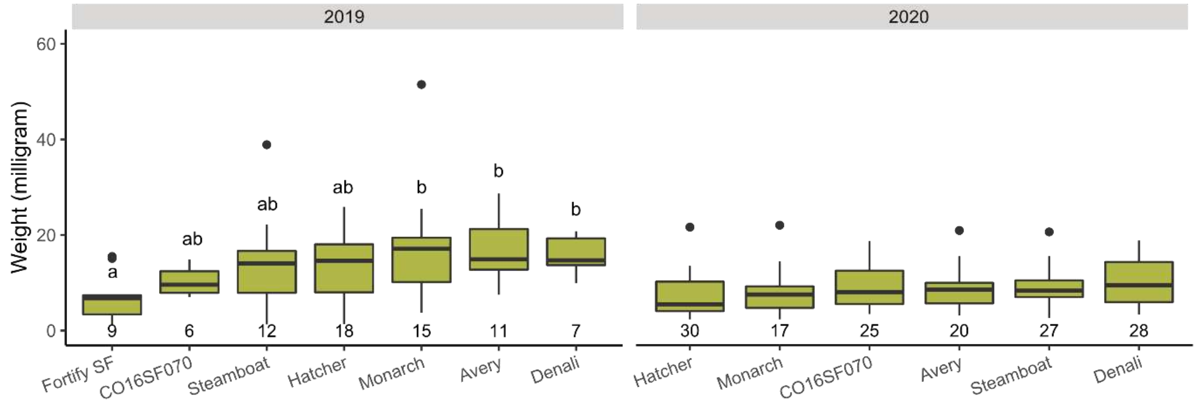

Figure 3.7. Effects of genotype on wheat stem sawfly larval weight. The total number of stems cut is indicated by the number below each boxplot. Letters indicate significant pairwise differences (*P* < 0.05, FDR corrected post hoc test). In 2020, Fortify SF was excluded from the larval head length and weight analyses, as only one living larva was found. For more in-depth pairwise comparisons and estimated marginal means, please see Figure 3.8.

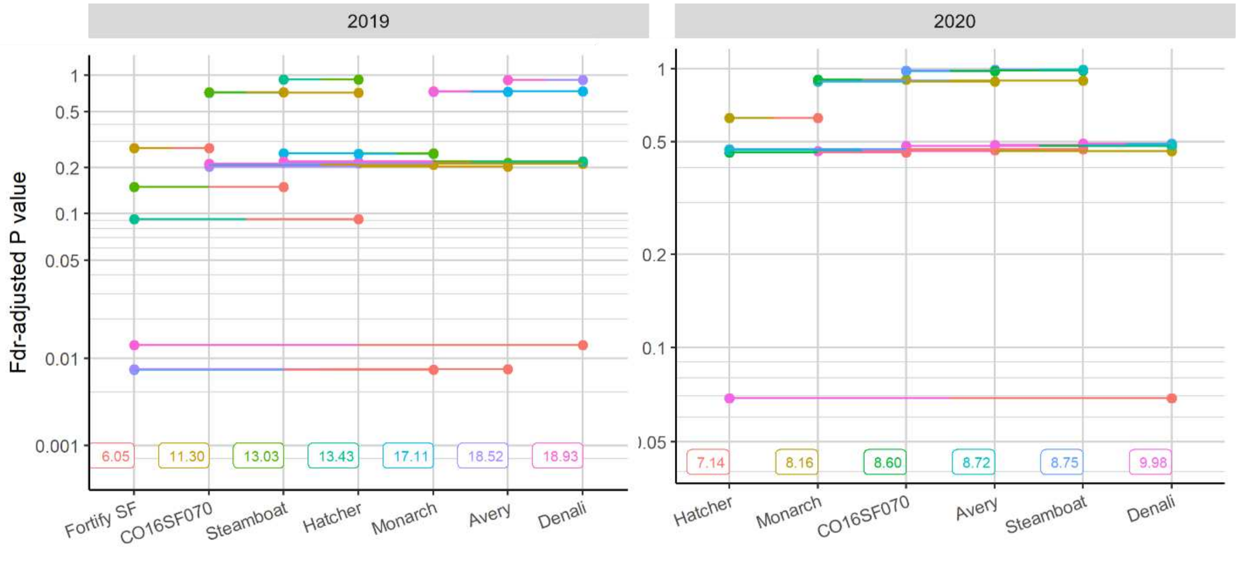

Figure 3.8: Estimated marginal means of the effects of genotype on wheat stem sawfly larval weight (g). The pairwise p-value plot shows FDR adjusted p-value comparisons. Each comparison is associated with a horizontal line segment that joins the scale positions of the two estimated marginal means being compared and whose vertical position is determined by the P-value of that comparison.

Discussion

Wheat stem sawfly continues to be a major pest of wheat across the U.S. and Canada. Host plant resistance is considered the most effective way of controlling this pest. However, visual field evaluations do not provide information on the mechanism of resistance, and current greenhouse choice tests are time-consuming and often lead to no-choices. Our results indicate that using cone-tainers to screen for wheat stem sawfly resistance may be a more effective way to identify mechanisms of host plant resistance, such as reduced larval survival and size.

This study suggests that the cone-tainer screening method is comparable to field experiments. In 2019, the percentage of infested stems was similar for genotypes in cone-tainers compared to stems collected from field plots. Solid stemmed genotypes produce smaller adult females (Cárcamo et al., 2005) and cause larval mortality (Holmes and Peterson, 1962; Cárcamo et al., 2016). We also found that Fortify SF, a genotype that expressed stem solidness, had the lowest infestation levels and smallest larva in 2019 and no living larva in 2020.

Interestingly, we found no significant differences among genotypes in the number of eggs present. This observation differs from previous studies, where the number of eggs found was different among genotypes (Weaver et al., 2009; Cárcamo et al., 2016). This discrepancy may be due to differences in infestation pressures. We placed the cone-tainer stands in a field with extremely high wheat stem sawfly infestation pressures in both years. The lack of differences among genotypes suggests that at high infestation pressures, wheat stem sawfly did not discriminate among the genotypes we tested or that the genotypes were placed so close together in a block, sawflies could not discriminate among the different plants.

Currently, greenhouse choice tests are used to examine the roles of plant volatiles, stem diameter, and other host traits on oviposition preference (Weaver et al., 2009; Buteler et al., 2010; Buteler and Weaver, 2012). However, choice tests take a considerable amount of time to conduct and often result in many instances where the adult fails to make a choice and low replications due to time taken to conduct experiments. The number of replicated plants per experiment can be easily manipulated using the cone-tainer method, allowing for more robust statistical analysis. Using cone-tainers, we can compare many lines in a fraction of the time required by greenhouse choice tests. Previous choice tests suggest the wheat stem sawfly should be exposed to plants for 2 or 3 days (Weaver et al., 2009; Buteler and Weaver, 2012). Using the cone-tainer method, it would take only 2-4 hours of exposure to sawflies to evaluate sawfly host preference and suitability.

While the cone-tainer method can be beneficial in screening for wheat stem sawfly resistance, there are potential pitfalls. First, the cone-tainer method may not be viable for areas or years that have low sawfly infestation levels. Additionally, it can be costly to drive the cone-tainers to the field if infested fields are far away. Costs to grow plants in the greenhouse should also be considered. Finally, there is a tradeoff between identifying host preference which would require low infestation pressure, and ensuring most of the stems are infested for the adequate sample size for larval development and infestation, requiring high infestation pressures.

Overall, we believe the cone-tainer method is best suited for studying host plant resistance mechanisms in situations where there is limited seed or plants are not adapted to the local environment, such as wheat land races. Since seeds and plants are provided optimal conditions for germination and growth in the greenhouse prior to field exposure, we can control plant maturity by altering planting dates to coincide with sawfly flight directly. Using the proposed screening method, wheat breeders and entomologists can screen host plants for various wheat stem sawfly resistance traits quickly and gather information on host preference and suitability for genotypes before further field evaluations.

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CHAPTER 4 - Effects of landscape composition on wheat stem sawfly (Hymenoptera: Cephidae) and its associated braconid parasitoids[2]

Introduction

Recent studies and reviews have highlighted the need to consider a landscape approach when designing agroecological and pest management strategies (Peterson et al., 2018; Gliessman and Ferguson, 2020) and that biodiversity and land management play important roles in pest control (Gliessman, 2015; Liere et al., 2017; Stenberg, 2017; Dara, 2019). Landscape complexity of both crop and non-crop habitats can bolster natural enemy populations and biological control (Landis et al., 2000; Tscharntke et al., 2007, 2016; Redlich et al., 2018). However, an extensive review of landscape studies shows that non-crop habitats do not always enhance pest control and can sometimes even increase pest densities (Karp et al., 2018).

Many polyphagous insect herbivores and natural enemies use multiple resources across different habitat types in their environment. Depending on the quality of resources they provide, different habitat types in the landscape can either be sources of increased pest and/or natural enemy densities or sink habitats that reduce densities on crops of interest (Kennedy and Storer, 2000). Increasing or decreasing the areas of these habitats, and their associated resources can thus impact populations of both natural enemies and insect pests. For example, densities of *Lygus*, a pest of cotton, varies in response to annual temporal variation in crop resource quality (Carrière et al., 2012). Similarly, both western corn rootworm (*Diabrotica virgifera*), and

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[2] Peirce, E. S., Rand, T. A., Cockrell, D. M., Ode, P. J., and Peairs, F. B. (2021). Effects of landscape composition on wheat stem sawfly (Hymenoptera: Cephidae) and its associated braconid parasitoids. J. Econ. Entomol. 114, 72–81. doi:10.1093/jee/toaa287.

northern corn rootworm (*D. barberi*) population densities were found to increase with increasing corn cover, while densities of the European corn borer (*Ostrinia nubilalis*) showed no response (O'Rourke et al., 2011). Generalist predators of *D. barberi*, *D. virgifera,* and *O. nubilalis* either decreased or did not change as corn cover increased. Furthermore, natural enemy and pest responses to changes in resources can vary across scales (O'Rourke et al., 2011). Understanding how specific species respond to non-crop habitats will be critical to developing management practices that promote natural enemy populations without increasing pest populations.

Wheat stem sawfly (Hymenoptera: Cephidae: *Cephus cinctus* Norton) can use a variety of grass species in addition to spring and winter wheat (Cockrell et al., 2017). Although originally described feeding on non-cultivated grass hosts, wheat stem sawfly began using spring wheat as it became more prevalent in the northern Great Plains (Ainslie, 1920). Wheat stem sawfly became a notable pest in winter wheat only in the 1990s (Morrill and Kushnak, 1996), with damage to winter wheat in Colorado occurring even more recently (Terri Randolph, unpublished data, 2010). In order to host-switch from non-cultivated grasses to winter wheat, adults must emerge earlier in the spring to begin oviposition (Morrill and Kushnak, 1996). The use of multiple host plants allows the wheat stem sawfly to use both non-crop and crop habitats.

Wheat stem sawfly larvae begin and end their development within a single host stem. As the larva reaches the final instar it creates a hibernaculum at the base of the stem where it overwinters as a final instar larva. The hibernaculum is formed by cutting the wheat stem above ground level just before harvest, which causes the stem to lodge leaving a 'stub' just above soil level (Ainslie, 1920). In Colorado, lodging occurs between late June and early August (Peairs et al., 2014). Cut stems are difficult to harvest and easily blown away. conservative estimates indicate grain-yield losses exceeding 30% and the economic losses surpassing $350 million annually (Beres et al., 2011). Estimated losses in Colorado, exceed $30 million per year and are expected to increase (Peairs and Haley, personal communication). Wheat stem sawfly has been a key wheat pest for over 100 years and there are still no effective controls. Current methods such as solid stem varieties and pesticides do not effectively control wheat stem sawfly (Delaney et al., 2010; Rand et al., 2012; Peairs et al., 2014). Biological control is predicted to be effective when populations of two parasitoids of wheat stem sawfly, *Bracon cephi* Gahan (Hymenoptera: Braconidae) and *B. lissogaster* Muesebeck, parasitize at least 68% of the wheat stem sawfly population within a field (Rand et al. 2017). However, this level of parasitism is only occasionally recorded in wheat (Rand et al., 2011, 2014, 2017).

Wheat stem sawfly has nine recorded parasitoids that are primarily encountered in non-cultivated grasses (Davis et al., 1955; Morrill et al., 1998). However, *B. cephi* and *B. lissogaster* are the only known parasitoids to parasitize wheat stem sawfly in spring and winter wheat in Canada (Nelson and Farstad, 1953; Cárcamo et al., 2012), Montana (Morrill et al., 1998; Runyon et al., 2002), and Nebraska (Thomas and Bradshaw, 2020). Both *Bracon* species are idiobiont ectoparasitoids that are known to parasitize multiple larval instars of the wheat stem sawfly(Nelson and Farstad, 1953; Rand et al., 2011). To date, parasitism in Colorado is rarely observed in cultivated wheat.

In this study, we explored habitat use patterns by Colorado populations of *B. cephi, B. lissogaster,* and wheat stem sawfly. To do so we conducted three separate studies. In our first study we sampled sawflies and parasitoids in non-cultivated grasses in non-crop habitats to address three questions, (1) how prevalent is parasitism by either species of *Bracon* in non-crop habitats in CO, (2) how do parasitoid and wheat stem sawfly populations in non-crop environments respond to changes in landscape composition, and (3) which non-cultivated grasses are particularly suitable hosts for and wheat stem sawfly or its parasitoids. In our second study, we conducted a statewide survey to determine if either species of parasitoid is attacking wheat stem sawfly in Colorado wheat. For our third study, we conducted weekly sweep samples at six sites to quantify seasonal patterns in *Bracon* adult emergence in Colorado. Results from these experiments are expected to provide insight into how land use can influence future parasitoid and pest populations.

Methods

Non-cultivated grass survey

We conducted surveys of both *Bracon* species in nine non-crop habitat sites containing non-cultivated grasses in CO (Figure 4.1). Sites were chosen to span a gradient of landscape composition on the land cover at local and landscape levels. We used 500 m as our local scale as the average wheat field in the United States ranges between 0.646 km^2 and 1.295 km^2 (Yan and Roy, 2016). The 5 km scale was chosen as the landscape scale as previous research suggests wheat stem sawfly populations are positively influenced by increasing proportions of wheat cover at a 5 km scale (Rand et al., 2014). Since wheat stem sawfly is restricted to feeding on non-cultivated grasses and wheat, the proportion of wheat and grass cover in a surrounding landscape are important measures of available habitat.

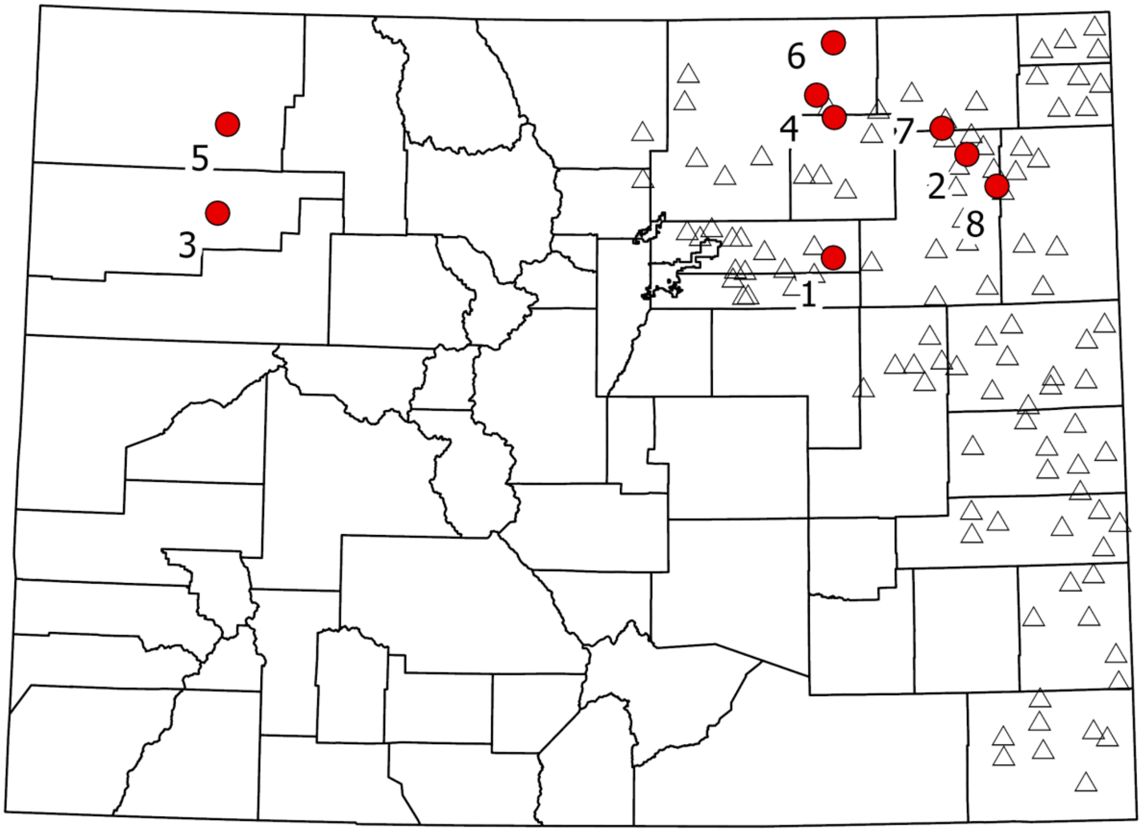

Figure 4.1: Map of the state of Colorado with counties outlined, showing collection locations for the noncultivated grass survey 2017–2019 (circles) and an example of the sample locations for the statewide wheat survey (triangles).

Using the geoprocessing software, ArcGIS Pro version 2.6.0 (Esri Inc.), and Cropland Data Layer (USDA, National Agricultural Statistics Service Cropland Data Layer 2017, 2018 and 2019; http://nassgeodata.gmu.edu/CropScape/), we calculated percent wheat and herbaceous cover at two scales: local (500 m) and landscape (5 km). Herbaceous landcover types that contained non-wheat hostplants for wheat stem sawfly included: Hay, Sod/Grass, Shrubland, Grass/Pasture, and Herbaceous Wetlands. Percent land cover was calculated using buffers around collection points, 500 m (local level), and 5 km (landscape level).

Nine locations were sampled mid-July and late August/early September in 2017 – 2019 after flight had concluded. Non-cultivated grasses (*Agropyron cristatum, Bromus inermis,* *Elymus trachycaulus, Elytrigia intermedium,* and *Pascopyrum smithii*) were chosen based on suitability as host plants to wheat stem sawfly and it's parasitoids (Cockrell et al., 2017). Sites were chosen such that two or more of the previously mentioned grass species were present. Three sites were surrounded by non-cultivated land and six sites were mostly surrounded by cultivated land within a 500 m radius. At each site, a minimum of 100 stems of each grass species present was collected. Stem diameter was measured at the first visible node using a digital caliper. The average stem diameter for each species was calculated using 10 random stems from each sample. Using an Exacto knife stems were bisected vertically to assess infestation and parasitism levels. We noted the presence of any insects in the stem and all insects were individually place into vials containing 95% ethanol. We also recorded the presence of wheat stem sawfly larvae, frass, and *Bracon* larvae. *Bracon cephi* and *B. lissogaster* cannot be differentiated based on larval morphology. Since both *Bracon* are ectoparasites larva can be easily found if present. In addition, evidence of parasitism included the presence of a *Bracon* larva, characteristic cocoons, and exit holes. To further ensure correct identification, uninjured *Bracon* larvae were placed in 35 mm shell vials and compared to larval voucher specimens. Insect vouchers were submitted to the C. P. Gillette Museum of Arthropod Diversity at Colorado State University.

Colorado State-wide tiller and sweep survey

As part of a larger study, we conducted a yearly state-wide survey of wheat fields. Surveys of the wheat-growing region of Colorado were conducted to assess the infestation and parasitism of wheat stem sawfly in winter wheat. One hundred sites were sampled yearly from 2012-2019. From 2017 – 2019 more emphasis on parasitoid presence was added to the larger study, which will be the focus of this study. From each site, 100 winter wheat tillers were collected from a field that bordered previous years' wheat-fallow. Tillers were stored at 2°C until they could be bisected. Using an Exacto knife, tillers were split in half, and the presence or absence of wheat stem sawfly, frass, and parasitoids were noted.

In 2017, 2018, and 2019 sweep net sampling for parasitoid adults was carried out from early-June to mid-July. Both parasitoids emerge after peak wheat stem sawfly flight which is around mid-May for Colorado (Holmes et al., 1963). We chose these dates as parasitoids in Montana and Canada start emerging before or synchronously with wheat stem sawfly which occurs in early-June and mid-July (Holmes et al., 1963; Morrill et al., 1998). To collect samples, we walked along the edge of a winter wheat field that bordered a fallow field. While doing so we swept 100 times using 180-degree pendulum sweeps with a standard 15-inch diameter sweep net. Sweep samples were conducted at the same sites as the tiller collections.

Seasonal phenology survey for Bracon

Weekly sweep net samples of *Bracon* adults also were collected at seven fields known to have heavy wheat stem sawfly infestation near New Raymer and Orchard, CO in 2017- 2019. The methodology was the same as that used for the statewide survey, described above. Collections started early in May and continued until mid-July.

Statistical analyses

Analysis of data was conducted using R (R-Core Team 2019, Version 3.6.2) and R packages lme4 (Bates et al., 2015) and emmeans (Lenth 2020).

Separate analyses were conducted for *Bracon* parasitism and sawfly infestation. Sawfly infestation was calculated as the number of stems infested with sawfly larvae out of the total sampled stems. *Bracon* parasitism was calculated as the number of stems with evidence of parasitism out of the total wheat stem sawfly infested stems. For both analyses, a Generalized Linear Mixed Model (GLMM) with a binomial error distribution and a logit link function was used. We treated grass species, wheat cover, and grass cover at 500 m (local scale) and 5 km (landscape scale) as fixed effects. The collection date was treated as a random effect to control for variance between years and collection dates. Since it is very common for landscape variables to be highly correlated, Pearson's correlations were calculated between all measured variables before conducting model selection. Full models were simplified to the best fit model using Akaike information criterion (AIC) selection. Models that did not converge were not considered. To examine the goodness of fit for the best model Pearson's residuals were plotted to ensure fixed variables did not deviate from a linear form. Also, the leverage of values was plotted against the Pearson residuals to ensure a small set of observations did not have a large influence. We conducted comparisons of the proportion of stems with evidence of parasitism and infested stems between grass species using the emmeans package on an odds ratio scale.

Results

Non-cultivated grass survey: Grass host suitability

We found wheat stem sawfly infestation at all sites across all three years (Figure 4.2 A-B). However, the proportion of infestation across all years and sites was variable (n = 2,256 infested stems, n = 9,518 total stems cut, mean: 0.25 ± 0. 20, max: 0.80, min: 0).

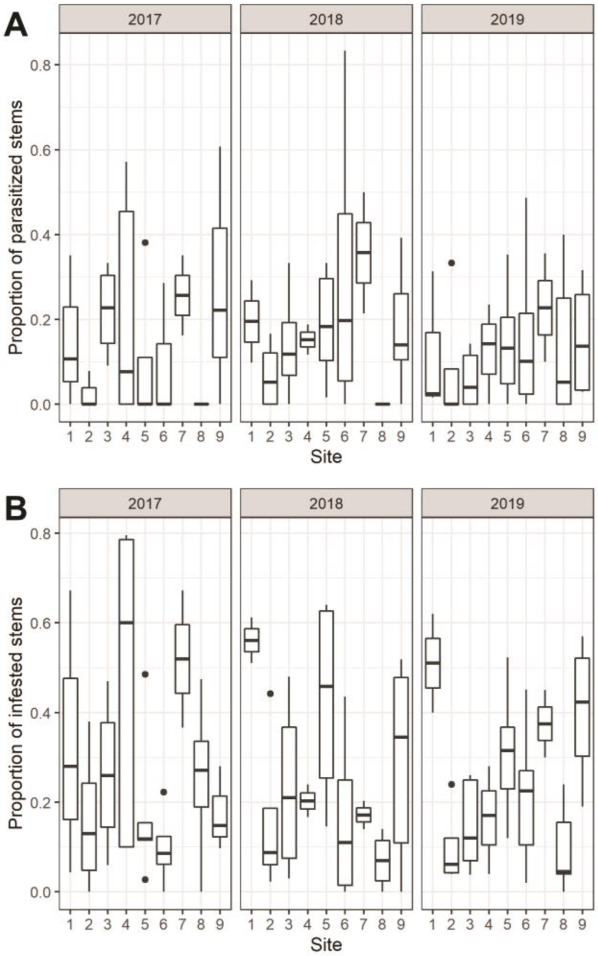

Figure 4.2: (A) Boxplot showing proportion *Bracon* in parasitized stems at each site separated by year sampled. (B) Boxplot showing proportion of wheat stem sawfly infested stems each site separated by year sampled. The boxes represent the 25th and 75th percentiles, the horizontal line in each box indicates the median. The whiskers signify maximum and minimum values, and circles indicate outliers.

Pascopyrum smithii had the highest proportion of stems infested by wheat stem sawfly across all years (n = 782 infested stems, n = 2129 total stems cut, 0.39 ± 0.19) while *A. cristatum* had the lowest proportion infested (n = 46 infested stems, n = 1224 total stems cut, 0.039 ± 0.049) (Figure 4.3B, D).

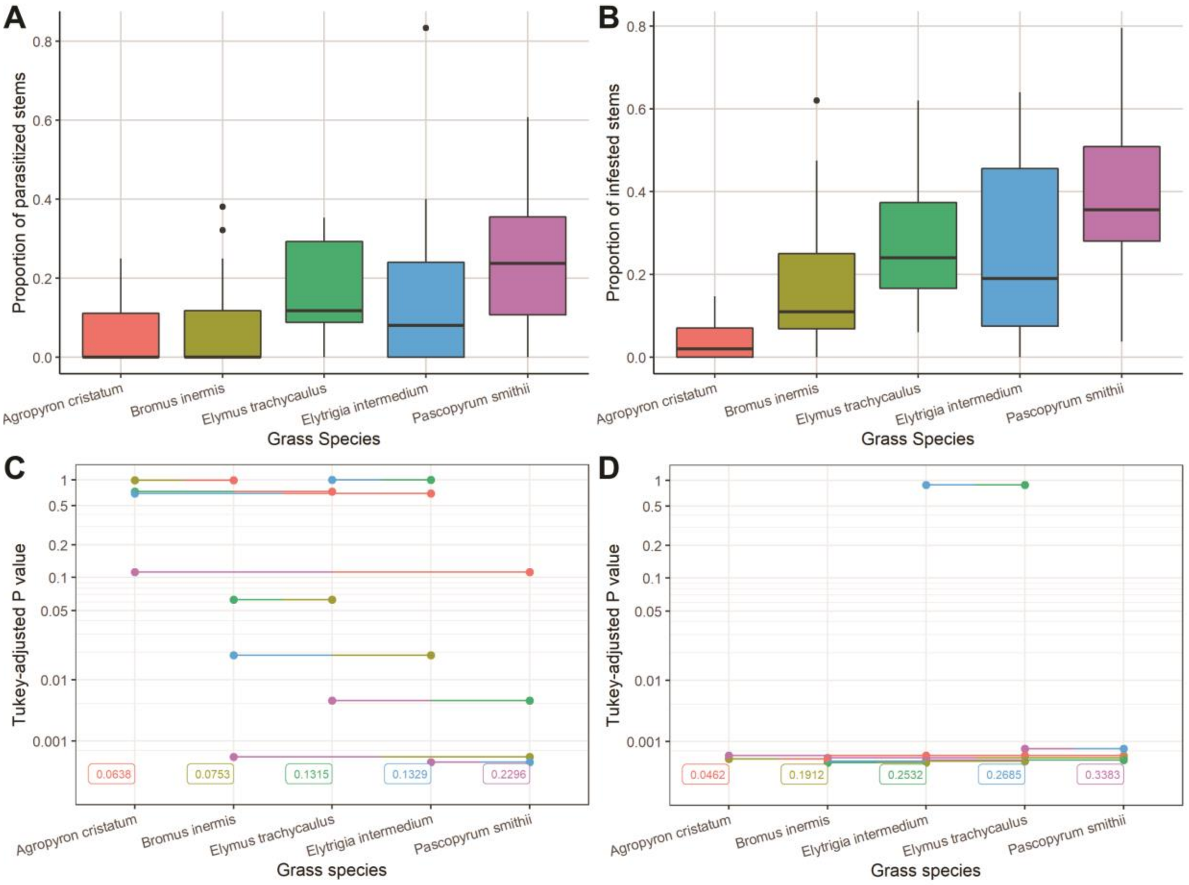

Figure 4.3: (A) Boxplot showing the proportion of parasitized stems by species. (B) Boxplot showing the proportion of wheat stem sawfly infested stems by species. The boxes represent the 25th and 75th percentiles, the horizontal line in each box indicates the median. The whiskers signify maximum and minimum values, and circles indicate outliers. (C) Pairwise comparisons between grass species based on the proportion of parasitized stems. (D) Pairwise comparisons between grass species based on the proportion of wheat stem sawfly infested stems. The pairwise *P*-value plot shows Tukey-adjusted *P*-value comparisons. Each comparison is associated with a horizontal line segment that joins the scale positions of the two estimated marginal means being compared, and whose vertical position is determined by the *P*-value of that comparison.

Across all three years *Bracon was* present at all but one site (Figure 4.2A). At site 8 we only found *Bracon* in 2019 (Figure 4.2A). The proportion of *Bracon* parasitism across all sites was also variable (n = 380 parasitized stems, n = 2,256 infested stems, mean: 0.16 ± 0. 17, max: 0.83, min: 0). Parasitism rates of wheat stem sawfly were highest on *P. smithii* (n = 197 parasitized stems, n = 782 infested stems, 0.25 ± 0.17) and lowest on *B. inermis* (n = 44 parasitized stems, n

= 519 infested stems, 0.07 ± 0.11) and *A. cristatum* (n = 3 parasitized stems, n = 46 infested stems, 0.07 ± 0.11) (Figure 4.3A, C).

Stem diameters for the five grass species were: *A. cristatum* (n = 493, 1.66 ± 0.47 mm), *B. inermis* (n = 883, 2.37 ± 0.66 mm), *E. trachycaulus* (n = 380, 1.81 ± 0.45 mm), *E. intermedium* (n = 708, 2.22 ± 0.54 mm), and *P. smithii* (n = 739, 1.58 ± 0.39 mm).

Non-cultivated grass survey: Landscape variables

Correlations between landscape variables were as follows: proportion wheat 500 m and herbaceous 500 m ($r = -0.63$, $P < 0.0001$); proportion wheat 500 m and herbaceous 5 km ($r = -0.44$, $P < 0.0001$); proportion wheat at 500 m and 5 km ($r = 0.55$, $P < 0.0001$); proportion herbaceous at 500 m and 5 km ($r = 0.76$, $P < 0.0001$). On the landscape level, the proportion of herbaceous 5 km and wheat 5 km were inversely correlated ($r = -0.91$, $P < 0.0001$). Due to the strong negative correlation between wheat cover at 5 km and herbaceous cover at 5 km, only herbaceous cover at 5 km was retained for model selection. We retained herbaceous cover rather than wheat cover because it was the habitat we were sampling and is a known habitat for both species of *Bracon* as well as wheat stem sawfly in Colorado.

Non-cultivated grass survey: Model Selection

Four predictor variables were retained in our best fit model for proportion parasitism by *Bracon*: grass species, proportion herbaceous cover, at both 500 m and 5 km, and proportion wheat cover at 500 m (Figure 4.4A-C). Odds ratios and 95% CI are presented in Table 4.1. Our best fit model suggested that an increase in wheat cover at 500 m results in the greatest increase in the odds of *Bracon* parasitism. For a one unit increase in proportion wheat cover at 500 m the odds of parasitism by either *Bracon* species increased by 9.14 times (2.83, 29.52 95% CI) with other variables held constant. However, it is important to note the wide confidence interval. The presence of grass species were also predictors of the presence of *Bracon*. However, the presence of *P. smithii* increased the odds of parasitism more than other grass species. If *P. smithii* is present, the odds of parasitism increase by 4.37 times (1.32, 14.50 95% CI).

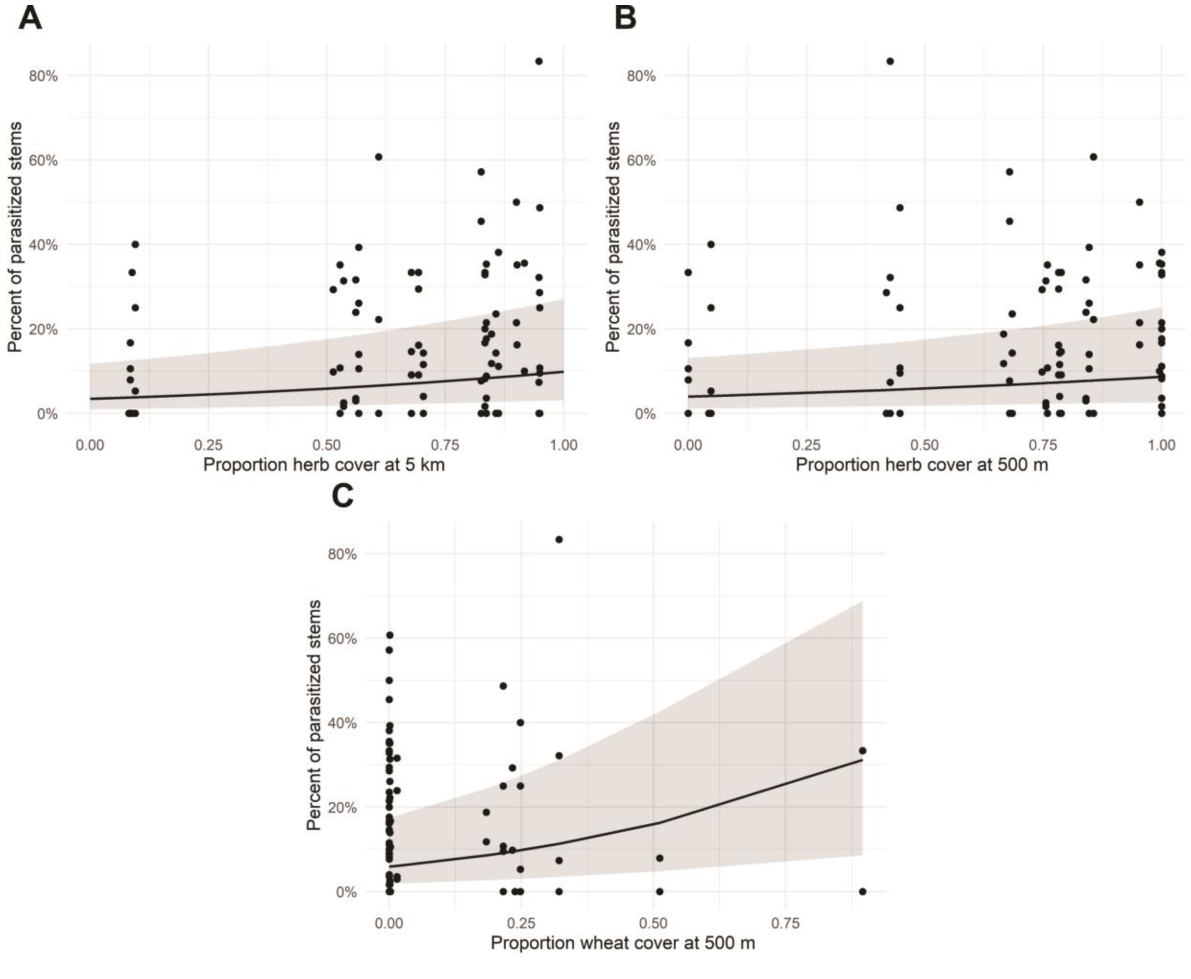

Figure 4.4: Plots showing data (points) alongside predictions of parasitized stems (line with standard error shaded in gray) based on the best fit model. (A) Predictions of parasitized stems based on proportion herbaceous cover at 5 km. (B) herbaceous cover at 500 m and (C) wheat cover at 500 m.

Table 4.1: Summary of best fit (lowest AIC) Generalized Linear Mixed Model (binomial distribution, logit link) examining the effects of various fixed effects predictor variables on the proportion parasitism of wheat stem sawfly larvae by braconid parasitoids with random effects being date sampled. Fixed effects are factors that have a predetermined set of levels that we expect to have an effect on our response variable, random effects are levels that are randomly selected from a population of possible levels, σ^2: total variance from dates in our test population. Intraclass correlation (ICC): ratio of between-cluster variance to the total variance.

***Bracon* parasitism best fit model**				
Predictors	*Fixed Effects Estimate*	*Odds Ratios*	*95% CI*	*Wald X^2*
(Intercept)	-4.1	0.02	0.00 – 0.06	34.98
wheat cover (500 m)	2.21	9.13	2.83 – 29.44	13.69
herbaceous cover (500 m)	0.84	2.32	1.05 – 5.11	4.33
herbaceous cover (5 km)	1.13	3.08	1.64 – 5.80	12.15
Grass species				54.13
Bromus inermis	0.179	1.2	0.35 – 4.10	
Elymus trachycaulus	0.8	2.22	0.64 – 7.72	
Elytrigia intermedium	0.81	2.25	0.67 – 7.60	
Pascopyrum smithii	1.47	4.37	1.32 - 14.5	
Random Effects				
σ^2				3.29
ICC				0.04
N_{Date}				6
Observations				111
AIC				Full Model: 492.8; Best Model: 492.8
Marginal R^2 / Conditional R^2				0.161 / 0.194

Three significant predictor variables were retained in our best fit model for the proportion of stems infested by sawfly: grass species, and proportion herbaceous cover at 500 m, and proportion herbaceous cover at 5 km (Figure 4.5A-B). Odds ratios and 95% CI are presented in Table 4.2. Our best fit model suggested an increase in herbaceous cover at 500 m is associated with the greatest increase in the odds of infestation. However, a decrease in herbaceous cover at

5 km led to an increase in the probability of infestation. The presence of *P. smithii* increased the odds of infestation more than other grass species. If *P. smithii* is present, the odds of infestation increase by 10.55 (7.68, 14.51 95% CI).

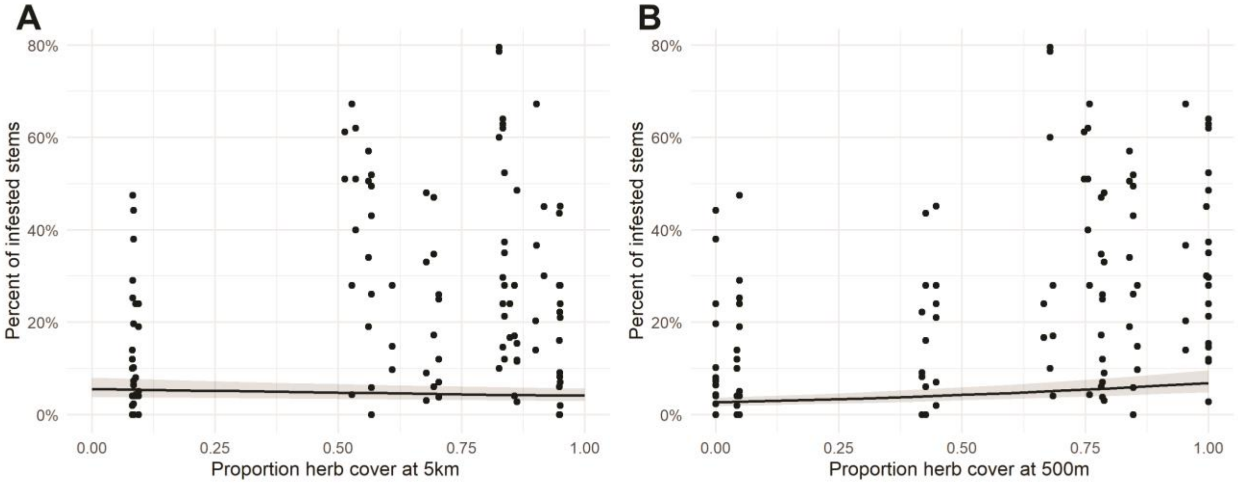

Figure 4.5: Plots showing data (points) alongside predictions of wheat stem sawfly infested stems (line with standard error shaded in gray) based on the best fit model. (A) Predictions of infested stems based on proportion herbaceous cover at 5 km and (B) herbaceous cover at 500 m.

Table 4.2: Summary of best fit (lowest AIC) Generalized Linear Mixed Model (binomial distribution, logit link) examining the effects of various predictor variables on the proportion of stems infested by wheat stem sawfly.

Wheat stem sawfly best fit model

Predictors	*Fixed Effects Estimate*	*Odds Ratios*	*95% CI*	*Wald X^2*
(Intercept)	-3.4137	0.03	0.02 – 0.05	351.6822
herbaceous cover (500 m)	1.0022	2.72	2.16 – 3.44	70.3703
herbaceous cover (5 km)	-0.3272	0.72	0.55 – 0.94	5.6291
Grass species				274.6872
Bromus inermis	1.5851	4.88	3.56 – 6.69	
Elymus trachycaulus	2.025	7.58	5.43 – 10.57	
Elytrigia intermedium	1.9455	7	5.10 – 9.61	

Pascopyrum smithii	2.3566	10.55	7.68 – 14.51
Random Effects			
σ^2	3.29		
ICC	0.01		
N_{Date}	6		
Observations	111		
AIC	Full Model: 1719.4; Best Model: 1717.6		
Marginal R^2 / Conditional R^2	0.176 / 0.186		

Weekly Bracon *sampling in cultivated wheat*

Low numbers of *Bracon* adults were found during weekly sweeps in wheat. A total of 17, 8, and 3 were found in 2017, 2018, and 2019. All the *Bracon* adults captured were identified as *B. lissogaster* except one *B. cephi* caught in 2017. Peak flight for both 2017 and 2018 occurred on the 19th and 20th of June (Figure 4.6). In 2019, *Bracon* adults were only caught in July.

Additionally, no evidence of parasitism by *Bracon* was observed in a wheat stem sawfly survey at the same locations in the same years (n= ca. 30,000 dissected stems) (Cockrell et al., 2021).

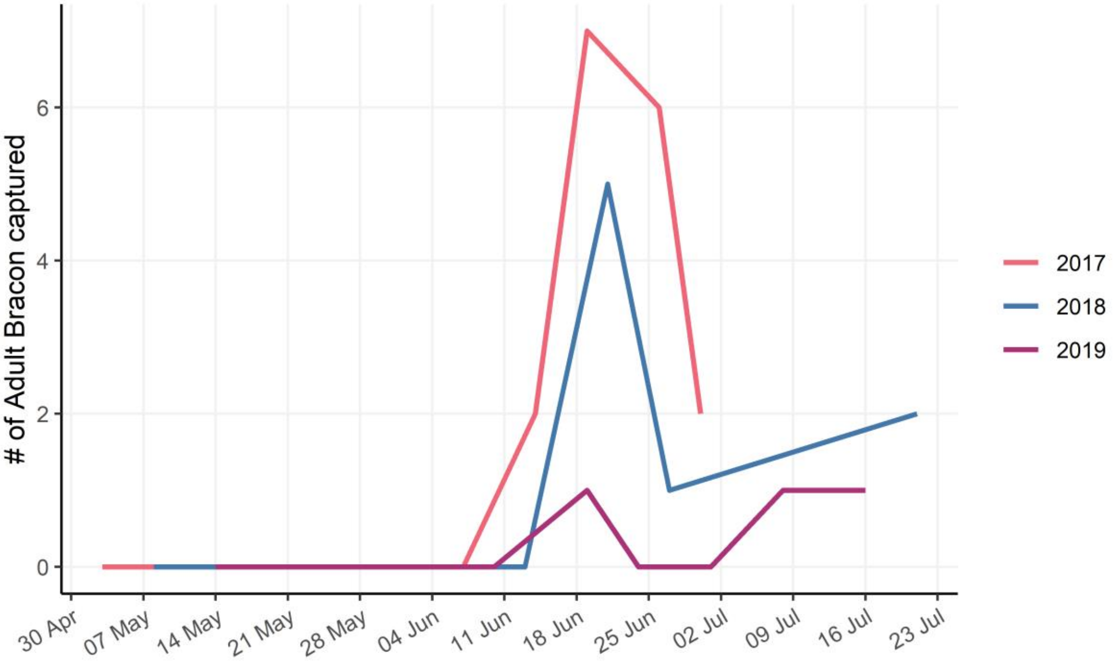

Figure 4.6: Line graph showing the total number of *Bracon* adults captured during weekly sweeps at seven fields known to have heavy wheat stem sawfly infestation near New Raymer and Orchard, CO. Samples were collected from early May and continued until mid-July during 2017–2019.

Discussion

Wheat stem sawfly infestation continues to increase in intensity across Colorado, devastating winter wheat production. Losses to wheat stem sawfly elsewhere in the Great Plains are the motivation to find effective and sustainable controls. In this study, we monitored *Bracon* and wheat stem sawfly populations in non-crop grasses, but also monitored *Bracon* adults in winter wheat. Concurrent wheat stem sawfly surveys at these same locations found no evidence of parasitism by *Bracon* despite sampling over 30,000 winter wheat stems. *Bracon* were found in natural habitats containing non-cultivated grasses, occurring at 100% of sampled sites across all three years with average parasitism rates of 14% and maximum parasitism rates of 83%. This raises questions about the underlying causes of the lack of parasitism of wheat stem sawfly in winter wheat in Colorado.

Parasitism in winter wheat

In other states, such as Montana and Nebraska, parasitism of wheat stem sawfly by *Bracon* has been documented in winter wheat (Morrill et al. 1994, Bradshaw and Peterson 2016). However, we have yet to find parasitism by either *Bracon* species in winter wheat in Colorado. Several factors might explain the lack of parasitism observed in Colorado. First, it is possible that *B. cephi* and *B. lissogaster* experience a phenological mismatch in emergence timing relative to their sawfly hosts. Non-crop hosts of wheat stem sawfly historically have similar development times to spring wheat. However, in order to infest winter wheat flight must occur around 20 days earlier (Morrill and Kushnak, 1996). Second, are three distinct genetic clusters of wheat stem sawfly across the Great Plains (Lesieur et al., 2016), and it is possible that genetic differences between wheat stem sawfly hosts may affect host acceptance by parasitoids in Colorado. These genetic clusters also may be reflected by genetic differences among *Bracon* populations however, this has not been evaluated. A study conducted by van Nouhuys et al. 2012 focused on differences in populations of the parasitoid, *Cotesia melitaearum,* and its host *Melitaea cinxia,* at several locations around the Baltic Sea. They found brood sizes, development rates, pupal sizes, and adult longevity were significantly different among populations. This suggests that populations of parasitoids attacking different host genotypes may not have the same survival rates. Regional differences in parasitism rates may occur if populations have differing survival rates on different wheat stem sawfly genotypes. Further research is needed to assess whether there are differences in populations of both *Bracon* species throughout the Great Plains. Third, *Bracon* may eventually move over into wheat in Colorado given sufficient time, as lags in the ability of natural enemies to exploit newly expanding or invading pests are commonly observed. For example, while little parasitism was seen during early range expansion of the Russian wheat aphid, *Diuraphis noxia*) (Wraight et al., 1993), overtime attack was observed mainly by parasitoids that were either endemic or from previous introductions (Brewer et al., 2005, 2019).

Alternatively, the lack of *Bracon* observed in winter wheat may be due to insufficient sampling. The most common and widely accepted measures monitoring wheat stem sawfly include sweep sampling and stem collection. It could be the case that parasitism in Colorado mainly occurs after the wheat stem sawflies have created their hibernacula, which occurs between the end of June and the beginning of August. A study conducted by Rand et al. (2011) found high levels of parasitism in the hibernaculum of the wheat stem sawfly. While conducting our weekly sweeps we found very low numbers of *Bracon* adults. To more accurately detect evidence of parasitism by *Bracon*, we suggest collecting stubble before and after wheat harvest.

Host plant use

A survey of grasses found 19 species to be suitable hosts for wheat stem sawfly (Cockrell et al., 2017). However, not all grasses are equally attractive to wheat stem sawfly. All of the grasses chosen for this study were good hosts for wheat stem sawfly and *Bracon*. *Pascopyrum smithii* on average had the highest infestation and parasitism rates, suggesting that it was the most suitable non-cultivated among the species sampled in this study. *Agropyron cristatum* experienced the lowest infestation rates and in turn the lowest parasitism. These two species of grasses had similar stem diameters but drastically different infestation levels. We measured stem diameters as a proxy for host suitability as previous research suggests that wheat stem sawfly prefer hosts with larger stem diameters (Farstad, 1940; Holmes and Peterson, 1960). However, we saw different infestation levels in *A. cristatum* and *P. smithii* which had similar stem diameters (*A. cristatum* (1.66 ± 0.47 mm), *P. smithii* (1.58 ± 0.39 mm), suggesting *P. smithii* is attractive for reasons other than stem diameter.

Pest responses to landscape variation

Our best fit model suggests the odds of infestation of wheat stem sawfly slightly decreased with herbaceous cover at a landscape scale (5 km). Since the proportion of herbaceous and wheat cover at a 5 km scale are highly inversely correlated, this could mean an increase in wheat cover leads to a higher chance of wheat stem sawfly infestation. Rand et al. (2014) similarly concluded that wheat stem sawfly infestation in cultivated wheat was positively impacted by the proportion wheat cover at a landscape scale (5 km). These comparable findings suggest that, in contrast to *Bracon*, wheat stem sawfly populations in crop and non-crop habitats may respond similarly to landscape composition.

We found that wheat stem sawfly infestation increased with decreasing herbaceous landcover, and the correlated increases in crop cover, suggesting that wheat stem sawfly populations from crop habitats may be moving into non-crop habitats, a cross-edge spillover (Rand et al., 2006). Most studies observing spillover focus on non-crop to crop habitat movement. Far fewer studies document movement in the opposite direction (Blitzer et al., 2012). The movement of crop insects into non-crop habitats have been found to directly impact non-crop species by damaging plants and limiting reproductive success (Kaiser et al., 2008; Squires et al., 2009). Long term damage to non-crop habitats could cause loss of ecosystem services and diversity. Whether spillover of wheat stem sawfly might affect native grasses and native ecosystems is unknown. Future studies could investigate the potential impacts of the movement of wheat stem sawfly into non-crop habitats.

Natural enemy responses to landscape variation

A meta-analysis by Attwood et al. 2008 suggests that semi-natural habitats in an agroecosystem can lead to increased biodiversity and abundance of arthropods, specifically natural enemies (Attwood et al., 2008). Another meta-analysis conducted in 2013 concluded that increasing semi-natural areas improved the biological control of agricultural insect pests (Veres et al., 2013). However, a separate data synthesis found non-crop habitat either has no effect on natural enemy densities or can even increase pest populations (Karp et al., 2018).

While we did not find instances of *Bracon* parasitism in winter wheat, we found small populations in non-cultivated grasses. Our study suggests that an increase in non-crop habitat increases the odds of parasitism by *Bracon* by2.3 and 3.1 at the local (500 m) and landscape (5 km) scales respectively. This suggests that non-crop populations of *Bracon* benefit from local and landscape level non-crop cover. However, our model further suggests that *Bracon* populations living in non-crop habitat are most positively affected by an increase in wheat cover at a local scale. This may be due to the increase in host availability as the proportion of wheat cover increases. As mentioned previously, the odds of wheat stem sawfly infestation in non-cultivated grasses decreases with increased herbaceous cover at a landscape scale (5 km).

A similar study conducted by Rand et al. 2014 examined the effects of landscape composition on parasitism by *Bracon* and wheat stem sawfly infestation in wheat, while our study examined populations in non-cultivated grasses. Rand et al. (2014) found no effects of landscape composition on parasitism rates by *Bracon*, whereas we found non-crop and crop habitats play a role in the likelihood of parasitism by *Bracon*. These conflicting results suggest natural enemy populations using non-crop and crop habitats may respond differently to landscape composition.

Management Implications

Our results suggest that an increase in herbaceous land cover could increase non-crop populations of *Bracon* which might eventually lead to spillover into cultivated wheat. Additionally, non-crop pest insect populations increase with increasing landscape scale cover of their host plants. A decrease in land cover of wheat could thus play a role in reducing pest populations and potentially increasing natural enemy populations. This could be done through landscape diversification either by planting alternative crops or converting cropland into non-cultivated or Conservation Reserve Program land (O'Rourke et al., 2011; Carrière et al., 2012). Future research could track population changes associated with changes in wheat landcover as the estimated acreage planted to wheat in the United States in 2020 was at its lowest level since 1919 (National Agricultural Statistics Service (NASS) and (USDA), 2020). Extensive surveys of winter wheat in Colorado from 2017-2019 did not detect either *Bracon* in wheat. We suggest focusing future research investigations on disrupting the pest through landscape diversification as an alternative to conserving natural enemies, which are not yet effectively moving into wheat.

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CHAPTER 5 - Triticale as a potential trap crop for the wheat stem sawfly (Hymenoptera: Cephidae) in winter wheat[3]

Introduction

Trap crops are used to attract insect pests away from the crop of interest. Typically, plants are planted in a small stand near the crop of interest and are destroyed or treated with pesticides once pest densities are high (Hokkanen, 1991). Some common methods of trap cropping include: perimeter planting where the trap crop is planted around the border of the main crop; sequential planting where the trap crop is planted earlier than the main crop; multiple planting where several other trap crops are used; and push-pull where the trap crop is highly attractive, and the main crop is not (Hokkanen, 1991; Shelton and Badenes-Perez, 2006). Using a trap crop can increase yield and an overall reduction in pesticide use (Hokkanen, 1991). Some examples of effective trap crops include using sunflowers as a trap crop for *Lygus pratensis* in Chinese cotton fields (Zhang et al. 2020). Cotton fields bordered by sunflower had 45% less damage to bolls and 28% less damage to leaves than fields not bordered by sunflower. Blue Hubbard squash as a trap crop for striped cucumber beetle, *Acalymma vittatum* F., in butternut squash reduced insecticide use by 94% in North American butternut squash fields (Cavanagh et al. 2009). Also, trap crops can increase insect diversity and promote natural enemies (Tiwari et al., 2020). For example, sweet alyssum, *Lobularia maritima*, improved predator abundance for green peach aphid, *Myzus persicae,* in radish in Nepal (Tiwari et al., 2020). Some trap crops may enhance pest control by producing semiochemicals (Shelton and Badenes-Perez, 2006), making the trap crop more attractive to pests (Khan et al., 2016). Additionally, trap cropping can be improved through

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various manipulations, mainly using sex and aggregation pheromones (Hokkanen, 1991). Yet, very few trap crop schemes have been widely adopted (Shelton and Badenes-Perez, 2006). Successful implementation often depends on the retention of the pest on the trap crop, the suitability of the system, and a minimal increase in supplemental management strategies (Holden et al., 2012; Sharma et al., 2019). A major pest of wheat (*Triticum aestivum* L.) known as the wheat stem sawfly (Hymenoptera: Cephidae: *Cephus cinctus* Norton), is considered to have life history attributes, such as low fecundity and a short adult lifespan, that make it suitable to be managed using trap crops (Morrill et al., 2001).

Grain-yield loss from the wheat stem sawfly exceeds $350 million annually in the northern Great Plains of North America (Beres et al., 2011b). Its life cycle makes it particularly difficult to control using conventional methods. The adult wheat stem sawfly lay their eggs within the stem of their host, and as the larvae develop, they consume parenchyma tissue (Ainslie, 1920). Towards the end of the season, the last instar larva creates a hibernaculum (stub) by cutting at the base of the stem, causing the seed head to fall to the ground. Cut stems are difficult to harvest and are easily blown over (Ainslie, 1920). Current management methods include biological control and solid stem genotypes (Delaney et al., 2010; Beres et al., 2011b; Rand et al., 2012; Peairs et al., 2014). A comprehensive review of wheat stem sawfly biology and management practices is reported by Beres, Dosdall, et al. 2011. Previous work dating back to 1922 suggests trap crops can control wheat stem sawflies (Criddle, 1922). In particular, winter wheat in Montana is potentially used as a trap crop for wheat stem sawflies in spring wheat cropping systems (Morrill et al., 2001; Beres et al., 2009; Buteler et al., 2010). The early planting of winter wheat attracted wheat stem sawfly adults from neighboring fallow wheat stubble and served as a sink for eggs. Later, the trap crop could be harvested or swathed to destroy larva developing within the stems (Morrill et al., 2001). Studies suggest stem height, stem solidness, and semiochemicals play a role in attractiveness and retention rates of winter wheat to wheat stem sawfly (Morrill et al., 2001; Beres et al., 2009; Buteler et al., 2010; Buteler and Weaver, 2012). A pheromone found to attract adult females of wheat stem sawfly could be introduced into trap strips to improve the attractiveness of trap crops. However, the two pheromones currently described are not sex discriminant (Bartelt et al., 2002; Cossé et al., 2002b).

Since most wheat grown in Colorado is winter wheat, we focused on assessing the retention, attractiveness, and host suitability of winter triticale (x *Triticosecale* Wittmack)*,* a cross between wheat and rye (*Secale cereale,* Will.), as a trap crop for the wheat stem sawfly in a winter wheat cropping system. Winter triticale has tall stems, good grain and forage yield potential, early maturity, and desirable forage quality (Oettler, 2005; Estrada-Campuzano et al., 2012; Randhawa et al., 2015), making this plant attractive to wheat farmers who also feed cattle (Harper et al., 2017; Coblentz et al., 2018).

In the current study, we evaluated the attractiveness and retention of wheat and triticale over two years using both cone-tainer and field experiments. The cone-tainer experiment was designed to assess three triticale genotypes as suitable hosts for the wheat stem sawfly compared to a highly attractive winter wheat genotype. The field experiments included one genotype of triticale compared to wheat and pheromone traps (Cossé et al., 2002a) to assess whether pheromones could increase attraction and retention in either wheat or wheat triticale plots.

Methods

Cone-tainer experiment to test host preference and performance on triticale versus wheat

In 2019 and 2020, we compared three winter triticale genotypes; 'NT 05421', 'NT 07403', and 'NE 426GT' to 'Avery', a winter wheat genotype known to be highly attractive to wheat stem sawfly (CSU 2020) (Table 5.1). All three triticale genotypes are grown predominantly for forage. We germinated seeds on 5 x 10 mm germination blotter paper (Anchor Paper Co. St. Paul, MN) with 5 ml of tap water. Seeds were kept at an average temperature of 24 °C for three days. Germinated seeds were then vernalized by placing them in a cold room and held at 4 °C for seven weeks. After vernalization, a single seedling was planted in a cone-tainer (Stuewe & Sons SC10U UV-stabilized cones: 3.8 cm diameter x 20.9 cm depth, 164 ml volume) with a soil mixture of 7 parts soil 2 parts perlite, and a cotton ball at the bottom to prevent soil loss. Due to changes in our soil distributor, we used Metro Mix (Sungro, Agawam, MA) in 2019 and 20B/30V (Lambert, Québec, Canada) in 2020. Both brands used had comparable compositions of sphagnum peat moss and bark.

Table 5.1. Winter triticale and wheat genotypes used in this study.

Genotype	**Cone-tainer stem diameters (mm)**	**Pedigree**	**Citation**
NT 05421	3.43 ± 1.30	Complex cross involving NE422T	((UNL) and (USDA) 2017)
NT 07403	2.98 ± 0.56	NE98T424/FLOOD//NT00418	((UNL) and (USDA) 2017)
NE 426GT	2.97 ± 0.46	WB-UW24/TxTd#50//Fain Triticale/Centurk 78/NE69150/Jenkins X Unknown	(Baenziger et al., 2005)
Avery	2.66 ± 0.94	TAM 112/Byrd	(Haley et al., 2018)
Snowmass	N/A	KS96HW94//‘Trego’/CO960293	(Haley et al., 2011)

Plants were grown in a greenhouse at Colorado State University with supplemental light (430W HPS (High-Pressure Sodium) fixtures - P.L. Light Systems, bulbs - GE Lucalox lu400 series, 400W). The greenhouse had a 16L: 8D photoperiod and the day: night temperature of 23:18 °C. Plastic trays were placed under 42 cone-tainers, and all trays were bottom watered as needed.

Germination and planting dates were staggered over two weeks in 2019 and three weeks in 2020. We did this as peak sawfly flight is highly variable across years and wanted to ensure uniform maturity of plants. In each tray, cone-tainers of triticale were planted in alternating rows to Avery, and trays were placed randomly in the greenhouse. One genotype of triticale was planted per tray. Plants were fertilized once a week, beginning one week after initial planting, with 300 ml 15-16-17 Peters General Purpose Fertilizer (J. R. Peters, Allentown, PA) at 296 ppm in an aqueous solution. To promote primary stem growth, we cut and removed secondary tillers leaving only the primary tiller. This was conducted 10 days before infestation so plants could recover from injury. In 2019 once plants started to tiller, tillers were cut twice two weeks apart. In 2020 we cut tillers once, three weeks after planting.

Wheat stem sawfly infestation and data collection

We transported wheat plants to the field for infestation once plants reached Zadoks growth stage 32-75 (Zadoks et al., 1974). The wheat stem sawfly requires a stem for larval development; hence we considered Zadoks 32 (when two to three nodes were visible) to Zadoks 75 (ripening of kernels) as the appropriate stages for oviposition (Criddle, 1923). This range was chosen due to large varietal differences in maturity. We only brought plants out on calm sunny days, conditions favorable for adult sawfly activity. We measured infestation pressure by sweeping 100 times using 180-degree pendulum sweeps with a standard 38 cm diameter sweep net. All infestations occurred on a field of infested wheat stubble bordering growing wheat at Wickstrom Farms, near Orchard, CO. Trays were placed 10 meters apart, and trays were set 5 meters apart within rows.

In 2019, cone-tainer trays of a single planting date were placed in the field for 24 hours on May 24 and for 1 hour 30 mins on May 30. In 2020, trays were placed in the field for 2 hours on May

22 and June 5 and for 4 hours on May 29. Exposure times were variable based on variable adult infestation pressures and accounted for during statistical analysis. Plants in each round of exposure were from the same planting date. All exposures started at 9 am. Before plants were brought back to the greenhouse, sawfly adults were removed, and plants left outside for 2-3 hours and checked again to ensure no foraging females were brought inside. Once we brought the trays back to the lab, half of the plants were dissected to examine stems for eggs, while the other half remained in the greenhouse to allow larvae to mature. While examining stems for eggs, we recorded stem diameter using a digital caliper (Pittsburgh, Model 47257) by measuring the first visible node's width, the stem's maturity on the Zadoks scale, and the number of eggs present.

Thirty days after infestation, the remaining stems were dissected to assess wheat stem sawfly presence. All stems were examined on the same day. We considered a stem infested if there were frass and/or larvae present in the stem. If larvae were present, they were weighed, and pictures of the head capsule were taken. Using ImageJ (Rueden et al., 2017), the length of the head capsule was measured. Body measurements were recorded as a proxy to compare growth and development among genotypes, as suggested by Kumar and Venkatesan 2019. We noted if stems had been cut to form hibernaculum chambers. Assessing whether a stem was cut or not enables us to determine the development time of the larvae when compared to other genotypes since the creation of a hibernaculum occurs during the last larval instar (Ainslie, 1920). In 2019 we only dissected plants with more than one visible node. To collect more precise measurements on stem development in 2020 we recorded the Zadoks growth stage of the stem during dissections. We only considered plants at Zadoks growth stages higher than 32 (plants with more than two nodes) for further analysis. If there were multiple stems in a cone-tainer, we dissected the oldest stem based on the Zadoks growth stage.

Statistical analysis for cone-tainer plants

Analysis of data was conducted using R (R-Core Team 2019, Version 3.6.2) and R packages lme4 (Bates et al., 2015), ggplot2 (Wickham, 2009), and emmeans (Lenth, 2020). We used a linear mixed model with the infestation date as a random effect and year, Zadoks growth stage, genotype, and stem diameter as fixed effects to analyze the number of eggs within a stem. We included year, Zadoks growth stage, and stem diameter in the model, all known confounding variables for oviposition rates. Analysis of stem diameters was conducted using a linear model with genotype and year as independent variables.

Stem infestation data were considered binomial since only one larva can survive to maturity per stem (Ainslie, 1920). The proportion of infested stems was calculated as the number of infested stems divided by the total number of stems sampled. Using a generalized linear mixed model (GLMM), these data were evaluated with a binomial error distribution and a logit link function. In the GLMM model, infestation date was a random effect, and genotype was a fixed effect. We also analyzed the number of stems where a hibernaculum (stub) was formed. The number of stubs per genotype was also analyzed using a generalized linear model with a binomial error distribution and a logit link function with genotype and year as independent variables. Larval head length and weight were analyzed using a linear mixed model with infestation date as a random effect and genotype as a fixed effect.

Field experiment to test effects of planting a triticale trap crop on wheat infestation

A winter wheat field bordering a heavily infested wheat stubble field near Orchard, CO was chosen for this experiment in 2019 and 2020. Fields in 2019 and 2020 were different but less than a 2 km apart. Plots were planted along one edge border of the focal crop next to a wheat fallow field (Figure 5.1). The treatments were winter wheat ‘Snowmass’, or winter triticale NT 05421. Snowmass was also the cultivar planted in the main field. Two treatments were planted in a Randomized Complete Block Design, replicated 12 times, so each block contained two plots of each treatment. Plots were 1/26 the length of the field edge (31 meters each) to allow for a buffer plot to be planted on either end (24 plots + 2 buffer plots). In 2019 only we placed pheromone traps in half of the winter wheat and triticale plots. We used a single pheromone lure consisting of 9-acetyloxynonanal (Chemtica, San Jose, Costa Rica). This pheromone is considered attractive to wheat stem sawfly (Cossé et al., 2002a).

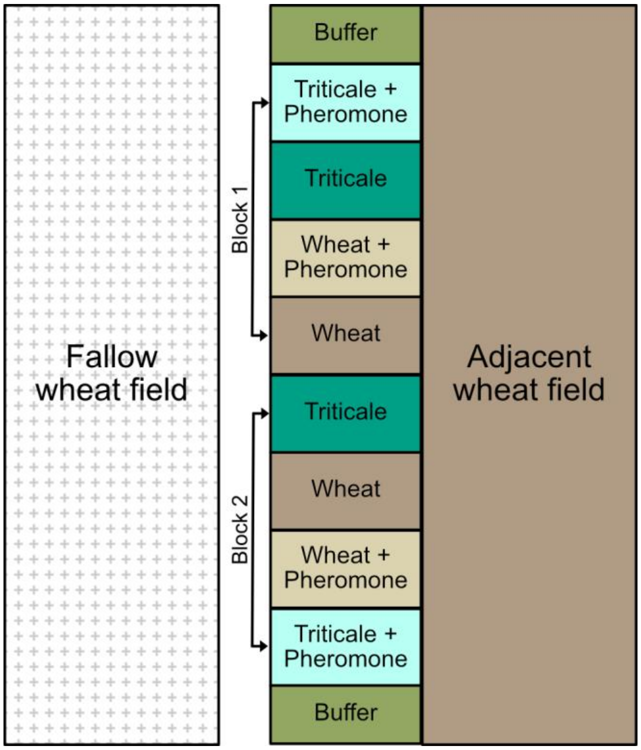

Figure 5.1. Field plot design in 2019, showing blocks 1 and 2 as examples. In 2020 pheromone was not added to plots but field design was otherwise unchanged.

Plots were visited weekly to accomplish the following in 2019 and 2020: (1) Ten sweeps using 180-degree pendulum sweeps with a standard 38 cm diameter sweep net were taken from the center of each plot to determine in-plot wheat stem sawfly abundance, and (2) ten tillers were taken from the middle of each plot and dissected to determine larval position over time. In 2019 pheromones were placed at the height of 1 meter in the center of the plots with pheromone treatment. Pheromones were replaced weekly. The larval position was calculated by measuring the larval distance from the root crown. On sample date 5/22/2020, 100 sweeps were conducted per plot, so sweep totals were divided by 10. The collaborating farmer swathed plots on (6/26/2019, 6/15/2020) at the height of 15-20 cm. Then 100 wheat stems were collected from the remaining stubble in each plot approximately one month later (7/20/2019, 7/20/2020) from each plot. To determine the final infestation levels, we dissected stems to look for signs of infestation, as described above. To determine infestation of the main crop, we collected and dissected 100 stems of stubble from the main field wheat adjacent to the plots (9/11/2019, 9/21/2020).

Statistical analysis for field experiment

We conducted separate analyses for 2019 and 2020 as the experimental design included a pheromone in 2019 but not in 2020. We used a linear model with crop type (wheat or triticale) and date sampled as independent variables for sweep net data and larval position. To examine the effects of pheromone presence on sweep samples, we used a linear model with treatment and date sampled as independent variables and the number of adults caught in sweeps as dependent variables. To analyze infestation of the plots and main field wheat adjacent plots we used a linear model with crop type and with or without pheromone treatment in 2019 and crop type in 2020 as independent variables.

Results

Cone-tainer experiment

Triticale varieties NT 07403, NE 426GT, and NT 05421 had larger stem diameters than Avery. The average stem diameter in 2019 and 2020 for the genotypes tested are presented in Table 5.1. There were differences in stem diameters between genotypes ($F_{3,425}$ = 20.867, *P* = < 0.0001). NT 05421 had the largest stem diameter when compared to the other genotypes tested (NT 07403: *P* = 0.001, NE 426GT: *P*= < 0.047, Avery: *P* = < 0.0001). Avery stem diameters were smaller than all triticale genotypes (NT 07403, NE 426GT, NT 05421: *P* = < 0.0001). NT 07403 and NE 426GT stem diameters were similar (*P* = 0.699). NE 426GT had more eggs inside stems than NT 07403 (*P* = 0.0126) (Figure 5.2). NT 07403, NE 426GT, NT 05421, and Avery did not have different egg counts (Figure 5.2). Pairwise comparisons and estimated marginal means, provided in Figure 5.3.

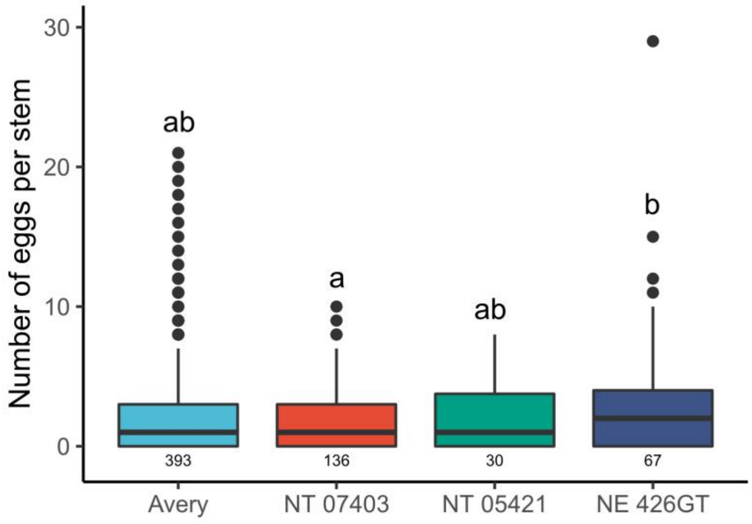

Figure 5.2. Effects of triticale genotypes and wheat on the number of wheat stem sawfly eggs found stems. Sample size is represented by the numbers beneath each boxplot (total stems cut). The boxes represent the 25th and 75th percentiles; the horizontal line in each box indicates the median. The whiskers signify maximum and minimum values, and circles indicate outliers. Letters indicate significant pairwise differences (*P* < 0.05, Tukey HSD corrected post hoc test). Pairwise comparisons and estimated marginal means, provided in Figure 3.

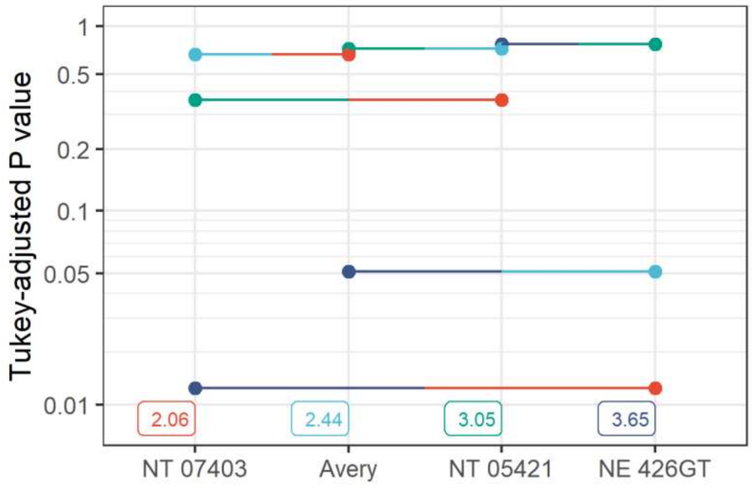

Figure 5.3. Estimated marginal means of the effects of triticale genotypes and wheat on the number of eggs laid in stems by the wheat stem sawfly. The pairwise p-value plot shows Tukey adjusted p-value comparisons. Each comparison is associated with a horizontal line segment that joins the scale positions of the two estimated marginal means being compared and whose vertical position is determined by the P-value of that comparison.

The number of adult sawflies found in sweep nets for each infestation date varied across dates. In 2019, 767 sawflies were captured on May 24 and 1,148 on May 30. In 2020, we collected 145 sawflies on May 22, 54 sawflies on May 29, and 4 sawflies on June 5.

Avery stems had the highest probability of infestation, higher than NT 0521 ($P = < 0.0001$) and NE 42GT ($P = < 0.0001$). Avery had a similar probability of infestation when compared to NT 07403 ($P = 0.933$) (Figure 5.4 (A)). Avery stems had the highest probability of being cut when compared with NT 7403 ($P = 0.010$). No other genotypes differed in the number of stems cut. (Figure 5.4 (B)).

Larvae in NT 05421 had larger head capsule lengths and body weights than Avery (head: $P = < 0.0001$, weight $P = 0.019$) and NT 07403 ($P = 0.026$, $P = 0.047$) (Figure 5.4 (C) and (D)). Pairwise comparisons and estimated marginal means, provided in Figure 5.5.

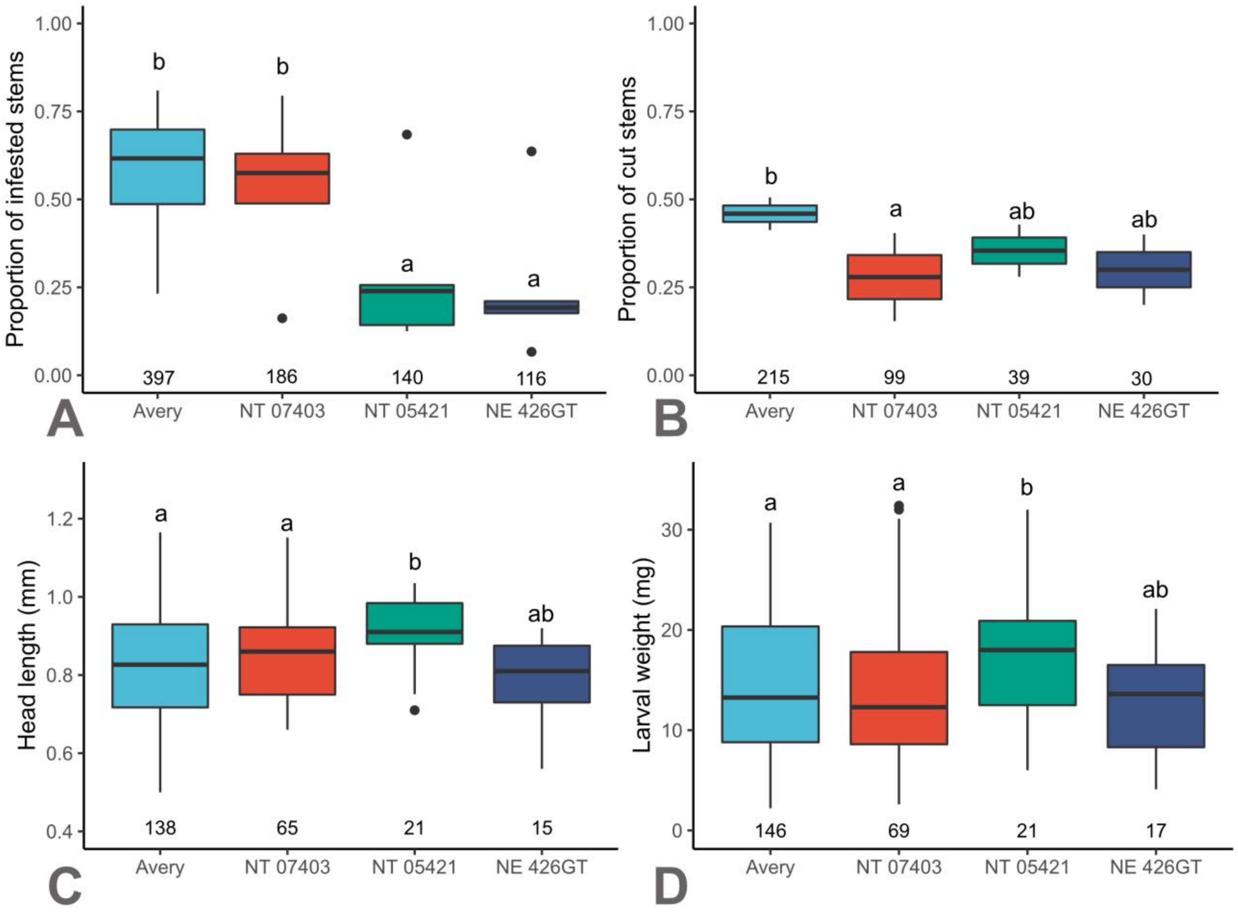

Figure 5.4 A-D. (A) Effects of triticale genotypes and wheat on the proportion of stems infested with wheat stem sawfly, (B) on the proportion of stems that contained frass and were cut by the wheat stem sawfly, (C) on wheat stem sawfly larval weight, (D) on wheat stem sawfly larval head capsule length. Sample size is represented by the numbers beneath each boxplot (total stems cut). Letters indicate significant pairwise differences (*P* < 0.05, Tukey HSD corrected post hoc test). Pairwise comparisons and estimated marginal means, provided in Figure 5.5.

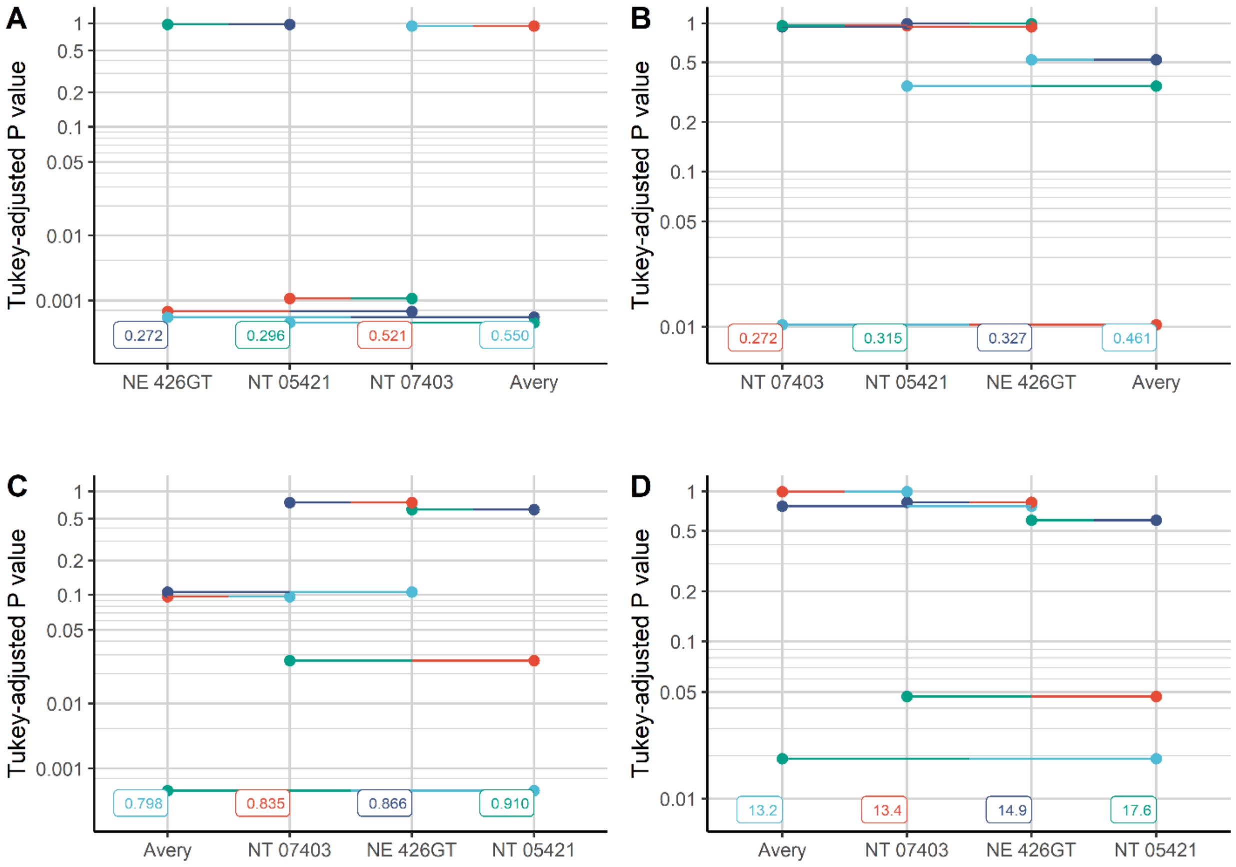

Figure 5.5 A-D. Estimated marginal means of the effects (A) triticale genotypes and wheat on the proportion of stems infested with wheat stem sawfly, (B) on the proportion of stems that contained frass and were cut by the wheat stem sawfly, (C) on wheat stem sawfly larval weight, (D) on wheat stem sawfly larval head capsule length. The pairwise p-value plot shows Tukey adjusted p-value comparisons. Each comparison is associated with a horizontal line segment that joins the scale positions of the two estimated marginal means being compared and whose vertical position is determined by the P-value of that comparison.

Field experiment

In 2019, adult abundance in plots with and without pheromones was similar (F (125) = 0.018, *P* = 0.892). More wheat stem sawfly adults were caught in sweeps in wheat plots than triticale plots on all dates in 2019 (*P* = 0.003) and 2020 (*P* = < 0.001) (Figure 5.6). In 2019 and 2020 larval positions were similar in triticale and wheat stems. However, in 2019 larvae were higher in position in triticale and wheat when sampled on July 16th compared to July 10th ($P = 0.039$) (Figure 5.7). In 2020, larvae were in a higher position in the stem on May 29th than on July 5th ($P = 0.003$) or July 11th ($P = 0.003$) for both triticale and wheat (Figure 5.7).

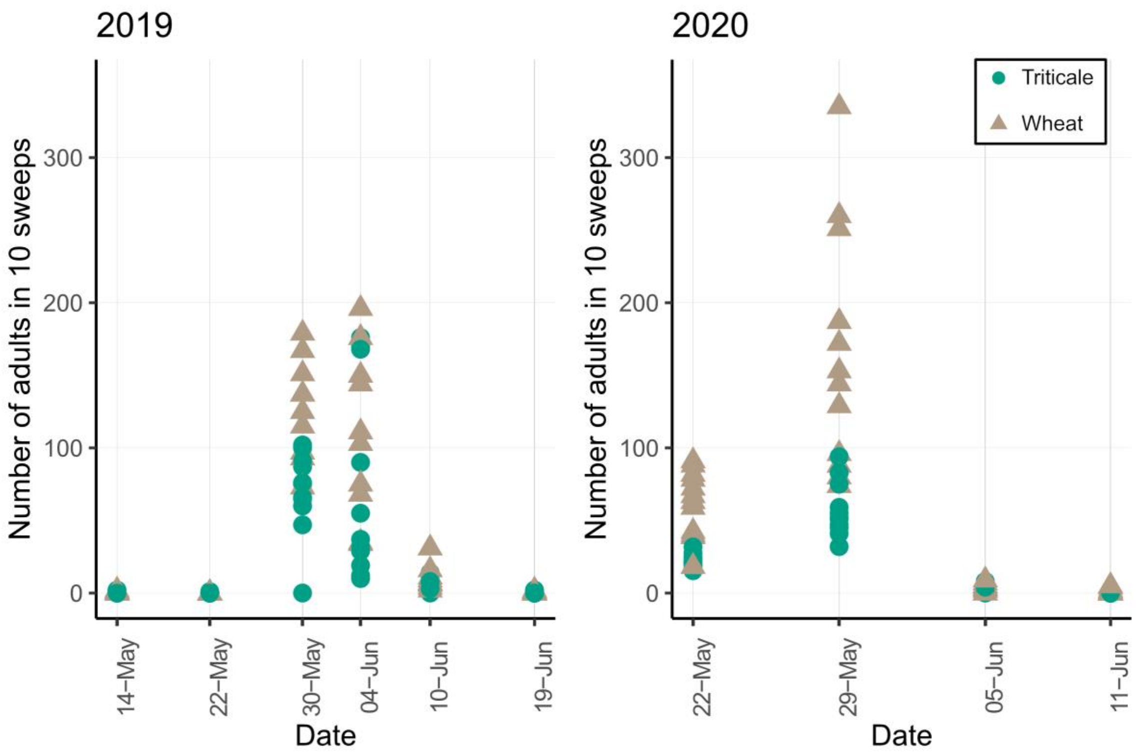

Figure 5.6. Number of adult wheat stem sawfly caught in 10 sweeps in triticale and wheat plots during the 2019 and 2020 trap crop field trial. Points represent adults caught in plots.

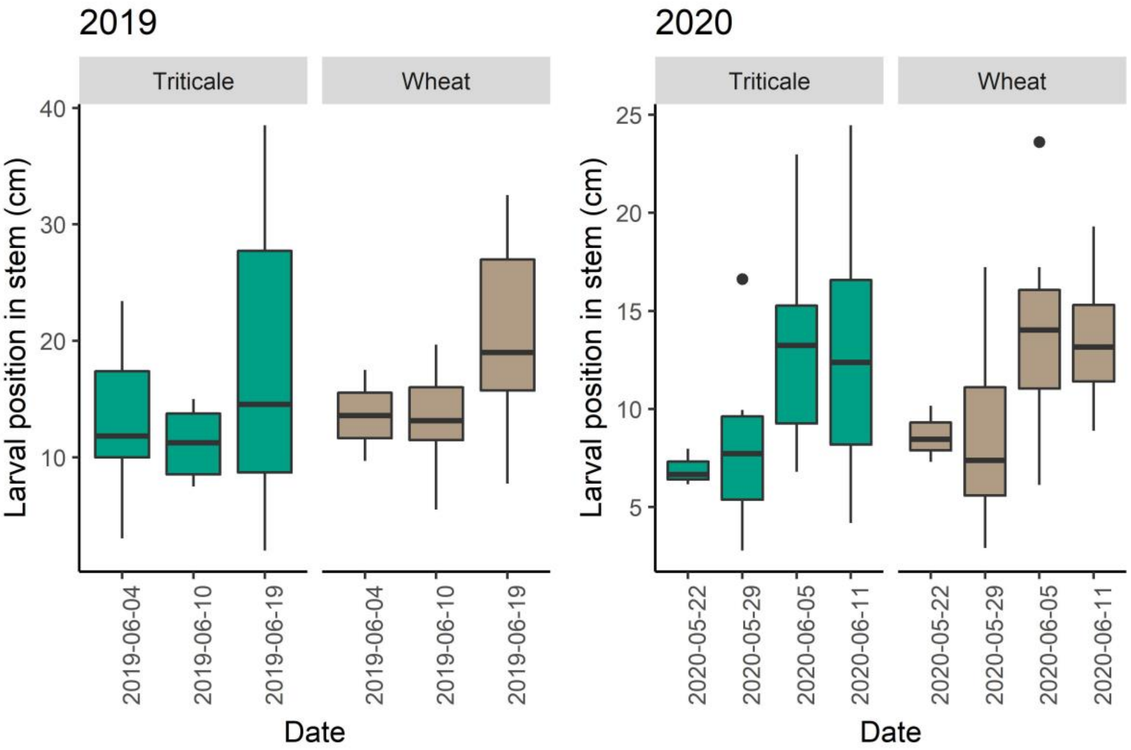

Figure 5.7. Changes in wheat stem sawfly larval position from the roots across dates and type of crop (wheat or triticale). Boxplots showing average larval position in the stem during collection dates in 2019 and 2020.

Stubble infestations in wheat and triticale plots were similar in both years (2019: $F_{1,21} = 0.334$, $P = 0.569$, 2020: $F_{1,22} = 0.744$, $P = 0.397$). In 2019 the presence of pheromone traps did not affect infestation rates ($F_{1,21} = 2.079$, $P = 0.164$). The infestation rates of the wheat adjacent to either crop was similar in both years (2019: $F_{1,21} = 1.403$, $P = 0.249$, 2020: $F_{1,22} = 4.013$, $P = 0.0576$) (Figure 5.8).

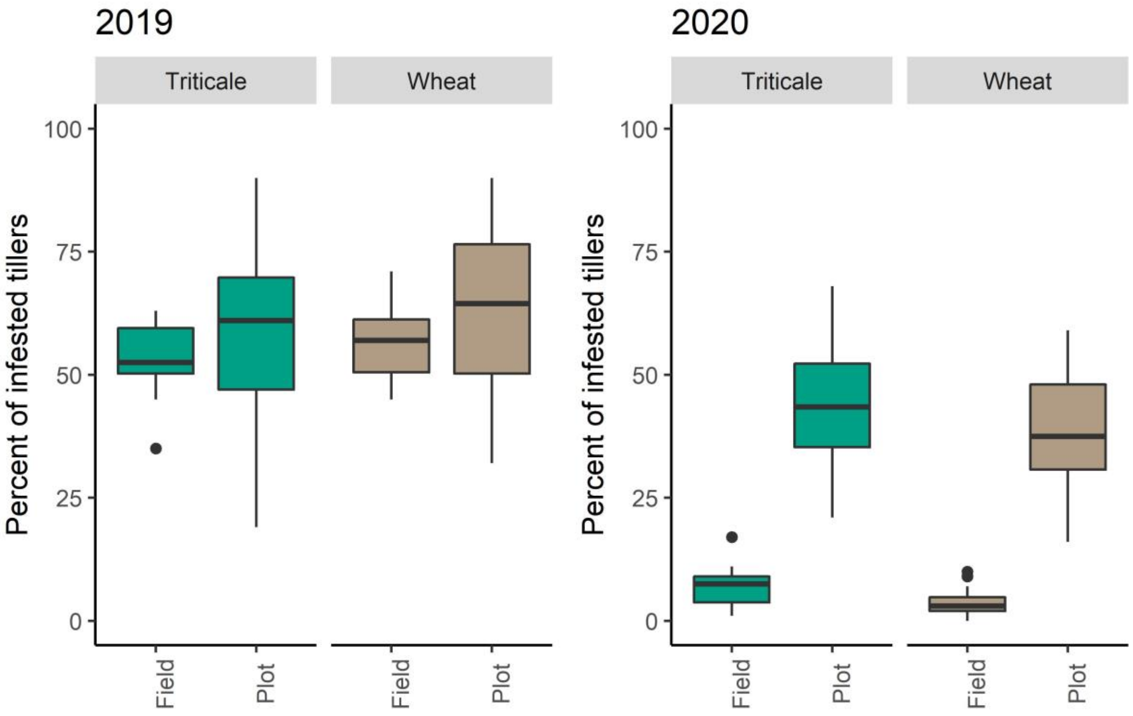

Figure 5.8. Differences in the number of infested stems between trial plots and adjacent wheat for 2019 and 2020.

Discussion

The winter triticale genotypes were as attractive as the winter wheat based on cone-tainer and field studies. Over the two years of this experiment, the field and greenhouse-reared plants were exposed to differing pest abundance. Still, infestation in triticale was consistently similar to that observed in Avery in the cone-tainer study and Snowmass in the field. There were slight differences in wheat stem sawfly attraction when looking at eggs laid to the three triticale genotypes in the cone-tainer study, which suggests using more attractive triticale varieties could improve the effectiveness of trap cropping, particularly when combined with less attractive wheat cultivars. Further exploration into using attractive triticale genotypes paired with less attractive wheat genotypes could potentially find triticale useful in a push-pull system.

Early studies in wheat stem sawfly trap crops found smooth brome, *Bromus inermis,* and fall rye, *Secale cereale,* could be effective for management (Criddle, 1922). Smooth brome was found to mature earlier than spring and winter wheat and have low larval survival and higher parasitism rates. Similarly, fall rye was harvested early in the summer, killing larvae before stubs could be formed. More recent studies suggest using certain winter wheat cultivars can be effective trap crops to spring wheat due to earlier maturities and taller stems (Morrill et al., 2001; Buteler et al., 2009; Weaver et al., 2009; Buteler and Weaver, 2012). For example, the spring wheat variety Reeder was preferred for oviposition over Conan during choice tests which may be due to greater amounts of attractive chemicals being emitted (Weaver et al., 2009). Our study found more adult sawflies in sweeps on wheat plots compared to triticale, which might suggest Snowmass is more attractive to sawflies than NT 05421 in the field. The addition of the sawfly pheromone did not have any effect on the number of adults captured. Attractiveness may have differed, but infestations of stubble collected in plots and adjacent wheat did not differ between wheat and triticale. Previous work examining winter wheat trap strip widths of 3, 6, 10 m and 0.6 km in length found no relation between wheat stem sawfly infestation and width of trap strip (Morrill et al., 2001).

In the cone-tainer experiment, we found sawfly larvae were much larger when developing in triticale stems when compared to wheat. Using the cone-tainer method, we could directly compare larvae on each planting date as we knew the exact date of infestation. This suggests wheat stem sawfly may spend less time in the larval stage in Avery than those in NT 07403. Slower development time could be helpful when considering the timing of swathing to destroy larval populations, possibly due to larger stem diameters.

Swathing has long been proposed as a method of control for wheat stem sawfly (Holmes and Peterson, 1965; Wallace and McNeal, 1966; Holmes, 1978). However, swathing is more expensive, labor-intensive and should only be used when a crop is heavily infested (Nansen et al., 2005; Beres et al., 2011a). Thus, it is often suggested to swath only field perimeters since the highest infestations occur at field edges (Weaver et al., 2005). We observed the average larval position moving up and down throughout the growing season in the field experiment. Often sawfly larvae are thought to only move towards the base of the stem during development (Ainslie, 1920). The average position in the stem varied between years in both wheat and triticale plots. We also saw much lower infestation rates in the adjacent wheat plots in 2020 than in 2019. In 2020, the experimental field was swathed earlier in the season than in 2019, which may have removed larvae higher up in stems. Timing of swathing has been shown to affect sawfly survival, and the yield trade-off is the loss to wheat stem sawfly or early swathing (Holmes and Peterson, 1965). However, since triticale is often used for forage early swathing may be less deterring for farmers.

Despite promising evidence of effectiveness for trap crops in managing wheat stem sawfly, few farmers have adopted the practice. A recent review on using trap crops for cereal crop management suggests lack of adoption may be due to increased management inputs, cost efficiency, and lack of trap crops that can meet broad-acre cereal crop production (Sharma et al., 2019). Triticale's desirable forage yield and quality could still be helpful for farms also growing forage even when field edges are swathed (Oettler, 2005; Estrada-Campuzano et al., 2012; Randhawa et al., 2015). However, while triticale is a suitable host for the wheat stem sawfly, the combinations of genotypes chosen for this study would not comprise an effective trap crop system for wheat stem sawfly. Genotype selection will play an essential role in creating an effective wheat-triticale push-pull trap cropping system. Based on our results, an effective triticale trap crop used for forage would have a large stem diameter, similar maturity to the main crop at adult sawfly flight, high infestation, and slow larval development reducing cutting. The main crop should be planted with a solid stem genotype or a genotype known to be less attractive than the triticale variety. Future research should examine combinations of triticale and wheat genotypes for their effectiveness as trap crops.

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