DISSERTATION

AN INTEGRATIVE APPROACH TO COMBAT THE WHEAT STEM SAWFLY, *Cephus cinctus* (HYMENOPTERA: CEPHIDAE)

Submitted by

Henrique Victor Vieira

Department of Agricultural Biology

In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Fall 2025

Doctoral Committee:

Advisor: Punya Nachappa

Jeff Bradshaw
Esten Mason
Tatyana Rand

ABSTRACT

AN INTEGRATIVE APPROACH TO COMBAT THE WHETA STEM SAWFLY, *Cephus cinctus* (HYMENOPTERA: CEPHIDAE)

The wheat stem sawfly (WSS) is a native grass-feeding insect and a major threat to wheat production across the Great Plains of North America. For over a century, WSS has caused significant damage to growers – not just by feeding within the stem and reducing yield, but particularly by causing wheat lodging. Economic losses are estimated at $350 million annually, with over $40 million in Colorado alone. Managing WSS has proven challenging and requires integrated tactics from multiple fronts. The goal of my PhD was to address WSS management through a combination of complementary, interdisciplinary approaches.

To sustainably manage a pest that threatens food security, entomologists must first understand its biology, life cycle, and management history. In Chapter 1, I conducted a comprehensive review of WSS, detailing its biological traits and tracing the evolution of control strategies.

Although WSS has long been a key pest, no predictive phenology model existed to guide management decisions. Phenology models are essential in pest management, helping forecast insect emergence and inform control timing. Therefore, in Chapter 2, I developed the first degree-day model (DDM) for WSS using 13 years of field data from Colorado. Wheat fields in northeastern Colorado were sampled annually from 2011 to 2023. Using local temperature data, we estimated the heat units (growing degree days) required for key life events. A generalized additive model (GAM) predicted adult emergence at 148 GDD, peak flight at 224 GDD, and

flight end at 354 GDD, using a base temperature of 10°C and upper threshold of 30°C. We also identified weather variables associated with population shifts. These findings provide a powerful tool to improve WSS monitoring and management.

I also explored resistance in wheat cultivars. While semi-solid stems have been the focus of past breeding efforts, growers reported potential resistance in a hollow-stem, herbicide-resistant cultivar – Clearfield Plus (CL Plus). I evaluated three cultivars: one resistant, one susceptible, and CL Plus. Across lab and field trials over three years, I measured oviposition, infestation, and cutting rates. Results showed that CL Plus cultivars exhibited resistance traits. To investigate further, I analyzed volatile organic compound (VOC) profiles using thermal desorption-gas chromatography/mass spectrometry (TD-GC/MS). One CL Plus cultivar had a VOC profile similar to the resistant line, suggesting possible chemical cues underlying resistance. This work opens avenues to explore the role of plant volatiles in WSS oviposition behavior and plant defense.

Chapter 3 addressed the effect of crop rotation. As rotations have become more diverse in northeastern Colorado, I assessed whether they influenced WSS infestation and larval survival. Post-harvest wheat stems were collected over two years from fields with different rotation histories, ranging from wheat–fallow to wheat–corn–millet–fallow. Surprisingly, crop rotation alone did not appear to reduce WSS pressure. However, fields with resistant cultivars showed lower infestation, reinforcing the role of host plant resistance as the most effective strategy—regardless of crop sequence.

Chapter 4 focused on biological control, specifically the role of braconid parasitoids. These wasps have historically played a key role in WSS suppression in the northern Great Plains, but their impact in Colorado has been minimal. I conducted cage experiments comparing parasitism rates among *Bracon spp*. populations from Colorado, Nebraska, and North Dakota.

All populations showed similar parasitism potential, suggesting no mismatch between Colorado *Bracon spp.* and local WSS.

To explore the low parasitism levels further, I performed a population genetics study. I extracted DNA from both WSS and their parasitoids to assess whether population structure or host-associated differentiation (HAD) could explain the pattern. Analyses of genetic diversity, phylogenetic trees, and haplotype networks revealed that one key parasitoid, *Bracon cephi*, may be undergoing population subdivision, possibly due to the rapid expansion of its host. In contrast, WSS populations in Colorado continue to expand, showing high haplotype diversity. I also investigated *Bracon lissogaster* populations from both wild grasses and wheat. Although higher genetic diversity was observed in wild grasses, there was no strong evidence of HAD. However, sample size needs to be increased substantially for a solid HAD analysis.

These chapters reflect a multi-faceted effort to understand and manage WSS through phenology modeling, cultivar resistance, crop rotation, and biological control. This work lays the groundwork for more precise, integrated, and regionally tailored pest management strategies.

ACKNOWLEDGMENTS

First and foremost, I want to thank my mentor and friend, Dr. Punya Nachappa. I remember emailing her to ask about joining her lab as a PhD student and sharing my hope to grow as a scientist – especially in writing and communicating research. From the beginning, she kept that goal in mind and supported me every step of the way. Under her guidance, I learned not only new scientific techniques, but also new ways of thinking and approaching research.

Beyond her research excellence, one of Punya's greatest strengths, often underrated, is her ability to build a strong, collaborative team. For that, I'm deeply grateful to the amazing group we've had here.

I'm thankful to my committee members – Esten Mason, Jeff Bradshaw, and Tatyana Rand – for their invaluable insights, thoughtful feedback, and ongoing support. I truly appreciate how attentive and responsive they've been, always taking the time to listen and help me work through challenges. I also want to thank the professors and staff in the Agricultural Biology department. It's been a privilege to learn from and work with them.

To my lab colleagues and collaborators: thank you. A special thanks to Adam Osterholzer, who helped me immensely with planting, insect infestations, and collections over the past four years. Felipe, that brought tremendous value to our parasitoid research by advancing it to the molecular level. MJ strengthened our host suitability and preference studies with her chemistry expertise, particularly in chemical analysis using the TD-GC/MS technique. Ben Bradford contributed his modeling skills – especially with GAMMs – which took our degree-day work to the next level. I'm truly grateful to have worked alongside such talented and generous scientists.

To all the lab assistants who supported the Nachappa Lab over the years—thank you. Whether it was planting, collecting data, or helping with experiments, your work was essential to this research.

I'm also thankful to the Colorado Wheat Research Foundation for funding our work and believing in its impact. That support, along with the dedication of wheat growers, motivated me to give my full effort to every part of this project. The Foundation plays a critical role—not just for Colorado State University and local farmers, but for society at large—by advancing food security and sustainable wheat production.

I also want to acknowledge the many mentors I've been fortunate to learn from throughout my education. Each one of them contributed in some way to my growth, and I carry their guidance with me as I continue this journey in research and in life.

To the friends I've made along the way, both inside and outside of work, thank you for your support and companionship over these four years.

I am especially grateful to my partner, Audrey Clavijo. She has been my biggest supporter and the person I could count on every day during this challenging and rewarding journey. Without her love, this experience would not have brought me the same joy and balance.

Finally, I'm eternally grateful to my family. My parents, Marcos Antonio Vieira and Silvia Helena Victor Vieira, did everything they could to give me the opportunity to pursue this dream. My brothers, Danilo Victor Vieira and Marcos Victor Vieira, have always been my closest friends and strongest supporters. My family is the foundation that made this achievement possible. We have been sharing this life together daily, after all.

DEDICATION

Dedicated to my family: Silvia, Marcos, Danilo and Marquinhos. Thank you for your love and endless support, always.

TABLE OF CONTENTS

ABSTRACT ii
ACKNOWLEDGMENTS v
DEDICATION vii
CHAPTER 1: WHEAT STEM SAWFLY: AGRICULTURAL CONTEXT, BIOLOGY, AND MANAGEMENT FRAMEWORK 1
Introduction 1
The life cycle of the WSS 3
Overwinter ability 5
Wheat stem sawfly damage and management 6
Host plant resistance 7
Biological control 8
Cultural control 9
Chemical control 10
Long-term goals and objectives 10
References 12
CHAPTER 2: A NEW GROWING DEGREE-DAY PHENOLOGY MODEL FOR WHEAT STEM SAWFLY (HYMENOPTERA: CEPHIDAE) IN COLORADO WHEAT FIELDS 23
Introduction 23
Methods 25
Data collection 25
Statistical analysis 27
Results 29
Observed WSS phenology 29
WSS degree-day models 32
Environmental factors affecting WSS population size 35
Discussion 36
References 41
CHAPTER 3: HOST SELECTION AND SUITABILITY OF HERBICIDE-TOLERANT CLEARFIELD® WHEAT CULTIVARS TO WHEAT STEM SAWFLY (*Cephus cinctus*) 49
Introduction 49
Methods 53
Greenhouse study 55
Field study 55
Herbicide spray study 56
Volatile analysis 57
Statistical Analysis 58
Results 59
Greenhouse study 59
Field study 60
Herbicide spray study 62
Stem Cutting 65
Volatile analysis 66
Discussion 69
Greenhouse and field analysis 69

Volatile analysis 72
Conclusion 76
References 78
CHAPTER 4: EVALUATING CROP ROTATION EFFECTS ON WHEAT STEM SAWFLY INFESTATION, DAMAGE AND LARVAL SURVIVAL 90
Introduction 90
Methods 92
Field sites 92
Rotations 92
Samples 93
Statistical methods 96
Results 97
Effect of crop rotations on WSS infestation, larval survival and stem cutting 97
Effect of cultivar within same rotation on WSS infestation, larval survival and stem cutting 100
Discussion 102
Conclusion 106
Acknowledgements 106
References 108
CHAPTER 5: ASSESSMENT OF PHYLOGEOGRAPHY AND HOST-ASSOCIATE DIFFERENTIATION IN *Bracon spp.* ACROSS THE GREAT PLAINS 116
Introduction 116
Methods 119
Greenhouse experiment 119
Molecular analysis 122
Results 124
Greenhouse experiment 124
Molecular analysis 126
Sequence variation 128
Haplotype network analysis 130
Population structure 131
Discussion 132
Recent WSS expansion in Colorado 133
Phylogenetic and haplotype patterns in *Bracon spp* 133
Biological contrasts between *B. cephi* and *B. lissogaster* 135
Implications for integrated pest management 136
Future research direction 137
References 139
APPENDIX 148
A1 Chapter 3 supplemental information 148

CHAPTER 1: WHEAT STEM SAWFLY: AGRICULTURAL CONTEXT, BIOLOGY, AND MANAGEMENT FRAMEWORK

Introduction

Wheat, *Triticum aestivum* (Poaceae), has been a staple and one of the most important sources of nutrition for over 10,000 years. It is the most widely cultivated plant worldwide, providing approximately 20% of the daily protein intake for the average person globally (Reynolds and Braun 2022, Kheiralipour et al. 2024). Grown across all five continents (Reynolds and Braun 2022), wheat stands as the most important food grain in the United States. To underscore its significance, in 2025 the U.S. had 45,478,000 acres planted and 36,636,000 acres harvested, generating a production value of $10.93 billion in 2024 (United States Department of Agriculture, National Agricultural Statistics Service 2025a). Within the US, the Great Plains region – composed by Colorado, Kansas, Nebraska, Oklahoma, Texas, and Wyoming – are responsible for over 60% of the wheat production in the country (Redmon et al. 1995, Paulsen and Shroyer 2008, Armenta et al. 2014). In Colorado, winter wheat is predominant over spring wheat (Paulsen and Shroyer 2008), positioning the state as the 10th largest wheat producer in the nation in 2024 (Colorado Wheat 2024). In 2024, Colorado reported over 2,000,000 planted acres and a production value of $331.66 million (United States Department of Agriculture, National Agricultural Statistics Service 2025b). Total wheat output reached 64 million bushels, valued at roughly $352 million (Colorado Wheat 2024). Due to the climate challenges of dryland systems, wheat producers in Colorado grow crops in fallow system to have better efficiency in capturing water, and commonly rotate due to multiple benefits that comes from diversification (Armenta et al. 2014).

There are over 30 known insect and mite pests affecting wheat in the United States (Hatchett et al. 1987, Armenta et al. 2014). Among these, the wheat stem sawfly - *Cephus cinctus* (WSS, Hymenoptera: Cephidae) – stands out as one of the most damaging, costing wheat growers in North America an estimated $350 million annually in losses (Beres et al. 2011). In addition, including $41 million in damage in 2022 to Colorado winter wheat production (Wasserman-Olin et al. 2024). Wheat stem sawfly populations are expected to expand and increase damage to wheat (Olfert et al. 2019, Cockrell et al. 2021) (**Figure 1.1**). Originally described in Colorado by Norton (Norton 1872), the first major outbreak was recorded in western Canada by Criddle (Criddle 1923). The pest's success in wheat systems has been linked to the widespread adoption of rust-resistant wheat cultivars, which remained healthy and thus became highly suitable hosts for WSS (Mcginnis 1950). While spring wheat was historically the primary host, winter wheat began experiencing significant infestations around the mid-1980s (Morrill and Kushnak 1996). Prior to that, the biology of the WSS was not well synchronized with the phenology of winter wheat (Lou et al. 1998).

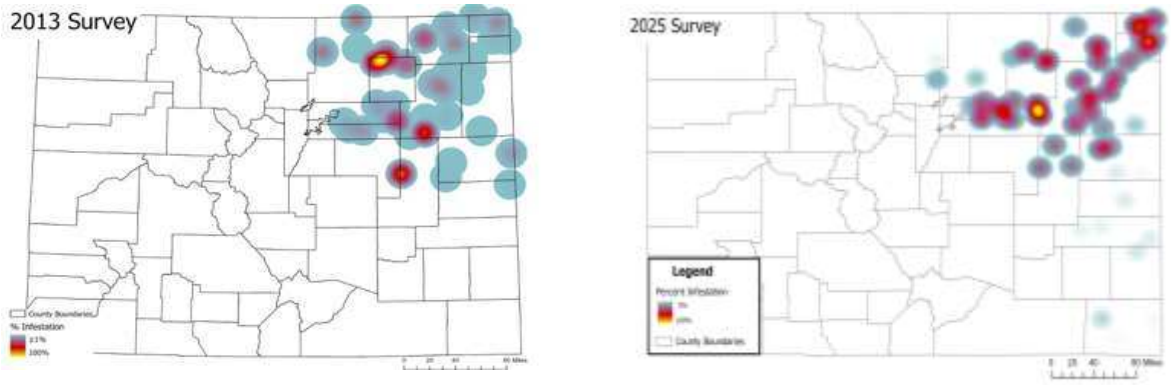

Figure 1.1: Image illustrating the intensification and spread of WSS populations over a twelve-year period in eastern Colorado.

The life cycle of the WSS

The life cycle of the WSS is heavily influenced by temperature (Perez-Mendoza and Weaver 2006, Vieira et al. 2025); as a result, latitude also plays a key role in regulating its phenology. Adults are approximately 12 mm long and are characterized by shiny black coloration, yellow abdominal bands, club-shaped antennae (with about 20 segments), prominent compound eyes, and a laterally compressed abdomen (**Figure 1.2**) (Fletcher 1896), and live for 5-8 days (Wallace and McNeal 1966).

Figure 1.2: Adult insect of the wheat stem sawfly. Photo credit: *Kathleen Hanson*

Reproduction occurs via arrhenotokous parthenogenesis, a form in which fertilized eggs develop into diploid females, while unfertilized eggs develop into haploid males (Mackay 1955). Eggs deposited earlier in the flight season are more likely to be fertilized and produce females, whereas eggs laid later (when males are less abundant) are more often unfertilized and result in

male offspring (Holmes 1979). Females tend to prefer oviposition sites with certain plant traits – thicker stem and taller plants (Weiss and Morrill 1992), but in the absence of abundant hosts, they may become less selective (Holmes and Peterson 1960). Using their saw-like ovipositor, females insert eggs into the elongating internodes of wheat stems, typically between the second and fourth internodes (**Figure 1.3**). They generally select hollow stem regions for deposition (Seamans 1945). A single female can carry an up to 50 eggs (Ainslie 1920). Eggs are crescent-shaped, milky white or translucent, and measure between 1.00-1.25 mm, with size depending on the female that laid them (Ainslie 1920). Around the sixth or seventh day post oviposition, the larva emerges from the egg sac and begins feeding inside the stem (Ainslie 1920). Adult sawflies typically live about one week (Wallace and McNeal 1966).

Figure 1.3: Wheat stem sawfly ovipositing on wheat stem.

Newly hatched larvae are transparent and colorless (**Figure 1.4A**) (Criddle 1923) but quickly change to a yellowish-brown after they begin feeding. Typically, the first larva to hatch gains a competitive advantage and is often the only one to survive (Criddle 1923), although it is still uncertain whether this is due to active cannibalism or simply a lack of discrimination during feeding (Holmes 1982). Notably, females are unable to distinguish between infested and uninfested stems when laying eggs (Buteler and Weaver 2012); therefore competition by cannibalistic activity is often present in WSS infested areas. The larval stage progresses through five instars, and sex can be determined during the final instar (Holmes 1979). The final instar enters an obligatory diapause, which requires approximately 90 days at 10°C to complete. To survive, larvae need an environment with about 12–15% moisture, though they can tolerate desiccation until they lose 40% of their body weight (Holmes 1979). If exposed to continuous light or temperatures of 35°C, the larva may re-enter diapause, restarting the requirement of 90 days at 10°C to resume development (Holmes 1979, 1982, Beres et al. 2011).

Overwinter ability

WSS larvae overwinter as fully developed fifth instars, enclosed within a hibernaculum. Their supercooling point ranges from –20°C to –28°C, allowing survival at subzero temperatures (Holmes 1979). However, because overwintering larvae remain close to the host plant's root crown, they benefit from thermal insulation – soil temperatures near the crown are typically 9-28°C warmer than ambient winter air temperatures and more stable than those measured just 15 cm above the surface (Morrill et al. 1993). Additionally, disturbances to wheat stubs (**Figure 1.4B**) and cocoons by tillage lead to higher mortality rates by disrupting the protective overwintering habitat (Morrill et al. 1993, Beres et al. 2011). Diapause completion requires

approximately 90 days at 10°C, typically ending by mid to late spring (Holmes 1979). The prepupal stage begins in early to mid-May, followed by pupation, which generally occurs within 21 days (Criddle 1923, Holmes 1979).

Figure 1.4: (**A**) Larvae of wheat stem sawfly feeding within wheat stem. (**B**) Wheat stubs where diapausing larvae overwinters.

Wheat stem sawfly damage and management

Plant injury is primarily caused by the larval stage of the WSS; adult sawflies inflict minimal damage to host plants (Beres et al. 2011). Larval feeding begins within the stem, and the first visible signs of herbivory are typically found in the subnodal region just below one or more nodes (Platt and Farstad 1946, Morrill et al. 1992). This area often becomes discolored or spotted as a result of boring activity. Damage from WSS larvae can reduce photosynthetic capacity (Weiss and Morrill 1992, Macedo et al. 2007), lead to reduced grain weight (Holmes 1977, Morrill et al. 1994), and cause significant yield losses by weakening the stems. Severely infested plants may lodge (Figure 1.5) before harvest (Ainslie 1920), creating serious challenges during mechanical harvesting (Holmes 1977, Morrill and Kushnak 1999, Nansen et al. 2005).

Effectively combating the aggressive damage caused by the WSS requires truly integrated management strategies that target the insect from multiple angles.

Figure 1.5: Lodged wheat due to wheat stem sawfly cutting.

Host plant resistance

A commonly used strategy to manage WSS in wheat is to improve plant defenses (Beres et al. 2011, Peirce et al. 2024). Stem solidness is one of the most effective resistance traits against WSS, significantly reducing both larval survival and stem cutting (Kemp 1934, Luginbill Jr. and McNeal 1958, Weiss et al. 1990, Varella et al. 2015). The primary source of this trait in

common wheat traces to the Portuguese landrace 'S-615' and is controlled by a major quantitative trait locus (QTL), Qss.msub-3BL, located on chromosome 3B (Cook et al. 2004). Additional resistance loci have since been identified, including those on chromosomes 2A, 3A, and 5B (associated with larval mortality), 2D (linked to reduced oviposition), and 4A (related to host attractiveness) (Varella et al. 2015, 2017). This genetic foundation enabled the development of semi-solid stem wheat, which offers a balance between WSS resistance and maintaining good grain yield (Bathini et al. 2023, Peirce et al. 2024). Numerous studies have demonstrated that solid-stemmed cultivars provide significantly better protection against WSS compared to hollow-stemmed varieties (Wallace et al. 1973, Beres et al. 2009, 2013, Cárcamo et al. 2016). This is currently the most effective and widely used defense against WSS attack by wheat farmers

Biological control

Nine species of Hymenoptera are known to parasitize the WSS (Morrill et al. 1998, Meers 2005). Among these, two species with similar life histories as sympatric idiobiont ectoparasitoids, in another words, they attack WSS larvae by stopping their development immediately and their offspring feed on the outside of the WSS larva (Runyon et al. 2001, 2002) have been identified as the most effective biological control agents: *Bracon cephi* (Hymenoptera: Braconidae), described by Gahan (Gahan 1918) and observed parasitizing WSS by Ainslie (Ainslie 1920) and Criddle (Criddle 1923); and *B. lissogaster*, described by Muesebeck (Muesebeck 1953) and reported as a parasitoid by Somsen and Luginbill (Somsen and Luginbill 1956). Those parasitoids will locate the closest approximation of their WSS larvae host, insert the ovipositor into the wheat stem, paralyzing the WSS larva and depositing their egg (Nelson and Farstad 1953, Holmes et al. 1963). The small larvae of *Bracon spp*. use their highly developed mandibles to feed on the larger WSS larvae host. Once feeding is complete, the

mature *Bracon spp*. larvae creates a cocoon where it will overwinter, and the adult will emerge the following spring (Nelson and Farstad 1953, Somsen and Luginbill 1956). After years of study, it is known that WSS population suppression via biological control is effective and holds high potential (Runyon 2001, Buteler et al. 2008, 2015, Sjolie et al. 2024). Amongst climate and natural field environment occurrences, the challenge is to provide ideal conditions for the parasitoid population's spread and establishment (Rand et al. 2019, Rand and Waters 2020, Peirce et al. 2021, Cavallini et al. 2022, 2023, Bradshaw et al. 2025).

Cultural control

Due to the biology of the WSS – larvae remain hidden inside the stem for most of their life cycle – early management efforts centered on cultural control practices such as burning (Fletcher 1904), tillage, and ploughing (Criddle 1922). However, these approaches proved ineffective (Ainslie 1920) and often resulted in serious negative consequences, including soil erosion. Other cultural tactics, such as trap crops, crop rotation, and row spacing adjustments have shown mixed results. For instance, decreasing row spacing and increasing plant density have been associated with reduced stem cutting and infestation (Luginbill Jr. and McNeal 1958). Perez-Mendoza et al. (Perez-Mendoza et al. 2006) reported that brome grass is highly attractive to WSS and that its presence in wheat fields can significantly alter population dynamics. Although some cultural practices may help combat WSS, they can also create or exacerbate other farming challenges, highlighting the complexity of implementing certain management tactics. Tillage, in particular, remains a subject of debate. Its effectiveness likely depends on both infestation level and timing, whether conducted in the fall or spring, for instance Goosey et al. (Goosey 1999) found no difference in sawfly mortality between intensely tilled and untilled fields. Meanwhile, strategies such as planting solid-stemmed border rows or adjusting row

spacing and plant density (Solie et al. 1991) have shown limited impact on damage reduction. Moreover, harvesting and tillage practices can influence not only water retention but also the survival and establishment of parasitoids (Weaver and Morrill 2001). Despite their limitations, cultural practices, as simple as they may seem, remain a key pillar of integrated WSS management.

Chemical control

Historically, chemical control has been a method that growers heavily rely on to manage crop pests. However, in the case of WSS, this approach has stagnated, showing little promise for advancement due to consistently poor performance and high costs. Additionally, its use raises sustainability concerns, as broad-spectrum insecticides can severely impact beneficial insects, including *Bracon spp*. Heptachlor is the only insecticide that has demonstrated satisfactory control of WSS (Wallace 1962), but its effectiveness depends on the larvae's position within the stem (Holmes and Peterson 1963) and the application of a precise dosage that avoids leaving residues in the grain (Wallace and Butler 1967). However, Heptachlor has been banned in the United States since 1988 due to its environmental persistence, high toxicity, and risks to human health (B. Beres et al. 2011). The use of chemical control is neither recommended nor encouraged for managing WSS, for several reasons. However, because it remains a component of integrated pest management (IPM), it is important to address it and provide accurate information and awareness regarding its use.

Long-term goals and objectives

The wheat stem sawfly does not have a "silver bullet" solution; therefore, this dissertation explores multiple approaches that can strengthen integrated pest management (IPM) strategies

for this insect-pest. First, I present a new forecasting tool based on degree-day models developed from a robust thirteen-year dataset. I then investigate winter wheat cultivars with potential plant defense mechanisms involving volatile organic compounds (VOCs) related to host selection, as well as the suitability of herbicide-resistant Clearfield® wheat cultivars to wheat stem sawfly. In addition, I examine the performance and genetic diversity of the parasitoid *Bracon cephi* collected from different regions across the Great Plains. Finally, I assess the potential effects of various crop rotation systems on wheat stem sawfly infestation and damage.

References

Armenta R, Leikam D, Burgener P, et al. 2014. Wheat production and pest management for the Great Plains region. Fort Collins, CO: Colorado State University Extension.

Bathini A, Mendu L, Singh NP, et al. 2023. A “solid” solution for wheat stem sawfly (Hymenoptera: Cephidae) resistance: genetics, breeding and development of solid stem wheat. Crop Science. 63(4):1934–1948. https://doi.org/10.1002/csc2.20998

Beres B, Dosdall L, Weaver D, et al. 2011. Biology and integrated management of wheat stem sawfly and the need for continuing research. The Canadian Entomologist. 143(2):105–125. https://doi.org/10.4039/n10-056

Beres BL, Cárcamo HA, Bremer E. 2009. Evaluation of alternative planting strategies to reduce wheat stem sawfly (Hymenoptera: Cephidae) damage to spring wheat in the northern Great Plains. Journal of Economic Entomology. 102(6):2137–2145. https://doi.org/10.1603/029.102.0617

Beres BL, Cárcamo HA, Byers JR, et al. 2013. Host plant interactions between wheat germplasm source and wheat stem sawfly *Cephus cinctus* Norton (Hymenoptera: Cephidae) I. Commercial cultivars. Canadian Journal of Plant Science. 93(4):607–617. https://doi.org/10.4141/CJPS2012-088

Beres BL, Cárcamo HA, Dosdall LM, et al. 2011. Do interactions between residue management and direct seeding system affect wheat stem sawfly and grain yield? Agronomy Journal. 103(6):1635–1644. https://doi.org/10.2134/agronj2011.0055

Bradshaw JD, Rand TA, Peirce ES, et al. 2025. ‘Beneficial Bug Baler’: A novel technique for mass relocation of *Bracon* in support of *Cephus cinctus* biocontrol in wheat, *Triticum aestivum*, 2024. Crow W, editor. Arthropod Management Tests. 50(1). https://doi.org/10.1093/amt/tsaf072

Buteler M, Peterson RKD, Hofland ML, et al. 2015. A multiple decrement life table reveals that host plant resistance and parasitism are major causes of mortality for the wheat stem sawfly. Environmental Entomology. 44(6):1571–1580. https://doi.org/10.1093/ee/nvv128

Buteler M, Weaver DK. 2012. Host selection by the wheat stem sawfly in winter wheat and the role of semiochemicals mediating oviposition preference. Entomologia Experimentalis et Applicata. 143(2):138–147. https://doi.org/10.1111/j.1570-7458.2012.01237.x

Buteler M, Weaver DK, Miller PR. 2008. Wheat stem sawfly-infested plants benefit from parasitism of the herbivorous larvae. Agricultural and Forest Entomology. 10(4):347–354. https://doi.org/10.1111/j.1461-9563.2008.00396.x

Cárcamo HA, Beres BL, Larson TR, et al. 2016. Effect of wheat cultivars and blends on the oviposition and larval mortality of *Cephus cinctus* (Hymenoptera: Cephidae) and parasitism by *Bracon cephi* (Hymenoptera: Braconidae). Environmental Entomology. 45(2):397–403. https://doi.org/10.1093/ee/nvv231

Cavallini L, Peterson RKD, Weaver DK. 2022. Dietary sugars and amino acids increase longevity and enhance reproductive parameters of *Bracon cephi* and *B. lissogaster*, two parasitoids that specialise on wheat stem sawfly. Physiological Entomology. https://doi.org/10.1111/phen.12399

Cavallini L, Peterson RKD, Weaver DK. 2023. Cowpea extrafloral nectar has potential to provide ecosystem services lost in agricultural intensification and support native parasitoids that

suppress the wheat stem sawfly. Dara S, editor. Journal of Economic Entomology. https://doi.org/10.1093/jee/toad083

Cockrell DM, Randolph T, Peirce E, et al. 2021. Survey of wheat stem sawfly (Hymenoptera: Cephidae) infesting wheat in eastern Colorado. Journal of Economic Entomology. 114(2):998–1004. https://doi.org/10.1093/jee/toab015

Colorado Wheat. 2024. Wheat production stats. https://coloradowheat.org/.

Cook JP, Wichman DM, Martin JM, et al. 2004. Identification of microsatellite markers associated with a stem solidness locus in wheat. Crop Science. 44(4):1397–1402. https://doi.org/10.2135/cropsci2004.1397

Criddle N. 1922. The western wheat stem sawfly in Canada. The Report of the Entomological Society of Ontario. 36:18–21.

Criddle N. 1923. Popular and practical entomology: the life habits of *Cephus cinctus* Nort. in Manitoba. The Canadian Entomologist. 55(1). https://doi.org/10.4039/Ent551-1

Fletcher J. 1896. The western wheat stem sawfly (*Cephus pygmaeus L.*). Dominion of Canada, Department of Agriculture, Report of the Dominion Entomologist.:147–149.

Fletcher J. 1904. Experimental farms reports: Report of the entomologist and botanist. Appendix to the Report of the Minister of Agriculture, Sessional Paper. 16:172–173.

Gahan A. 1918. Description of a new Hymenopterous parasite (Braconidae). Proceedings of the Entomological Society. 20(1):18–19.

Goosey HB. 1999. In-field distributions of the wheat stem sawfly (Hymenoptera: Cephidae), and evaluation of selected tactics for an integrated management program [Master of Science thesis]. Montana State University.

Hatchett JH, Starks KJ, Webster JA. 1987. Insect and mite pests of wheat. In: Heyne EG, editor. Agronomy Monographs. Madison, WI, USA: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America. p. 625–675. https://doi.org/10.2134/agronmonogr13.2ed.c34

Holmes N. 1979. The wheat stem sawfly. 26th Annual Meetings of the Entomological Society of Alberta and Entomological Society of Saskatchewan. 26.

Holmes N. 1982. Population dynamics of the wheat stem sawfly, *Cephus cinctus* (Hymenoptera:Cephidae), in wheat. The Canadian Entomologist. 114(9):775–788.

Holmes N, Nelson W, Peterson L, et al. 1963. Causes of variations in effectiveness of *Bracon cephi* (Gahan) (Hymenoptera: Braconidae) as a parasite of the wheat stem sawfly. The Canadian Entomologist. 95(2):113–126.

Holmes ND. 1977. The effect of the wheat stem sawfly, *Cephus cinctus* (Hymenoptera: Cephidae), on the yield and quality of wheat. The Canadian Entomologist. 109(12):1591–1598. https://doi.org/10.4039/Ent1091591-12

Holmes ND, Peterson LK. 1960. The influence of the host on oviposition by the wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae). Canadian Journal of Plant Science. 40(1):29–46. https://doi.org/10.4141/cjps60-004

Holmes ND, Peterson LK. 1963. Heptachlor as a systemic insecticide against the wheat stem sawfly, *Cephus cinctus* Nort. The Canadian Entomologist. 95(8):792–796. https://doi.org/10.4039/Ent95792-8

Kemp HJ. 1934. Studies of solid stem wheat varieties in relation to wheat stem sawfly control. Scientific Agriculture. 15(1):30–38. https://doi.org/10.4141/sa-1934-0076

Kheiralipour K, Brandão M, Holka M, et al. 2024. A review of environmental impacts of wheat production in different agrotechnical systems. Resources. 13(7):93. https://doi.org/10.3390/resources13070093

Lou KF, Weiss MJ, Bruckner PL, et al. 1998. RAPD variation within and among geographic populations of wheat stem sawfly (*Cephus cinctus* Norton). The American Genetic Association. 89:329–335. https://doi.org/10.1093/jhered/89.4.329

Luginbill Jr. P, McNeal FH. 1958. Influence of Seeding Density and Row Spacings on the Resistance of Spring Wheats to the Wheat Stem Sawfly1. Journal of Economic Entomology. 51(6):804–808. https://doi.org/10.1093/jee/51.6.804

Macedo TB, Weaver DK, Peterson RKD. 2007. Photosynthesis in wheat at the grain filling stage is altered by larval wheat stem sawfly (Hymenoptera: Cephidae) injury and reduced water availability. Journal of Entomological Science. 42(2):228–238. https://doi.org/10.18474/0749-8004-42.2.228

Mackay MR. 1955. Citology and parthenogenesis of the wheat stem sawfly, *Cephus cinctus* Nort. (Hymenoptera: Cephidae). Canadian Journal of Zoology. 33(3):161–174. https://doi.org/10.1139/z55-011

Mcginnis AJ. 1950. Sex ratio studies on the wheat stem sawfly, *Cephus cinctus* Nort. Montana: Montana State University.

Meers SB. 2005. Impact of harvest operations on parasitism of the wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae). Montana State University. https://scholarworks.montana.edu/xmlui/handle/1/1853.

Morrill WL, Gabor JW, Hockett EA, et al. 1992. Wheat stem sawfly (Hymenoptera: Cephidae) resistance in winter wheat. Journal of Economic Entomology. 85(5):2008–2011. https://doi.org/10.1093/jee/85.5.2008

Morrill WL, Gabor JW, Wichman D. 1993. Mortality of the wheat stem sawfly (Hymenoptera: Cephidae) at low temperatures. Environmental Entomology. 22(6):1358–1361.

Morrill WL, Kushnak GD. 1996. Wheat stem sawfly (Hymenoptera: Cephidae) adaptation to winter wheat. Environmental Entomology. 25(5):1128–1132.

Morrill WL, Kushnak GD. 1999. Planting date influence on the wheat stem sawfly (Hymenoptera: Cephidae) in spring wheat. Journal of Agriculture and Urban Entomology. 16(2):123–128.

Morrill WL, Kushnak GD, Bruckner PL. 1994. Wheat stem sawfly (Hymenoptera: Cephidae) damage, rates of parasitism, and overwinter survival in resistant wheat lines. Journal of Economic Entomology. 87:1373–1376.

Morrill WL, Kushnak GD, Gabor JW. 1998. Parasitism of the wheat stem sawfly (Hymenoptera: Cephidae) in Montana. Biological Control. 12(3):159–163. https://doi.org/10.1006/bcon.1998.0629

Muesebeck CFW. 1953. Three new reared Braconidae (Hymenoptera). Proceedings of the Entomological Society of Washington. 55(3):149–151.

Nansen C, Payton ME, Runyon JB, et al. 2005. Preharvest sampling plan for larvae of the wheat stem sawfly, *Cephus cinctus* (Hymenoptera: Cephidae), in winter wheat fields. The Canadian Entomologist. 137(5):602–614. https://doi.org/10.4039/n04-087

Nelson WA, Farstad CW. 1953. Biology of *Bracon cephi* (Gahan) (Hymenoptera: Braconidae), an important native parasite of the wheat stem sawfly, *Cephus cinctus* Nort. (Hymenoptera: Cephidae), in western Canada. The Canadian Entomologist. 85(3):103–107. https://doi.org/10.4039/Ent85103-3

Norton E. 1872. Notes on North American Tenthredinidæ, with descriptions of new species. Transactions of the American Entomological Society (1867-1877). 4:77. https://doi.org/10.2307/25076265

Olfert O, Weiss RM, Catton H, et al. 2019. Bioclimatic assessment of abiotic factors affecting relative abundance and distribution of wheat stem sawfly (Hymenoptera: Cephidae) in western Canada. Canadian Entomologist. 151(1):16–33. https://doi.org/10.4039/tce.2018.46

Paulsen GM, Shroyer JP. 2008. The early history of wheat improvement in the Great Plains. Agronomy Journal. 100(S3). https://doi.org/10.2134/agronj2006.0355c

Peirce ES, Evers B, Winn ZJ, et al. 2024. Identifying novel sources of resistance to wheat stem sawfly in five wild wheat species. Pest Management Science. https://doi.org/10.1002/ps.8008

Peirce ES, Rand TA, Cockrell DM, et al. 2021. Effects of landscape composition on wheat stem sawfly (Hymenoptera: Cephidae) and its associated braconid parasitoids. Journal of Economic Entomology. 114(1):72–81. https://doi.org/10.1093/jee/toaa287

Perez-Mendoza J, Weaver DK. 2006. Temperature and relative humidity effects on postdiapause larval development and adult emergence in three populations of wheat stem sawfly (Hymenoptera: Cephidae). Environmental Entomology. 35(5):1222–1231. https://doi.org/10.1093/ee/35.5.1222

Perez-Mendoza J, Weaver DK, Morrill WL. 2006. Infestation of wheat and downy brome grass by wheat stem sawfly and subsequent larval performance. Environmental Entomology. 35(5):1279–1285.

Platt AW, Farstad CW. 1946. The reaction of wheat varieties to wheat stem sawfly attack. Scientific Agriculture. 26(6):231–247. https://doi.org/10.4141/sa-1946-0028

Rand TA, Titus EF, Waters DK. 2019. Do floral resources benefit the herbivorous sawfly, *Cephus cinctus* (Hymenoptera: Cephidae), a major pest of wheat in North America? Journal of Economic Entomology. 112(2):565–570. https://doi.org/10.1093/jee/toy408

Rand TA, Waters DK. 2020. Aphid honeydew enhances parasitoid longevity to the same extent as a high-quality floral resource: implications for conservation biological control of the wheat stem sawfly (Hymenoptera: Cephidae). Journal of Economic Entomology. 113(4):2022–2025. https://doi.org/10.1093/jee/toaa076

Redmon LA, Horn GW, Krenzer EG, et al. 1995. A review of livestock grazing and wheat grain yield: boom or bust? Agronomy Journal. 87(2):137–147. https://doi.org/10.2134/agronj1995.00021962008700020001x

Reynolds MP, Braun H-J. 2022. Global trends in wheat production, consumption and trade. In: Reynolds MP, Braun H-J, editors. Wheat improvement, food security in a changing climate. Cham: Springer International Publishing. p. 3–15. https://doi.org/10.1007/978-3-030-90673-3_1

Runyon J, Hurley R, Morrill W, et al. 2001. Distinguishing adults of *Bracon cephi* and *Bracon lissogaster* (Hymenoptera: Braconidae), parasitoids of the wheat stem sawfly (Hymenoptera: Cephidae). The Canadian Entomologist. 133(2):215–217.

Runyon JB. 2001. Wheat stem sawfly parasitism in varying field sizes and tillage systems in dryland wheat in Montana. Montana State University.

Runyon JB, Morrill WL, Weaver DK, et al. 2002. Parasitism of the wheat stem sawfly (Hymenoptera: Cephidae) by *Bracon cephi* and *B. lissogaster* (Hymenoptera: Braconidae) in wheat fields bordering tilled and untilled fallow in Montana. Journal of Economic Entomology. 95(6):1130–1134. https://doi.org/10.1603/0022-0493-95.6.1130

Seamans HL. 1945. A preliminary report on the climatology of the wheat stem sawfly (*Cephus cinctus* Norton.) on the Canadian prairies. Scientific Agriculture. 25(7):432–457. https://doi.org/10.4141/sa-1945-0012

Sjolie DM, Willenborg CJ, Vankosky MA. 2024. The causes of wheat stem sawfly (Hymenoptera: Cephidae) larval mortality in the Canadian prairies. The Canadian Entomologist. 156. https://doi.org/10.4039/tce.2023.29

Solie JB, Solomon Jr SG, Self KP, et al. 1991. Reduced row spacing for improved wheat yields in weed-free and weed-infested fields. Transactions of the ASAE. 34(4):1654–1660. https://doi.org/10.13031/2013.31784

Somsen HW, Luginbill P. 1956. *Bracon lissogaster* Mues: A parasite of the wheat stem sawfly. U.S. Department of Agriculture (Technical bulletin (United States. Department of Agriculture)). https://books.google.com/books?id=Go3i5AKCfa8C.

United States Department of Agriculture, National Agricultural Statistics Service. 2025a. Wheat: acreage, yield, and production summary. USDA NASS. https://www.nass.usda.gov/Statistics_by_Subject/result.php?CD15D6FF-E7AB-3B60-B7B7-4F7599B38A27&sector=CROPS&group=FIELD%20CROPS&comm=WHEAT.

United States Department of Agriculture, National Agricultural Statistics Service. 2025b. Colorado State agriculture overview. https://www.nass.usda.gov/Quick_Stats/Ag_Overview/stateOverview.php?state=COLORADO.

Varella AC, Weaver DK, Peterson RKD, et al. 2017. Host plant quantitative trait loci affect specific behavioral sequences in oviposition by a stem-mining insect. Theoretical and Applied Genetics. 130(1):187–197. https://doi.org/10.1007/s00122-016-2805-0

Varella AC, Weaver DK, Sherman JD, et al. 2015. Association analysis of stem solidness and wheat stem sawfly resistance in a panel of North American spring wheat germplasm. Crop Science. 55(5):2046–2055. https://doi.org/10.2135/cropsci2014.12.0852

Vieira HV, Bradford BZ, Osterholzer A, et al. 2025. A new growing degree-day phenology model for wheat stem sawfly (Hymenoptera: Cephidae) in Colorado wheat fields. Fand BB, editor. PLoS ONE. 20(4):e0320497. https://doi.org/10.1371/journal.pone.0320497

Wallace L, McNeal F. 1966. Stem sawflies of economic importance in grain crops in the United States. Entomology Research Division.

Wallace LE. 1962. Field-plot tests of chemicals for wheat stem sawfly control. Journal of Economic Entomology. 55(6):908–912. https://doi.org/10.1093/jee/55.6.908

Wallace LE, Butler LI. 1967. Residues of heptachlor and heptachlor epoxide in wheat treated to control the wheat stem sawfly. Journal of Economic Entomology. 60(1):254–256. https://doi.org/10.1093/jee/60.1.254

Wallace LE, McNeal FH, Berg MA. 1973. Minimum stem solidness required in wheat for resistance to the wheat stem sawfly. Journal of Economic Entomology. 66(5):1121–1124. https://doi.org/10.1093/jee/66.5.1121

Wasserman-Olin R, Hill R, Osterholzer A, et al. 2024. The economic impact of wheat stem sawfly on Colorado wheat. Colorado State University Report No.: TR24-3. https://webdoc.agsci.colostate.edu/csucrops/reports/winterwheat/wheatreport_2024_WFD.pdf.

Weaver DK, Morrill WL. 2001. Potential impact of cultural practices on wheat stem sawfly (Hymenoptera: Cephidae) and associated parasitoids. Journal of Agricultural and Urban Entomology. 18(1):35–45.

Weiss MJ, Morrill WL. 1992. Wheat stem sawfly (Hymenoptera: Cephidae) revisited. American Entomology. 38(4):241–245. https://doi.org/10.1093/ae/38.4.241

Weiss MJ, Riveland NR, Reitz LL, et al. 1990. Influence of resistant and susceptible cultivar blends of hard red spring wheat on wheat stem sawfly (Hymenoptera: Cephidae) damage and wheat quality parameters. Journal of Economic Entomology. 83(1):255–259. https://doi.org/10.1093/jee/83.1.255

CHAPTER 2: A NEW GROWING DEGREE-DAY PHENOLOGY MODEL FOR WHEAT STEM SAWFLY (HYMENOPTERA: CEPHIDAE) IN COLORADO WHEAT FIELDS

Introduction

The wheat stem sawfly (WSS), *Cephus cinctus* Norton (Hymenoptera: Cephidae), is a native grass-feeding insect and is considered to be one of the most economically important pests of wheat in North America (Beres et al. 2011, Irell and Peairs 2014). Plant injury is mainly caused by stem boring and stem cutting by WSS larvae. In addition, WSS can reduce photosynthetic activity (Macedo et al. 2007), cause grain weight reduction (Holmes 1977, Morrill et al. 1992), and cause significant yield loss by toppling stems (Ainslie 1920), causing difficulties in harvesting. WSS has traditionally infested spring wheat, but over the last few decades, the damage has become increasingly common in winter wheat (Irell and Peairs 2014, Cockrell et al. 2021). WSS is univoltine with adult emergence from overwintering pupae influenced by temperature (Villacorta et al. 1972, Perez-Mendoza and Weaver 2006). Diapause requires approximately 90 days below 10°C, with many individuals remaining in or re-entering diapause at temperatures of 10°C or below and 35°C or above (Salt 1947, Perez-Mendoza and Weaver 2006). WSS thrives in dry weather but requires adequate moisture for adult emergence (Criddle 1923, Olfert et al. 2019). Temperature and moisture significantly influence flight length and success (Seamans 1945). Warmer conditions and calm winds enhance population growth by facilitating mating and oviposition (Wallace and McNeal 1966).

Several pest control strategies are employed to reduce losses from WSS, but none by themselves have been shown to provide completely effective control (Irell and Peairs 2014). Amongst different management strategies, cultural control methods (Ainslie 1920, Lafond et al. 1996, Lal 1997, Beres et al. 2011), biological control (Nelson and Farstad 1953, Runyon et al.

2002, Buteler et al. 2008, 2015, Peterson et al. 2011), and solid and semi-solid stem cultivars (Eckroth and McNeal 1953, Holmes 1984, Delaney et al. 2010, Beres et al. 2012, 2017, Nilsen et al. 2016, Subedi et al. 2021, Bathini et al. 2023, Peirce et al. 2024) have achieved varying success, but not fully effective at mitigating losses from WSS. Wheat stem sawfly is extremely difficult to control with insecticides because the insects spend most of their life cycle protected inside the stem as larvae, and managing adult populations has proven to be an ineffective way to reduce the population so far (Beres et al. 2011). Due to the varying effectiveness of current WSS management strategies, growers and researchers continue to seek more reliable methods.

One such strategy involves accurate monitoring and forecasting as one of the five key steps for a holistic IPM program, right after proper identification and diagnosis (Pedigo et al. 2021). Forecast can be achieved by modeling the seasonal phenology of WSS from field monitoring data. Accumulated GDD have assisted farmers in various ways such as predicting insect emergence and population peak, anticipating damage, determining optimal sampling times, and guiding appropriate management actions (Leybourne et al. 2024). This approach has potential management implications, such as timing insecticide treatments to coincide with predictions of high pest pressure. Phenology models based on GDD have been successfully established and are very effective in supporting management strategies for insect pests (Teixeira and Polavarapu 2001, Frost et al. 2013, Kamiyama et al. 2020, Ebbenga et al. 2022). For example, the oviposition phenology model for grape berry moth helped reduce insecticide applications, increasing the efficacy of a single spraying in grape (Teixeira et al. 2009). Similarly, using the GDD model instead of a calendar-based insecticide application for billbugs resulted in population reduction (Van Dyke et al. 2023).

To date, several studies have examined the impact of weather on WSS phenology and ecology. Achhami et al. (Achhami et al. 2020) found that GDD correlated with pre-diapause larval mortality in barley cultivars. Beres et al. (Beres et al. 2011) demonstrated that GDD was more effective than calendar days in estimating WSS population peaks in Montana wheat cultivars. In addition, Perez-Mendoza and Weaver (Perez-Mendoza and Weaver 2006) demonstrated that temperature and humidity affect WSS larval development and adult emergence, using data from three Montana WSS populations to identify optimal conditions for shorter developmental times and extreme conditions where development ceases. These complementary studies provide valuable resources that can potentially help mitigate WSS damage. Here, we use a long-term data set based on multiple years of adult WSS monitoring in Colorado to predict the seasonal phenology of WSS and determine the effects of temperature and precipitation on WSS populations in Colorado winter wheat.

Methods

Data collection

Field sites. Adult WSS monitoring was performed in winter wheat fields located at New Raymer and Orchard, CO (**Table 2.1**). These sites were chosen because WSS outbreaks were first reported here in 2011. These sites were monitored every year from 2011 to 2023 except for 2015, when data was not collected due to adverse weather conditions, which prevented sampling. The data includes 6-9 sites per year; the variation in the number of fields sampled per year is attributed to factors such as crop rotation or low WSS population density. Throughout much of the High Plains Ecoregion, growers use a wheat-fallow and wheat-corn-fallow rotations, which consists of leaving a portion of the land without sowing for one crop season; this helps retain soil

moisture for the next field season as most precipation occurs as snow throughout this region (Unger 1992, Schlegel et al. 2019). Therefore, the fields sampled had little variation in their geographical location through the 13 years.

Table 2.1: Wheat fields sampled and their corresponding coordinates.

Field	Location	Latitude	Longitude
1	New Raymer, CO	40.595973	−103.898422
2	New Raymer, CO	40.591324	−103.897172
3	New Raymer, CO	40.591277	−103.898672
4	New Raymer, CO	40.587007	−103.898172
5	New Raymer, CO	40.584113	−103.896798
6	New Raymer, CO	40.582215	−103.889426
7	Orchard, CO	40.468039	−104.066917
8	Orchard, CO	40.482110	−104.075203
9	Orchard, CO	40.474342	−104.102564

Samples. Sampling occurred on private land and permission to conduct research was granted by each landowner of the sites. At each site, 100, 180-degree sweeps were taken close to field edges using a canvas sweep net. Sampling would typically start by the middle or end of April and continue bi-weekly until mid-June. Wheat stem sawflies are more active at higher temperatures, so samples were collected after 11 am during the warmest part of the day. Wheat stem sawfly adult samples were collected and brought back to the laboratory to be counted.

Temperature and precipitation. We used CoAgMET (https://coagmet.colostate.edu/) to access temperature and precipitation data for the past 12 years from weather stations near the field sites. Stations “New Raymer 21” and “Briggsdale” were chosen, both located within approximately 22 miles of the fields where the surveys were conducted. Minimum, mean, and maximum temperatures were retrieved for each day from January 1st to December 31st of each year and cumulative growing degree days (GDDs) were generated with a biofix (specific date when monitoring of GDD begins for an insect) of January 1st, a base temperature of 10°C, and an upper threshold of 30°C (Salt 1947, Villacorta et al. 1972, Perez-Mendoza and Weaver 2006).

A base temperature of 10°C was chosen because it is commonly used in phenological models, and previous research has shown that roughly 90 days of development at 10°C are required to break the WSS obligatory larval diapause (Salt 1947, Holmes 1979). The upper-temperature threshold of 30°C was again selected because it is commonly used in phenological models, and previous research has indicated this is an optimal developmental temperature for WSS larvae (Perez-Mendoza and Weaver 2006). A single sine method was used to generate DD from daily minimum and maximum temperatures, as this method has been demonstrated to better approximate true daily thermal accumulation from minimum and maximum temperatures than the traditional simple average GDD method, which assumes linear temperature change and can over or underestimate DD, especially when temperatures fall below the developmental threshold (Allen 1976, Pruess 1983).

Statistical analysis

Two approaches were used to characterize the phenology of the spring WSS flight based on degree-day accumulation: a GLM/GAM (Generalized Linear Model and Generalized Additive Model) approach, using the methods previously described (Jagemann et al. 2018). GLMs assume a linear relationship between predictors and the response, while GAMs capture more complex, nonlinear relationships, providing flexibility to model unique biological patterns. The GLM/GAM method allows for including other factors in the initial model construction before isolating the coefficients associated with the degree-day term and constructing a phenology model based on those coefficients, representing relative abundances at given degree-day accumulations. The GAM model fit to those isolated coefficients can be used to identify the start, peak, and end points of insect emergence such as WSS in Colorado. However, this model is not easily used to characterize the DD accumulation associated with a specific fraction of the WSS

flight (such as 10% or 90%), which would be valuable when communicating pest management recommendations to stakeholders. Transforming insect catches into a cumulative proportion of total captures and modelling the distribution with a probit function allows the model to predict degree-day accumulations associated with specific times in the WSS flight. Statistical analysis was performed with R version 4.4 (R Core Team 2025) using packages lme4 (Bates et al. 2015) and mgcv (Wood 2010).

Generalized additive mixed (GAM) phenology model. Based on the methods detailed in Frost et al. (2013), we constructed a GAM model with cumulative DD, cumulative precipitation, site ID, and site-by-year interaction terms, all included as random effects, and WSS capture per 100 sweeps as the response variable to ultimately generate a phenological relationship between WSS captures and cumulative DD. A Poisson distribution was chosen for the model because it closely fit the distribution of observed WSS captures (full dataset, n = 669). The inclusion of terms other than DD in the GAM model serves only to isolate the effect of DD on WSS captures from other possible influences. Next, the random effect coefficients for the DD term were collected from this full GAM model and used as the basis for generating a second GAM model that fits a smooth curve through these GDD coefficients. Points on this smooth fit curve were identified where the value crossed from negative to positive (start of WSS emergence), reached a maximum (peak emergence), and crossed back to negative (end of flight). The GAM model is wss_capture ~ 1 | gdd + 1 | precip + 1 | location_ year + 1 | site_id, where each term is included as a random effect due to the absence of an assigned experimental design.

Cumulative proportion generalized linear phenology model (GLM). As an alternative statistical approach for characterizing WSS flight based on accumulated DD, we merged all years of WSS sweep captures, sorted the dataset by the cumulative DD associated with each

capture, and transformed each WSS capture into a cumulative proportion of all WSS captures (cumulative capture divided by total capture). The GLM was then fit with the formula wss_cumulative_proportion ~ cumulative_degree_days, and a quasibinomial distribution was chosen with a probit link function to account for overdispersion in the proportional response.

Environmental factors GAM model. While both the GAM and GLM models provided insights into the timing of the flight, we lacked a framework for understanding environmental conditions either before or during the WSS flight that could affect the magnitude or severity of the emergence. To investigate these relationships, we constructed a GAM model that initially contained summary terms generated from the WSS dataset that were hypothesized to influence the magnitude of the WSS population. These terms included: the day of the year of first detection, the number of days over which the flight took place, the mean GDD per day during the flight, the total snow accumulation during the prior winter, the total rainfall before the WSS flight, the incremental rainfall during the WSS flight, and the minimum and maximum temperature before or during the flight. In addition to these fixed terms, random effect smooth terms were added to the model to account for unexplained variance associated with the year and the field where sampling occurred. After fitting the full model, backward selection was used to incrementally drop the least significant terms from the model until only significant effects remained.

Results

Observed WSS phenology

The calendar date of emergence and peak WSS population were highly variable across years. Wheat stem sawfly populations began to increase in early to mid-May and peaked in late

May to early June except for 2012 (**Figure 2.1**). In 2012, unusually warm and dry winter and spring conditions led to much earlier WSS emergence, causing the population peak to occur up to 20 days earlier than usual (**Table 2.2**). This high degree of calendar variability in WSS emergence makes anticipating the flight very difficult. It underscores the need for a GDD-based phenological model that models past flights effectively and provides a framework for predicting current-year flight initiation.

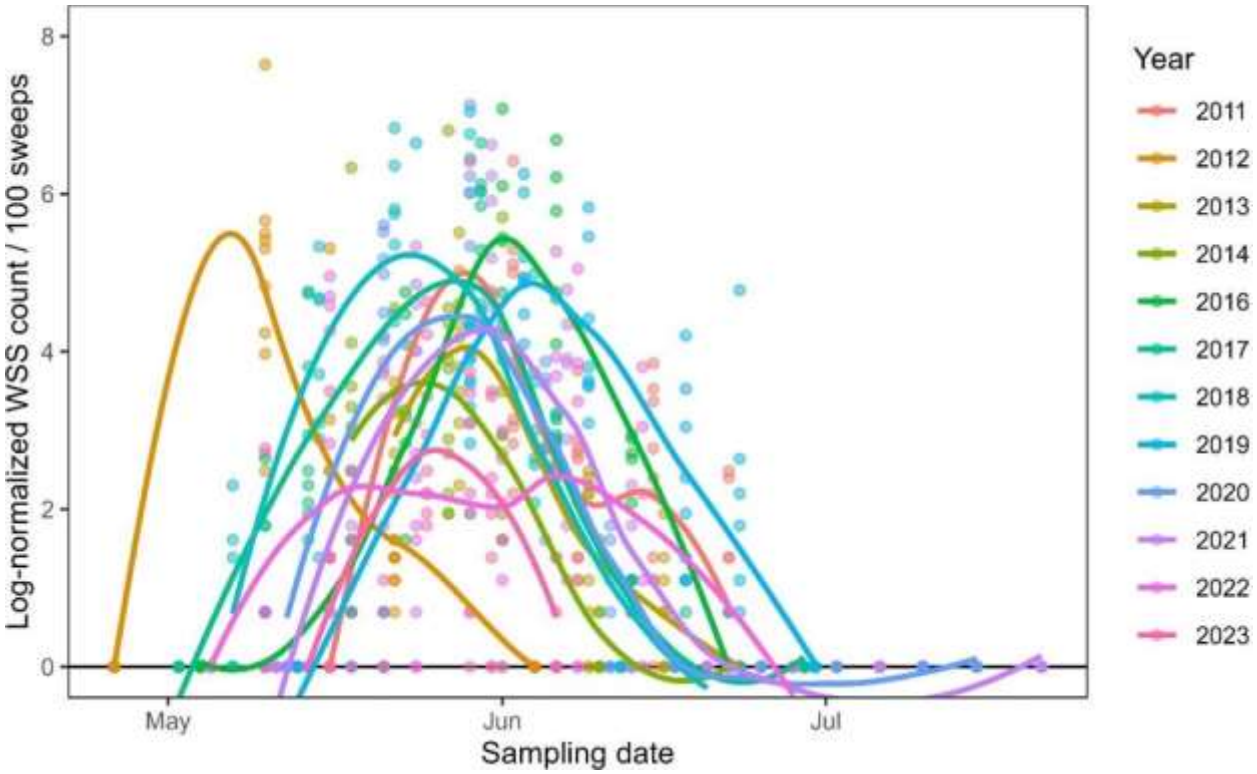

Figure 2.1: Wheat stem sawfly (WSS) seasonal phenology from 2011 to 2023. Smooth fit curves illustrate the average number of WSS adults caught per 100 sweeps by date and year. Depending on the year, the timing of the WSS flight relative to the calendar date is highly variable.

Table 2.2: Summary of average temperature, rainfall, snowfall, and wheat stem sawfly counts per year.

Year	Avg Temperature (□C)	Avg Rainfall (mm)	Avg Snowfall (mm)	Avg WSS Count	Avg WSS Count (Log)
2011	1.89	1.11	3.87	115	4.74
2012	4.89	0.71	2.15	340	5.83
2013	1.88	0.94	7.90	65	4.17
2014	1.92	1.05	7.82	110	4.70
2016	3.53	1.31	6.81	325	5.78
2017	4.15	1.44	2.40	300	5.70
2018	2.81	1.43	5.81	360	5.89
2019	1.09	1.24	5.73	200	5.30
2020	2.81	0.64	4.27	415	6.03
2021	1.89	1.10	5.16	285	5.65
2022	2.25	0.49	4.04	120	4.79
2023	0.84	1.48	7.50	90	4.50

Using cumulative GDD significantly improves overlap in observed WSS annual phenology curves across years relative to using simple calendar dates (**Figure 2.2**). While the magnitude of each year's flight (e.g., total WSS captures) does vary, the timing of the flight's start, peak, and end points are much more closely aligned when using GDD instead of calendar day. While there is still some remaining annual variability in phenology timing, and significant variation in total WSS captures per year, we attempt to explain these characteristics using environmental data, explained later in the results.

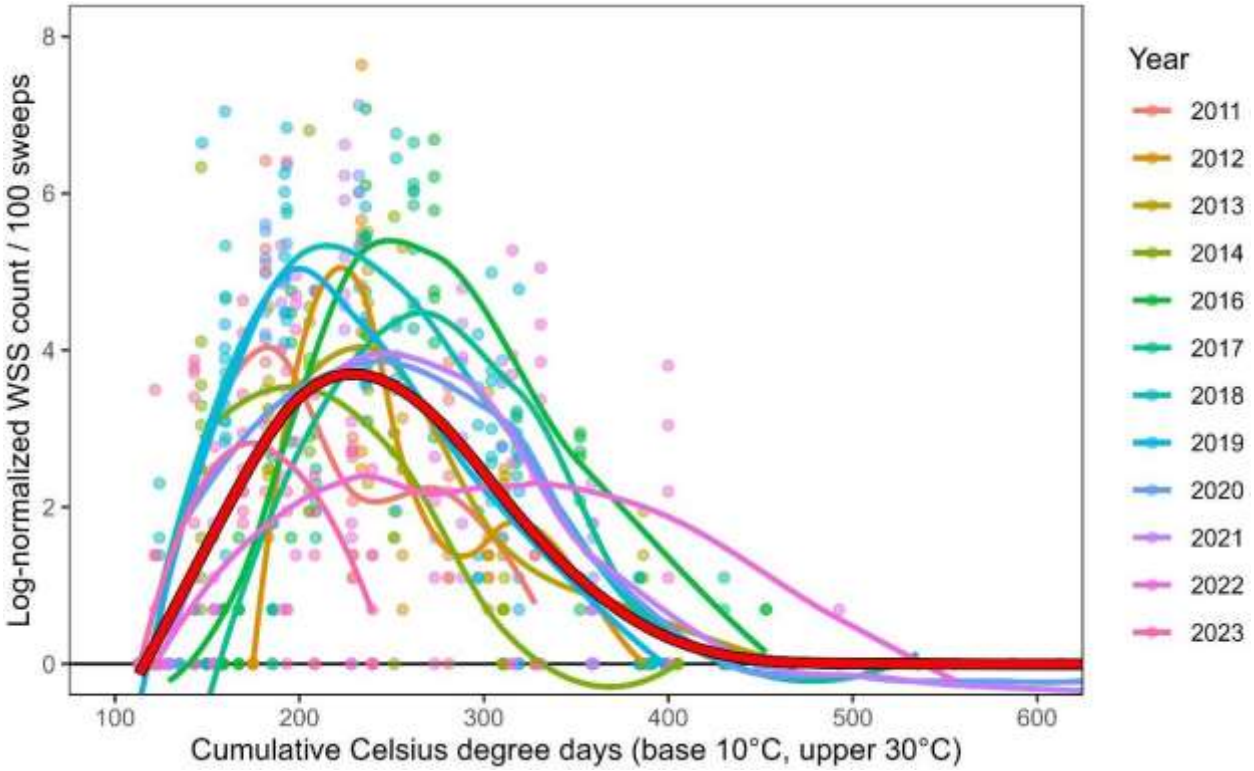

Figure 2.2: Relationship between log-normalized adult wheat stem sawfly in 100 sweeps made weekly and cumulative GDD over the thirteen years of sampling. Each individual year's WSS flight is illustrated with colored smooth-fit lines (method: loess), while the combined dataset is modeled using a GAM and shown in a bold red line. This model is the basis for generating the GAM degree-day model.

WSS degree-day models

Using the GLM/GAM modeling approach, the start of the WSS flight was estimated to be 148 GDD (base 10°C, upper 30°C), the peak at 224 GDD, and the end at 354 GDD (**Figure 2.3**). Based on actual WSS sweep net captures, we found that 4.7% (1,723) occurred before 148 GDD, 39.8% (14,496) before 224 GDD, 60.2% (21,957) after 224 GDD, and 0.4% (129) after 354 GDD. Expanding the peak to ± 10 GDD (214–234 GDD), 23.3% (8,493) of captures occurred (approximately 4 days at an average GDD accumulation of 5.4 per day).

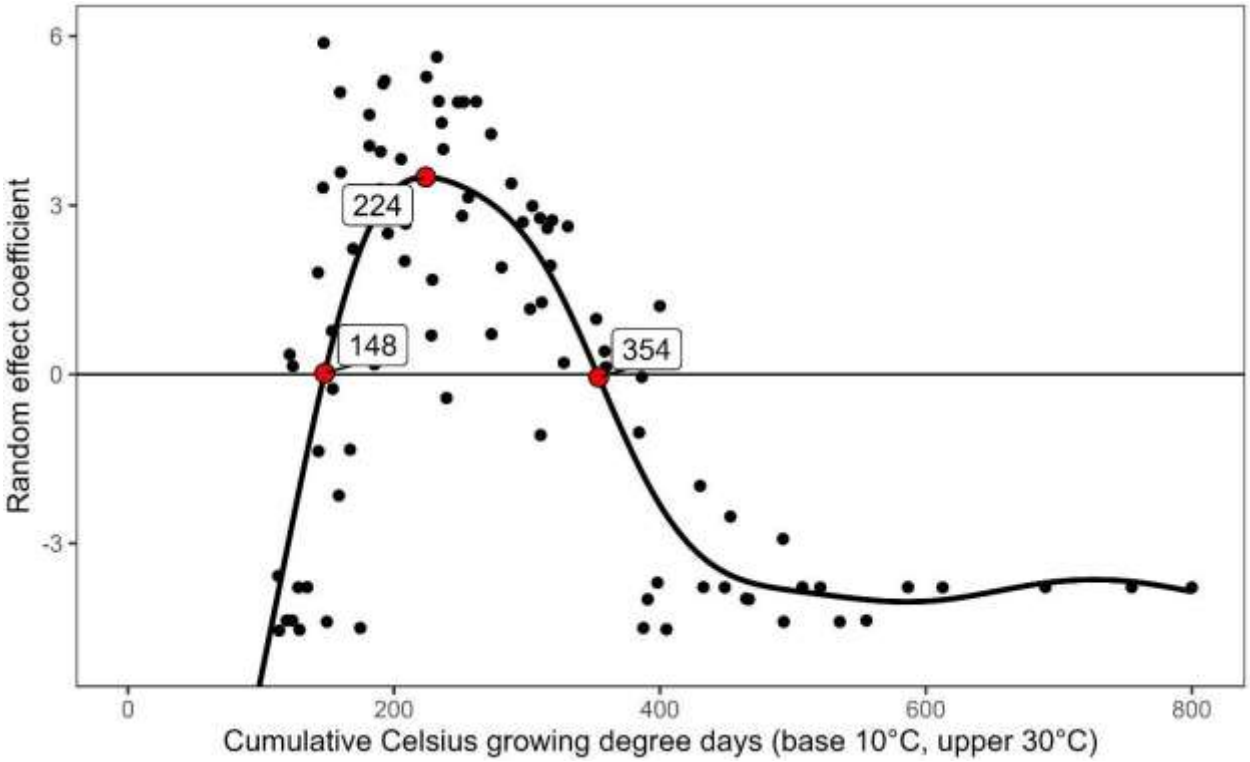

Figure 2.3: GLM/GAM-derived WSS emergence phenology model. Random effect coefficients associated with the cumulative growing degree-day term in the initial GLM are modeled using GAM. These coefficients represent relative over/ under abundance of WSS captures after controlling for variance associated with year and location, and the points where the modeled fit crosses the axis or reaches a local maximum are used to identify the start, peak, and end of the WSS emergence period. Here, emergence is predicted to begin at 148 cumulative Celsius growing DD (GDD), peak at 224 GDD, and decline at 354 GDD, with a biofix of Jan 1.

Using a cumulative proportion probit fit, our second phenology modeling approach produced a similar result to the GLM/GAM approach. In addition, it allowed for the characterization of additional points along the WSS emergence distribution. The results of this model generally aligned with the previously described GAM model, though this GLM model predicted a slightly earlier initiation to the flight relative to the GAM. Using this curve, we find that 1% of WSS emerge by 125 GDD, 5% emerge by 154 GDD (roughly equivalent to the start point in the GLM/GAM model), 10% emerge by 169 GDD, 50% emerge by 223 GDD (peak emergence), 90% by 276 GDD, 95% by 291 GDD, and 99% by 320 GDD (**Figure 2.4**). This model has an R2 of 0.987. The cumulative GDD term is highly significant ($t = 74.77$, df = 623, P

< .0001). When the cumulative GDD to proportion of adult captures over the past five years were plotted, we observed a good fit for the model and the phenology events (**Figure 2.5**).

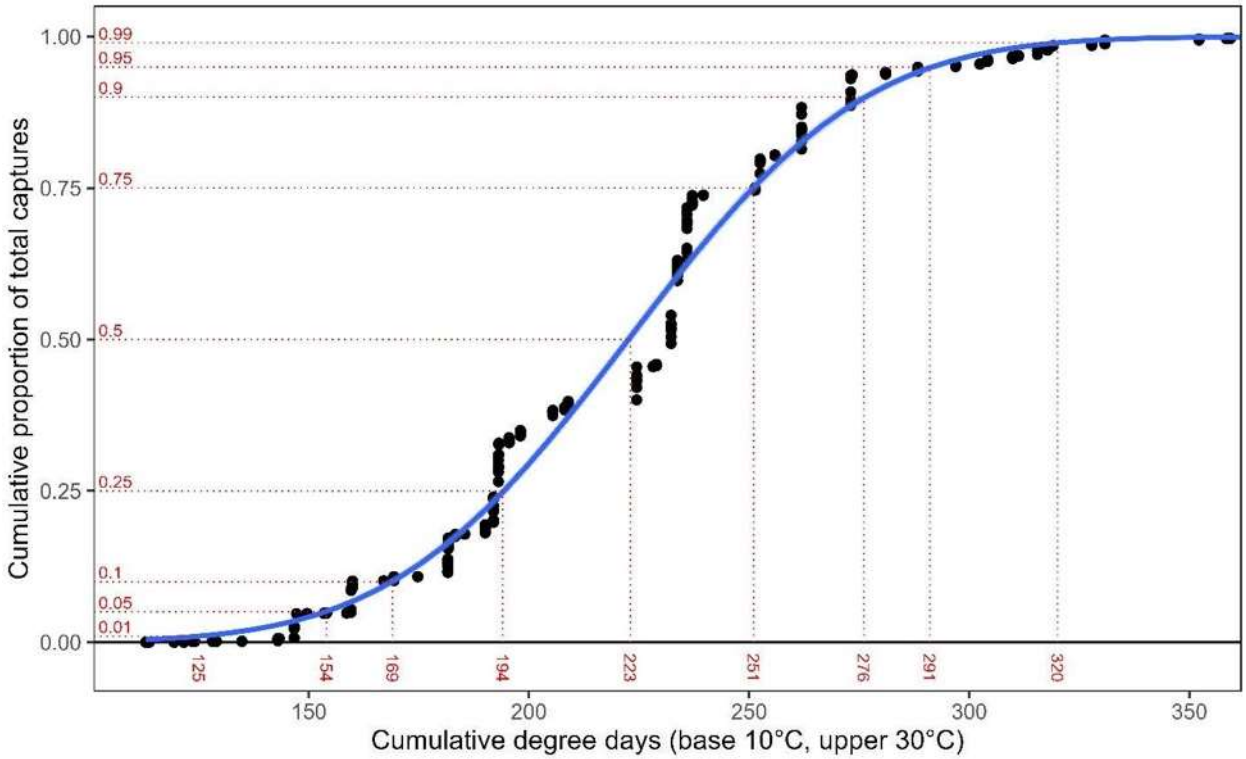

Figure 2.4: Probit fit of the cumulative proportion of total WSS captures over cumulative growing DD. Observed WSS captures (black points) from 2011–2023 were arranged by cumulative growing degree-day (base 10°C, upper threshold 30°C, biofix Jan 1), and captures were converted into a cumulative proportion of all captures. As shown in red, a GLM with a quasibinomial distribution and a probit link function was used to characterize specific points along the modeled WSS emergence period.

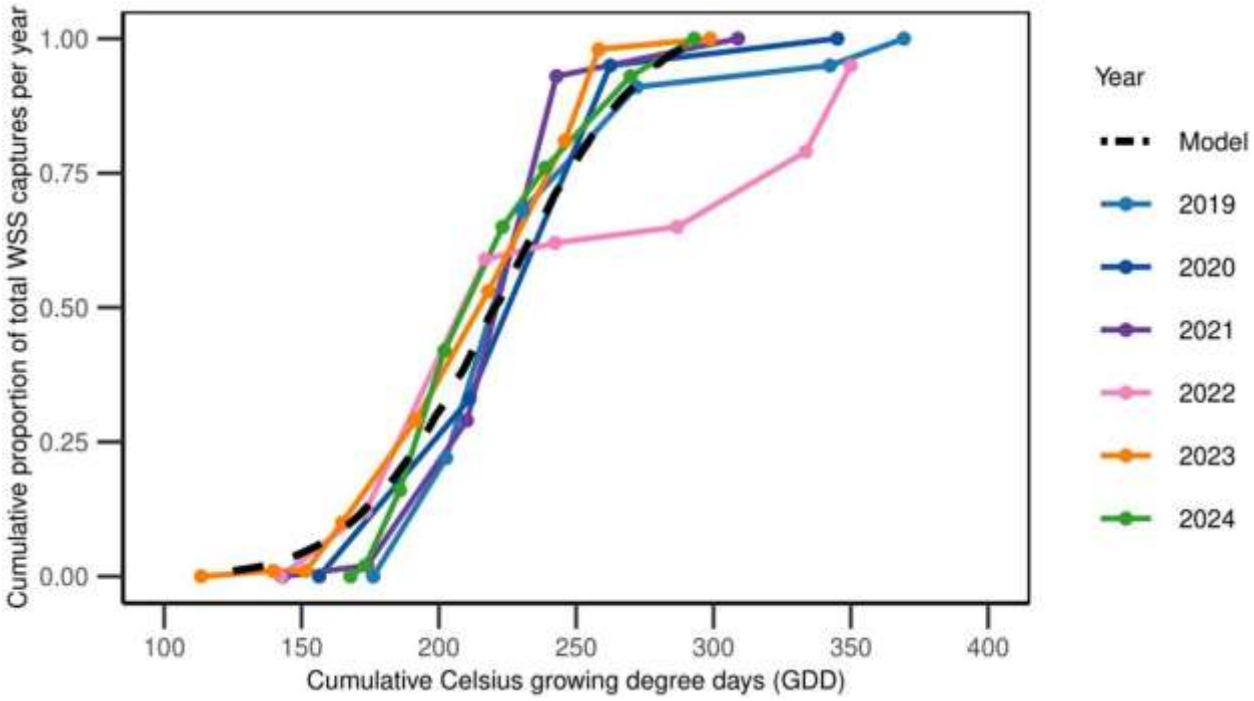

Figure 2.5: Comparison of observed WSS phenology and GAM model predictions. This figure illustrates the cumulative proportion of WSS captures from 2019 to 2024 in relation to cumulative Growing Degree Days (GDD; base 10°C, upper threshold 30°C, biofix Jan 1). Observational data for each year is shown as colored lines, while the model predictions are represented by the dashed black line.

Environmental factors affecting WSS population size

Significant factors influencing mean WSS capture were the length of the emergence period (positive effect, $t = 2.03$, $P = 0.046$), the amount of rain accumulated between Jan 1 and the start of the WSS emergence (negative effect, $t = -3.53$, $P = 0.0007$), the minimum temperature during the emergence period (positive effect, $t = 3.11$, $P = .003$), the maximum temperature before WSS emergence (positive effect, $t = 2.21$, $P = 0.03$), and the maximum temperature during the emergence (negative effect, $t = -5.72$, $P < 0.0001$). Factors that were not significant and were dropped from the reduced model included the day of year of first emergence, the mean daily GDD accumulation during the emergence period, the amount of snowfall during the prior winter, the amount of rainfall during the emergence period, and the minimum temperature before the start of emergence. The model also accounts for the random

effects of the year ($F = 16.6$, df = 11, $P < .0001$) and site ($F = 9.68$, df = 13, $P < .0001$) using smooth terms. The reduced model has an R2 of 0.744 and explains 79.7% of the variance in mean WSS captures between sites and years. See **Table 2.3** for a summary of significant model terms. These results suggest that higher WSS populations are associated with longer emergence periods, less precipitation before emergence, milder low temperatures during emergence, and milder maximum temperatures before and during emergence.

Table 2.3: GAM model results show environmental factors influencing mean wheat stem sawfly (WSS) captures.

Term	Min	Max	Mean	Coefficient	Std. Error	*t*-value	*Pr*(>|t|)	Sig.
(Intercept)				7.283	4.453	1.64	0.106	
Emergence length (days)	6	43	21	0.058	0.029	2.03	0.046	*
Prior rainfall (mm)	68.6	256.5	147.1	−0.021	0.006	−3.53	0.0007	***
Min temp during emergence (°C)	−1.7	6.7	4.5	0.348	0.112	3.11	0.0026	**
Max temp before emergence (°C)	27.2	32.8	30.1	0.300	0.136	2.21	0.0299	*
Max temp during emergence (°C)	24.4	37.2	29.4	−0.407	0.071	−5.72	<.0001	***

Discussion

Wheat stem sawfly (WSS) has long been considered the most important pest of wheat in the northern Great Plains of North America. In recent years, damage from WSS has expanded to areas of Nebraska, Wyoming, Colorado, and Kansas. With climate change, WSS populations are predicted to increase and expand to new geographical areas (Olfert et al. 2019). The WSS is a

stem-boring insect where the larvae spend most of their time inside the stem and then undergo diapause in the wheat stubble (Ainslie 1920, Beres et al. 2011), which makes the development of forecasting tools a challenge. Previous models attempted to predict WSS cutting (Beres et al. 2017) and found that stem cutting is influenced by precipitation. Other models generated data on larval mortality to provide information for future phenology models that can estimate WSS population density (Sjolie et al. 2024). There is limited information on the phenology of WSS, especially in areas where infestations are more recent, such as Colorado. Here, we leveraged a thirteen-year dataset of adult WSS captures to predict the seasonal phenology of WSS and determine the effects of temperature and precipitation on WSS populations in Colorado. We found that WSS populations in Colorado wheat fields follow predictable patterns based on growing degree- day (GDD). The GLM model analyzed the weekly adult WSS captures over the thirteen-year period, which is useful for predicting field populations based on unique GDD estimates. The model provided GDD approximations for WSS seasonal population trends across the thirteen-year period, with WSS first adult appearance predicted at 148 GDD, population peak at 224 GDD, and decline at 359 GDD. This represents the first published phenology model for WSS in Colorado.

When interpreting the data, it is recommended to start scouting for WSS before 148 GDD. Since spring GDD accumulation is variable, beginning scouting at when temperatures are starting to rise – typically mid-April for Colorado – is advisable to prepare for years like 2012, when emergence occurred 20 days earlier than average. From emergence until population peak, it typically takes around two weeks in Colorado. The average date of population peak across the 13-year period is May 28th, which closely fits in the two-week average between emergence and peak for the WSS population. This average date concurs with previous research from

McCullough et al. (2020) that sampled winter wheat fields for two years in Nebraska and recorded peak flight at the end of May and beginning of June in the Central Great Plains. The second phenology modeling approach, using a cumulative proportion probit fit, produced results that were in alignment with the GLM/GAM approach but offered an enhanced characterization of WSS emergence distribution. The cumulative proportion probit fit model identified key emergence points, which provide a more nuanced understanding of the WSS emergence pattern, offering critical data for early intervention and monitoring. The probit function confirms that the peak emergence at 223 GDD is consistent with the GLM/GAM model, validating the robustness of our model. The benefit of this model is that it can be used to predict the degree-day accumulation associated with any given fraction of the WSS flight, such as the 1% or 5% initial accumulation points, the 50% accumulation point (peak flight), and the end of the flight (95% or 99%). Understanding the distribution of emergence can help develop adaptive management strategies responsive to annual variations in weather conditions, particularly temperature. In addition, identifying specific GDD emergence timing can aid in cultural control methods for wheat such as swathing timing or trap crop cutting timing to reduce the WSS population.

Several published and unpublished studies have shown that insecticides are often ineffective against WSS because the adult sawfly emergence window spans over a month, reducing exposure for adults emerging after spraying (Wallace 1962, Holmes and Peterson 1963, Wallace and Butler 1967, Beres et al. 2011). Our model indicates when adult WSS populations are emerging in significant numbers, allowing for optimal timing of insecticide applications, enhancing their efficacy. Therefore, this research would suggest targeting chemical control tactics during the initial population build-up. Further, males typically emerge first (Holmes 1979, Perez-Mendoza and Weaver 2006), so phenology models such as the one presented here can be

used to reduce the number of males before mating takes place. This strategy might reduce female populations for the following year since WSS are haplodiploid, and unfertilized eggs will produce male offspring (Holmes 1979).

The amount of rainfall accumulated preceding WSS emergence negatively impacted WSS captures. This is in agreement with previous research that found that dry conditions preceding adult emergence could lead to higher WSS captures (Criddle 1923, Olfert et al. 2019). In contrast, rainfall had a positive impact on larval survival with larval survival increasing with increasing weekly rainfall. Further, the minimum temperature during the emergence period positively impacted WSS captures. The maximum temperature before emergence can positively affect the WSS population, whereas the maximum temperature during emergence has a negative effect. Previous research demonstrated that post-diapause larvae (preceding adult emergence) cannot complete development or will reenter diapause under low temperatures (< 10°C), and adult WSS may have high mortality under high temperatures (> 35°C) (Salt 1947, Villacorta et al. 1972, Perez-Mendoza and Weaver 2006). Moreover, increasing temperatures preceding emergence potentially influences the suitability of winter wheat host plants. The model can be influenced by climate variability or extreme conditions but given the large data set (13 years), we were able to account for some of that climatic variation in outlier years such as 2012. In 2012, unusually warm and dry winter and spring conditions led to much earlier WSS emergence, causing the population peak to occur up to 20 days earlier than usual. However, extreme climate can affect the larvae during diapause, which can have an impact on the emergence of adults and thereby the model. Hence, atypical years, such as those with abnormally cold winters or extreme heat in late summer, can affect the accurate estimation of population sizes for the following field season. Overall, by incorporating 13 years of data from the same locations, we developed a

robust GDD model to forecast WSS emergence and population growth, leading to more timely and effective management strategies.

Our model is region-specific, having been derived from WSS data from specific locations and environmental factors pertaining to eastern Colorado winter wheat fields. This is the first effort towards improving management for WSS in eastern Colorado. We have not validated the model at other locations because we do not have phenological data for any additional sites across different years. The applicability of the model in other wheat-growing regions affected by WSS will require model validation in each region, along with an evaluation of GDD accumulation rates that consider these regional differences. Additionally, genetic variability among WSS populations (Lesieur et al. 2016) may influence developmental thresholds and temperature responses, posing a challenge to applying the model across different regions, especially since the biological characteristics of WSS were originally studied using populations from the northern Great Plains. Future research may consider interactions between GDD, precipitation, and WSS emergence across wheat cultivars in different locations, which will allow for more precise field population trend predictions and the development of preventative management options to control WSS.

References

Achhami BB, Reddy GVP, Sherman JD, et al. 2020. Effect of precipitation and temperature on larval survival of *Cephus cinctus* (Hymenoptera: Cephidae) in barley cultivars. Journal of Economic Entomology. 113(4):1982–1989. https://doi.org/10.1093/jee/toaa097

Ainslie CN. 1920. The western grass-stem sawfly. Entomological Assistant, Cereal and Forage Insect Investigations. 841.

Allen JC. 1976. A modified sine wave method for calculating degree days. Environmental Entomology. 5(3):388–396. https://doi.org/10.1093/ee/5.3.388

Bates D, Mächler M, Bolker B, et al. 2015. Fitting linear mixed-effects models using lme4. J. Stat. Soft. 67(1). https://doi.org/10.18637/jss.v067.i01

Bathini A, Mendu L, Singh NP, et al. 2023. A "solid" solution for wheat stem sawfly (Hymenoptera: Cephidae) resistance: genetics, breeding and development of solid stem wheat. Crop Science. 63(4):1934–1948. https://doi.org/10.1002/csc2.20998

Beres B, Dosdall L, Weaver D, et al. 2011. Biology and integrated management of wheat stem sawfly and the need for continuing research. The Canadian Entomologist. 143(2):105–125. https://doi.org/10.4039/n10-056

Beres BL, Cárcamo HA, Dosdall LM, et al. 2011. Do interactions between residue management and direct seeding system affect wheat stem sawfly and grain yield? Agronomy Journal. 103(6):1635–1644. https://doi.org/10.2134/agronj2011.0055

Beres BL, Hill BD, Carcamo HA, et al. 2017. An artificial neural network model to predict wheat stem sawfly cutting in solid-stemmed wheat cultivars. Canadian Journal of Plant Science. 97(2):329–336. https://doi.org/10.1139/cjps-2016-0364

Beres BL, McKenzie RH, Cárcamo HA, et al. 2012. Influence of seeding rate, nitrogen management, and micronutrient blend applications on pith expression in solid-stemmed spring wheat. Crop Science. 52(3):1316–1329. https://doi.org/10.2135/cropsci2011.05.0239

Buteler M, Peterson RKD, Hofland ML, et al. 2015. A multiple decrement life table reveals that host plant resistance and parasitism are major causes of mortality for the wheat stem sawfly. Environmental Entomology. 44(6):1571–1580. https://doi.org/10.1093/ee/nvv128

Buteler M, Weaver DK, Miller PR. 2008. Wheat stem sawfly-infested plants benefit from parasitism of the herbivorous larvae. Agricultural and Forest Entomology. 10(4):347–354. https://doi.org/10.1111/j.1461-9563.2008.00396.x

Cockrell DM, Randolph T, Peirce E, et al. 2021. Survey of wheat stem sawfly (Hymenoptera: Cephidae) infesting wheat in eastern Colorado. Journal of Economic Entomology. 114(2):998–1004. https://doi.org/10.1093/jee/toab015

Criddle N. 1923. Popular and practical entomology: the life habits of *Cephus cinctus* Nort. in Manitoba. The Canadian Entomologist. 55(1). https://doi.org/10.4039/Ent551-1

Delaney KJ, Weaver DK, Peterson RKD. 2010. Photosynthesis and yield reductions from wheat stem sawfly (Hymenoptera: Cephidae): interactions with wheat solidness, water stress, and phosphorus deficiency. Journal of Economic Entomology. 103(2):516–524. https://doi.org/10.1603/EC09229

Ebbenga DN, Hanson AA, Burkness EC, et al. 2022. A degree-day model for forecasting adult phenology of *Popillia japonica* (Coleoptera: Scarabaeidae) in a temperate climate. Frontiers in Insect Science. 2. https://doi.org/10.3389/finsc.2022.1075807

Eckroth EG, McNeal FH. 1953. Association of plant characters in spring wheat with resistance to the wheat stem sawfly. Agronomy Journal. 45(9):400–404. https://doi.org/10.2134/agronj1953.00021962004500090002x

Frost KE, Esker PD, Van Haren R, et al. 2013. Seasonal patterns of aster leafhopper (Hemiptera: Cicadellidae) abundance and aster yellows phytoplasma infectivity in Wisconsin carrot fields. Environmental Entomology. 42(3):491–502. https://doi.org/10.1603/EN12240

Holmes N. 1979. The wheat stem sawfly. 26th Annual Meetings of the Entomological Society of Alberta and Entomological Society of Saskatchewan. 26.

Holmes ND. 1977. The effect of the wheat stem sawfly, *Cephus cinctus* (Hymenoptera: Cephidae), on the yield and quality of wheat. The Canadian Entomologist. 109(12):1591–1598. https://doi.org/10.4039/Ent1091591-12

Holmes ND. 1984. The effect of light on the resistance of hard red spring wheats to the wheat stem sawfly, *Cephus cinctus* (Hymenoptera: Cephidae). The Canadian Entomologist. 116(5):677–684. https://doi.org/10.4039/Ent116677-5

Holmes ND, Peterson LK. 1963. Heptachlor as a systemic insecticide against the wheat stem sawfly, *Cephus cinctus* Nort. The Canadian Entomologist. 95(8):792–796. https://doi.org/10.4039/Ent95792-8

Irell B, Peairs F. 2014 Oct. Wheat stem sawfly: A new pest of Colorado wheat. Colorado State University Extension. [accessed 2024 Apr 18]. https://extension.colostate.edu/topic-areas/insects/wheat-stem-sawfly-a-new-pest-of-colorado-wheat-5-612/.

Jagemann SM, Juzwik J, Tobin PC, et al. 2018. Seasonal and regional distributions, degree-day models, and phoresy rates of the major sap beetle (Coleoptera: Nitidulidae) vectors of the oak wilt fungus, *Bretziella fagacearum*, in Wisconsin. Environmental Entomology. 47(5):1152–1164. https://doi.org/10.1093/ee/nvy080

Kamiyama MT, Bradford BZ, Groves RL, et al. 2020. Degree day models to forecast the seasonal phenology of *Drosophila suzukii* in tart cherry orchards in the Midwest U.S. PLoS ONE. 15(4). https://doi.org/10.1371/journal.pone.0227726

Lafond GP, Boyetchko SM, Brandt SA, et al. 1996. Influence of changing tillage practices on crop production. Canadian Journal of Plant Science. 76:641–649. https://doi.org/10.4141/cjps96-114

Lal R. 1997. Residue management, conservation tillage and soil restoration for mitigating greenhouse effect by CO2-enrichment. Soil & Tillage Research. 43:81–107. https://doi.org/10.1016/S0167-1987(97)00036-6

Lesieur V, Martin JF, Weaver DK, et al. 2016. Phylogeography of the wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae): implications for pest management. PLoS ONE. 11(12). https://doi.org/10.1371/journal.pone.0168370

Leybourne DJ, Storer KE, Marshall A, et al. 2024. Thresholds and prediction models to support the sustainable management of herbivorous insects in wheat. A review. Agronomy for Sustainable Development. 44(3). https://doi.org/10.1007/s13593-024-00965-5

Macedo TB, Weaver DK, Peterson RKD. 2007. Photosynthesis in wheat at the grain filling stage is altered by larval wheat stem sawfly (Hymenoptera: Cephidae) injury and reduced water availability. Journal of Entomological Science. 42(2):228–238. https://doi.org/10.18474/0749-8004-42.2.228

McCullough CT, Hein GL, Bradshaw JD. 2020. Phenology and dispersal of the wheat stem sawfly (Hymenoptera: Cephidae) into winter wheat fields in Nebraska. Journal of Economic Entomology. 113(4):1831–1838. https://doi.org/10.1093/jee/toaa093

Morrill WL, Gabor JW, Hockett EA, et al. 1992. Wheat stem sawfly (Hymenoptera: Cephidae) resistance in winter wheat. Journal of Economic Entomology. 85(5):2008–2011. https://doi.org/10.1093/jee/85.5.2008

Nelson WA, Farstad CW. 1953. Biology of *Bracon cephi* (Gahan) (Hymenoptera: Braconidae), an important native parasite of the wheat stem sawfly, *Cephus cinctus* Nort. (Hymenoptera: Cephidae), in western Canada. The Canadian Entomologist. 85(3):103–107. https://doi.org/10.4039/Ent85103-3

Nilsen KT, Clarke JM, Beres BL, et al. 2016. Sowing density and cultivar effects on pith expression in solid-stemmed durum wheat. Agronomy Journal. 108(1):219–228. https://doi.org/10.2134/agronj2015.0298

Olfert O, Weiss RM, Catton H, et al. 2019. Bioclimatic assessment of abiotic factors affecting relative abundance and distribution of wheat stem sawfly (Hymenoptera: Cephidae) in western Canada. Canadian Entomologist. 151(1):16–33. https://doi.org/10.4039/tce.2018.46

Pedigo, L. P., M. E. Rice, and R. K. Krell. 2021. Entomology and pest management. Waveland Press, Long Grove, IL

Peirce ES, Evers B, Winn ZJ, et al. 2024. Identifying novel sources of resistance to wheat stem sawfly in five wild wheat species. Pest Management Science. https://doi.org/10.1002/ps.8008

Perez-Mendoza J, Weaver DK. 2006. Temperature and relative humidity effects on postdiapause larval development and adult emergence in three populations of wheat stem sawfly (Hymenoptera: Cephidae). Environmental Entomology. 35(5):1222–1231. https://doi.org/10.1093/ee/35.5.1222

Peterson RKD, Buteler M, Weaver DK, et al. 2011. Parasitism and the demography of wheat stem sawfly larvae, *Cephus cinctus*. BioControl. 56(6):831–839. https://doi.org/10.1007/s10526-011-9357-7

Pruess KP. 1983. Day-Degree methods for pest management. Environmental Entomology. 12(3):613–619. https://doi.org/10.1093/ee/12.3.613

R Core Team. 2025. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/

Runyon JB, Morrill WL, Weaver DK, et al. 2002. Parasitism of the wheat stem sawfly (Hymenoptera: Cephidae) by *Bracon cephi* and *B. lissogaster* (Hymenoptera: Braconidae) in wheat fields bordering tilled and untilled fallow in Montana. Journal of Economic Entomology. 95(6):1130–1134. https://doi.org/10.1603/0022-0493-95.6.1130

Salt R. 1947. Some effects of temperature on the production and elimination of diapause in the wheat stem sawfly, *Cephus cinctus* Nort. Canadian Journal of Research. 25:66–86. https://doi.org/10.1139/cjr47d-004

Schlegel, A. J., Y. Assefa, L. A. Haag, C. R. Thompson, and L. R. Stone. 2019. Soil water and water use in long-term dryland crop rotations. Agronomy Journal. 111: 2590–2599

Seamans HL. 1945. A preliminary report on the climatology of the wheat stem sawfly (*Cephus cinctus* Norton.) on the Canadian prairies. Scientific Agriculture. 25(7):432–457. https://doi.org/10.4141/sa-1945-0012

Sjolie DM, Willenborg CJ, Vankosky MA. 2024. The causes of wheat stem sawfly (Hymenoptera: Cephidae) larval mortality in the Canadian prairies. The Canadian Entomologist. 156. https://doi.org/10.4039/tce.2023.29

Subedi M, Cárcamo HA, Knodel JJ, et al. 2021. Stability analysis of stem solidness, grain yield, and grain protein concentration in spring wheat. Canadian Journal of Plant Science. 101(4):456–475. https://doi.org/10.1139/cjps-2020-0089

Teixeira LAF, Mason K, Isaacs R. 2009. Control of grape berry moth (Lepidoptera: Tortricidae) in relation to oviposition phenology. Journal of Economic Entomology. 102(2):692–698. https://doi.org/10.1603/029.102.0230

Teixeira LAF, Polavarapu S. 2001. Postdiapause development and prediction of emergence of female blueberry maggot (Diptera: Tephritidae). Environmental Entomology. 30(5):925–931. https://doi.org/10.1603/0046-225X-30.5.925

Unger, P. W. 1992. Infiltration of simulated rainfall: tillage system and crop residue effects. Soil Science Society of America Journal. 56: 283–289

Van Dyke A, Wickwar D, Dupuy MM, et al. 2023. Evaluating insecticide application timings against billbugs (*Sphenophorus spp*.) using a degree-day model and calendar-based approach in

the Intermountain West. Crop, Forage and Turfgrass Management. 9(2). https://doi.org/10.1002/cft2.20252

Villacorta A, Bell RA, Callenbach JA. 1972. Respiratory activity during development and diapause of *Cephus cinctus* (Hymenoptera: Cephidae), with emphasis on effect of temperature. Annals of the Entomological Society of America. 65(2):419–422. https://doi.org/10.1093/aesa/65.2.419

Wallace L, McNeal F. 1966. Stem sawflies of economic importance in grain crops in the United States. Entomology Research Division.

Wallace LE. 1962. Field-plot tests of chemicals for wheat stem sawfly control. Journal of Economic Entomology. 55(6):908–912. https://doi.org/10.1093/jee/55.6.908

Wallace LE, Butler LI. 1967. Residues of heptachlor and heptachlor epoxide in wheat treated to control the wheat stem sawfly. Journal of Economic Entomology. 60(1):254–256. https://doi.org/10.1093/jee/60.1.254

Wood SN. 2010. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. Journal of the Royal Statistical Society Series B: Statistical Methodology. 73(1):3–36. https://doi.org/10.1111/j.1467-9868.2010.00749.x

CHAPTER 3: HOST SELECTION AND SUITABILITY OF HERBICIDE-TOLERANT CLEARFIELD® WHEAT CULTIVARS TO WHEAT STEM SAWFLY (*Cephus cinctus*)

Introduction

Wheat stem sawfly (WSS), *Cephus cinctus* Norton, is a native grass-feeding insect and persistent pest for over a century (Ainslie 1920, Weiss and Morrill 1992, Beres et al. 2011). Wheat stem sawfly can cause severe damage to wheat via grain weight reduction and plant lodging (Ainslie 1920, Holmes 1977, Morrill et al. 1992, Cárcamo et al. 2016). Among integrated pest management strategies for suppressing WSS populations, enhancing plant defense through genetic resistance has proven the most effective. Plant defenses fall into two categories: direct and indirect. Direct defenses cause harm to herbivores through mechanical traits or chemical substances that disrupt insect growth and development. Indirect defenses, on the other hand, involve signaling to natural enemies or neighboring plants to either boost their effectiveness or "alert" other plants (Price et al. 1980, Dicke 2000, Schoonhoven et al. 2005, Arimura et al. 2009, Gols 2014, Aljbory and Chen 2018). These defenses can be constitutive (always present) or induced (triggered by herbivory or wounding) (Dudareva et al. 2006, Heil 2008, Kaplan et al. 2008, War et al. 2012).

Plants produce both primary and secondary metabolites. Primary metabolites support core functions like growth and development, while secondary metabolites are often linked to defense against biotic and abiotic stressors (Dicke 2000, Schoonhoven et al. 2005). Many of these defense-related compounds are considered "infochemicals" due to their key roles in plant-plant and plant-insect communication, and known as volatile organic compounds (VOCs) (Vet and Dicke 1992, Rudgers and Whitney 2006, Cha et al. 2011). For instance, some plants respond to insect oviposition by emitting specific volatiles that attract natural enemies of the herbivore. In

Scots pine, *Pinus sylvestris*, egg deposition by *Diprion pini* Linnaeus triggers the release of volatiles that lure the egg parasitoid *Chrysonotomyia ruforum*, offering a proactive defense before larvae emerge (Hilker et al. 2002, 2005). Similarly, feeding by mirid bugs on tomato plants induces the release of herbivore-induced plant volatiles (HIPVs) that activate defense genes in nearby plants, repel additional pests, and attract beneficial parasitoids (Pérez-Hedo et al. 2021). Moreover, infestation by rice striped stem borer (*Chilo suppressalis*) larvae triggers rice plants to emit specific VOCs that repel adult female rice striped stem borer from laying eggs, and attract natural enemies of the pest (Shen et al. 2024).

In the case of WSS management, increasing stem solidness remains the most widely adopted tactic for resistance (Wallace et al. 1973, Beres et al. 2011, Varella et al. 2015, Subedi et al. 2021, Wong et al. 2022 Nov 23, Bathini et al. 2023). In addition to the mechanical protection provided by stem solidness – which contributes to larval mortality through antibiosis – the quantitative trait locus (QTL) on chromosome 3B associated with this trait has also been shown to influence host preference. This antixenosis effect alters WSS oviposition behavior, adding another layer of plant resistance by discouraging egg-laying (Varella et al. 2017). However, stem solidness is typically expressed in early developmental stages (Hager et al. 2025), making it ineffective during years with late or extended WSS flights (Vieira et al. 2025). In addition, solidness is highly variable, influenced by environmental conditions, and is linked to a noticeable yield drag in regions with low WSS pressure (Beres et al. 2012, 2013, Szczepaniec et al. 2015, Nilsen et al. 2016, 2017, Subedi et al. 2021), which limits its effectivness. These limitations reinforce the need to continue the search for new sources of WSS resistance (Peirce et al. 2024).

Several studies have examined host preference in WSS, particularly in relation to different wheat and barley cultivars (Buteler et al. 2010, Buteler and Weaver 2012, Achhami et al. 2020). This behavior is typical of oligophagous insects like WSS, which often specialize within a single plant family (Schoonhoven et al. 2005, Gripenberg et al. 2010). Previous research identified VOCs from wheat plants associated with WSS attraction, including (Z)-3-hexenyl acetate, (Z)-3-hexenol, β-ocimene, linalool, and 6-methyl-5-hepten-2-one (Peck 2004, Piesik et al. 2008, Weaver et al. 2009, Buteler et al. 2010, Buteler and Weaver 2012, Achhami et al. 2021). Lavergne et al. (2020) further demonstrated that WSS infestation triggers cultivar-specific changes in wheat stem metabolites, including benzenoids and phenolics, many of which are known to function as insect-deterring VOCs or their precursors. More recently, Hager et al. (2024) found that oat stems differ significantly from wheat in their baseline levels of lipids, defense compounds, and metabolites related to cell structure, highlighting key biochemical features underlying oat's resistance to WSS.

Alongside to WSS, wheat farmers must still contend with other persistent agronomic challenges, such as weed competition (Jabran et al. 2017). One response to this has been the adoption of Clearfield® (CL) technology, introduced through a mutagenesis targeting the acetolactate synthase (ALS) gene (BASF 2010). This mutation-based trait enabled selective herbicide application, such as imazamox, to control grassy weeds without damaging wheat (Jiménez et al. 2015, Nakka et al. 2019). Interestingly, some farmers report reduced WSS adult activity and less lodging in fields planted with 'CL Plus' cultivars, suggesting potential added benefits beyond weed control (personal communication).

CL wheat carries a single point mutation (Ser653Asn) in the ALS gene – key enzyme in the biosynthesis of branched-chain amino acids – on chromosome 6D, conferring herbicide

resistance (Jimenez et al. 2016, Nakka et al. 2019). Clearfield Plus (CL Plus) wheat combines two independent ALS mutations across different sub-genomes (A and D or B and D), resulting in stronger and more stable tolerance (Rojano-Delgado et al. Clearfield Plus (CL Plus) wheat combines two independent ALS mutations across different sub-genomes (B and D), resulting in stronger and more stable tolerance (Rojano-Delgado et al. 2015, Jimenez et al. 2016). Beyond target-site resistance, these mutations are also linked to enhanced metabolic detoxification related to imazamox herbicide application, including hydroxylation and glucosylation of herbicides via cytochrome P450 monooxygenases and glucosyltransferases (Rojano-Delgado et al. 2015, Jimenez et al. 2016, Soni et al. 2022). Further, Kraus and Stout (2019) found that herbicide applications can directly and indirectly (via plant defense activation) impact pest dynamics in rice, including ALS inhibitor herbicides.

Notably, ALS mutations have been shown to alter amino acid metabolism in rice plants (Endo et al. 2013). Endo et al. showed that it disrupt feedback inhibition by branched-chain amino acids, leading to elevated levels of valine, leucine, and isoleucine (Endo et al. 2013). Increases in other amino acids, such as phenylalanine, methionine, and citrulline, have also been reported. These compounds play key roles in defense pathways mediated by salicylic acid, ethylene, and reactive oxygen species (Endo et al. 2013). These amino acids and their derivatives are known to be precursors of strong plant defense pathways (jasmonic acid) in rice plants (Ruan et al. 2019, Fu et al. 2022), and in the biosynthesis of VOCs involved in plant signaling (Divekar et al. 2022), providing a strong biochemical link to defense mechanisms. These metabolic shifts may help explain anecdotal reports from Colorado of reduced WSS activity and lodging in CL Plus wheat fields. While untested, this raises the possibility that CL Plus cultivars carry

unrecognized traits that affect WSS behavior or suitability, an idea that warrants formal investigation.

Given the central role of plant-insect chemical interactions in shaping host preference and suitability (Atkins 1980, Gripenberg et al. 2010), we hypothesized that CL Plus wheat may be a less favorable host for WSS – either by deterring adult oviposition (antixenosis) or impairing larval survival (antibiosis) – and that these effects may be linked to differences in chemical signaling, such as VOCs and secondary metabolites. To test this hypothesis, we conducted a multi-year investigation combining one year of greenhouse experiments and two years of replicated field trials, followed by VOC profiling using thermal desorption and gas chromatography–mass spectrometry (TD-GC-MS). Our objectives were: (1) to evaluate whether WSS exhibits exhibits host preference for CL Plus cultivars relative to WSS-susceptible hollow-stem cultivars – including assessment of herbicide spray-treated plants to explore whether herbicide metabolism influences WSS interactions; and (2) to identify VOCs potentially contributing to resistance in CL Plus wheat. By integrating behavioral data with chemical profiles, this study seeks to clarify the mechanisms of WSS non-preference and support the development of cultivars with enhanced resistance.

Methods

We evaluated five winter wheat cultivars: one highly susceptible hollow-stem cultivar, Byrd (PlainsGold, 2011); two semi-solid, WSS-resistant cultivars, Fortify SF (PlainsGold, 2019) and Amplify SF (PlainsGold, 2021); and two CL Plus cultivars, Byrd CL Plus (PlainsGold, 2018) and Brawl CL Plus (PlainsGold, 2011). It is worth noting that Byrd CL Plus is a backcross (four backcrosses) derivative of Byrd, possessing a Byrd pedigree of 93.25%.

Seeds were sourced from Colorado State University's wheat breeding program, and germination began in late January. To account for temperature-driven variability in WSS emergence (Vieira et al. 2025) and their preference for wheat at the stem elongation stage (Holmes and Peterson 1960, Beres et al. 2011), we staggered germination across three planting dates, each spaced one week apart. The experimental protocol from followed one used by Peirce et al. (2022); seeds were germinated on 5 × 10 mm blotter paper (Anchor Paper Co., St. Paul, MN) moistened with 5 ml of a 10 ppm aqueous solution of Dividend XL RTA seed treatment (Syngenta, Greensboro, NC) to prevent fungal growth. Seeds were held at 25 °C in the laboratory (Insectary, Colorado State University) for five days to allow for sprouting.

Germinated seeds were then transferred to a walk-in refrigerator set at 2 °C for a period of eight weeks. After vernalization, a single seedling was planted in a conetainer (Stuewe & Sons SC10U UV-stabilized cones: 3.8 cm diameter × 20.9 cm depth, 164 ml volume) filled with a soil mixture of seven parts germination blend (Lambert 20B/30V, Québec, Canada) and two parts perlite. A cotton ball was placed at the bottom of each container to prevent soil loss and support water retention. Plants were grown in a greenhouse at Colorado State University with day/night temperatures of 23 °C/18 °C and a 16:8 light:dark photoperiod. Conetainers were arranged in trays (42 per tray) and bottom-watered as needed. Plants were regularly fertilized using Peters Excel 15-15-15 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. To manage aphids and mites, biological control agents (ladybugs and green lacewings) were released. While plants developed in the greenhouse, WSS sampling was conducted twice weekly beginning in mid-April. Monitoring WSS emergence and flight activity

at the field sites allowed infestations to be timed with peak population periods to improve infestation success.

Greenhouse study

In 2023, we collected infested wheat (cultivar Amplify SF) from past field season stubble at New Raymer, CO, and transferred it to the laboratory under controlled conditions (25 °C) to promote WSS emergence for use in artificial infestations. Each cultivar – Byrd, Fortify SF, Amplify SF, Brawl CL Plus, and Byrd CL Plus – was placed in a separate meshed cage (30 × 18 in) to assess susceptibility to infestation. Each cage contained 42 plants, and 23-25 female WSS were introduced per cage. Half of the plants were immediately dissected to count oviposited eggs; the remaining plants were left to mature for larval survival assessments.

Dissected plants were evaluated for traits linked to WSS oviposition: Zadoks stage, stem diameter, plant height, stem solidness, egg count, larval presence, frass, plant cutting, and larval status (alive or dead).

Field study

In 2024 and 2025, we used natural WSS infestations. When the plants reached appropriate development stage (2024: average Zadoks 57 and 2025 average Zadoks 39) and WSS adult populations were active, conetainers were transported to WSS-infested field sites: New Raymer, CO (2024), and Byers, CO (2025). Because WSS emergence is weather-dependent, plant physiological stage (Zadoks et al. 1974) at the time of infestation varied between years. Field sites were selected based on historically high WSS pressure. Plants were placed directly into wheat stubble from the previous season to ensure emerging females had access to oviposition targets without competition from current season wheat. Plants were

exposed to natural WSS infestation for 4-6 hours, with timing adjusted for weather conditions. After exposure, plants were returned to the lab for analysis.

Across both years, we evaluated eggs per plant and infestation rates by cultivar. Given the climate sensitivity of WSS emergence, we also assessed how wheat physiological stage influenced oviposition in certain cultivars. Additionally, we measured stem cutting (lodging) in 2025 to assess cultivar standability; an important trait for wheat growers regarding harvest efficiency. Dissected plants were evaluated for traits linked to WSS oviposition: Zadoks stage, stem diameter, plant height, stem solidness, egg count, larval presence, frass, plant cutting, and larval status (alive or dead). In 2025, our sample size was reduced by half due to failed infestation in the second planting batch, despite proper exposure to field conditions. The cause for no infestation by WSS is unknown.

Herbicide spray study

Across all years, we tested whether herbicide application influenced WSS host preference and plant suitability. To simulate typical grower management practices, herbicide was applied just before stem elongation. In 2023, we conducted the study indoors using meshed cages, each containing 42 plants. Female WSS (23-25 per cage) were introduced when wheat reached the heading stage (average Zadoks 50). Each cultivar was placed in a separate cage, with herbicide-treated and untreated plants kept in different cages, even within the same cultivar. This setup was similar to the design of the 2023 indoor cultivar comparison trial.

In 2024 and 2025, the same herbicide application protocol was used, but infestations occurred under natural conditions in WSS-infested wheat stubble fields. In all three years, herbicide was applied at the four-leaf stage (plants ~15 cm tall) using imazamox (Beyond®; BASF) at 53 g ai ha^{-1}, plus 1% (v/v) methylated seed oil (MSO) and 5% (v/v) urea ammonium

nitrate (UAN). Applications were made using a laboratory chamber single-nozzle sprayer calibrated to deliver 187 L ha^{-1}. As in other experiments, we evaluated egg counts and infestation rates across cultivars, comparing herbicide-treated and untreated plants to assess potential effects on WSS behavior and plant susceptibility. Dissected plants were evaluated for traits linked to WSS oviposition: Zadoks stage, stem diameter, plant height, stem solidness, egg count, larval presence, frass, plant cutting, and larval status (alive or dead).

Volatile analysis

We used the same greenhouse protocols described previously to grow four winter wheat cultivars: Byrd, Amplify SF, Byrd CL Plus, and Brawl CL Plus. A total of 32 plants were grown - 8 per cultivar. Of these, four plants per cultivar were artificially infested with WSS between stem elongation and booting stages (Zadoks 34-45). Although all cultivars were infested on the same date, minor differences in physiological development resulted in slight variation in Zadoks stage at infestation. At the time of volatile sampling, plants ranged from swollen boot (Zadoks 45) to half inflorescence emergence (Zadoks 55), with most around first spikelet visible (Zadoks 50). From the plants submitted to infestation, we dissected the stems after TD-GC/MS analysis and determined the true infestation numbers: 50% for Byrd, Brawl CL Plus, and Byrd CL Plus, and 25% for Amplify SF.

Plants were enclosed in a 0.23 m3 polytetrafluoroethylene (PTFE) fumigation chamber for one hour. Air flow into the chamber was held at 4.1 L/min. CO_2 levels within the chamber were regulated using a 60 L CO2 cylinder and monitored using a CO_2 /H2O gas analyzer (1.2 L/min, LI–840A, LI-COR, Intl.). Chamber temperature was maintained at 25 °C, relative humidity ranged from 40-50%, and average CO_2 concentration was ~500 ppm. Two brushless fans mixed the air within the chamber. Two inert-coated Tenax sorbent tubes (C1-CXXX-5003,

Markes Intl.) were mounted inside the chamber to capture emitted volatiles. Two low-flow handheld pumps pulled air through the tubes at ~200 mL/min. Between samples, the chamber was flushed with lab air to remove residual volatiles and re-establish a clean baseline, minimizing background interference. To confirm effective volatile capture, we initially tested the setup using two plants, then increased to four plants per sample. The four-plant configuration yielded stronger VOC signals, so only those samples were used for analysis. At the end of each cultivar's run, a blank sample containing only soil and conetainers was collected to account for background noise. Each sorbent tube was labeled accordingly and submitted for GC/MS analysis.

Sorbent tubes were run using a Unity-xr thermal desorption unit (Markes, Intl.) coupled with a single quadrupole GC/MS (Trace 1310 and ISQ QD, Thermo Scientific). High purity helium carrier gas was used for both tube desorption and GC/MS analysis. Tubes were heated at 300 °C for six minutes, then recollected onto a graphitized carbon cold trap before being transferred to the GC/MS. The oven ramp included: 8 min at 90 °C, 20 °C/min until 280 °C, then 2 min at 280 °C. Data was analyzed using Chromeleon™ Chromatography Data System (version 7.3.2, Thermo Fisher Scientific).

Statistical Analysis

We evaluated oviposition, infestation rates, and cutting damage across wheat cultivars and treatments using generalized linear models (GLMs) and negative binomial regression models in R version 4.4.3 (R Core Team 2025). Count data such as egg oviposition per plant exhibited overdispersion and were modeled using negative binomial GLMs via the glm.nb () function from the MASS package. To evaluate oviposition responses to herbicide treatment, the same model structure was used on a separate dataset. Binary response data – infestation rates

(presence/absence of WSS) and cutting damage (presence/absence of stem stubs) – were analyzed using binomial GLMs with a logit link function (glm (..., family = binomial)). Infestation differences among cultivars were modeled as glm (Infested ~ Cultivar, family = binomial), and herbicide effects on infestation were assessed with the same structure applied to the herbicide dataset. Cutting damage was analyzed on a subset of survival trial data using the model glm with binomial distribution.

To examine interaction effects between cultivar and plant developmental stage (Zadoks scale) on egg deposition, we fit a GLM with interaction terms: glm (Eggs ~ Cultivar * Zadoks), accommodating both categorical and continuous predictors. All models were subjected to ANOVA to assess the significance of fixed effects. Where significant differences were detected, Tukey's Honest Significant Difference (HSD) test was used for pairwise comparisons. This was implemented through the multcomp or emmeans packages, depending on model structure. Statistics used were rigorous to detect cultivar-specific differences and treatment effects while accounting for distributional properties of the data (overdispersion, non-normality) and the experimental design structure.

Results

Greenhouse study

In 2023, no significant differences were observed between the susceptible cultivar Byrd and the Clearfield cultivars in the number of eggs per plant (χ^2 (4) = 14.96, p = 0.005; **Figure 3.1A**). Similar patterns were observed for the infestation rate, although there were differences between Byrd and the semi-solid cultivars Fortify SF and Amplify SF (χ^2 (4) = 15.99, p = 0.003; **Figure 3.1B**).

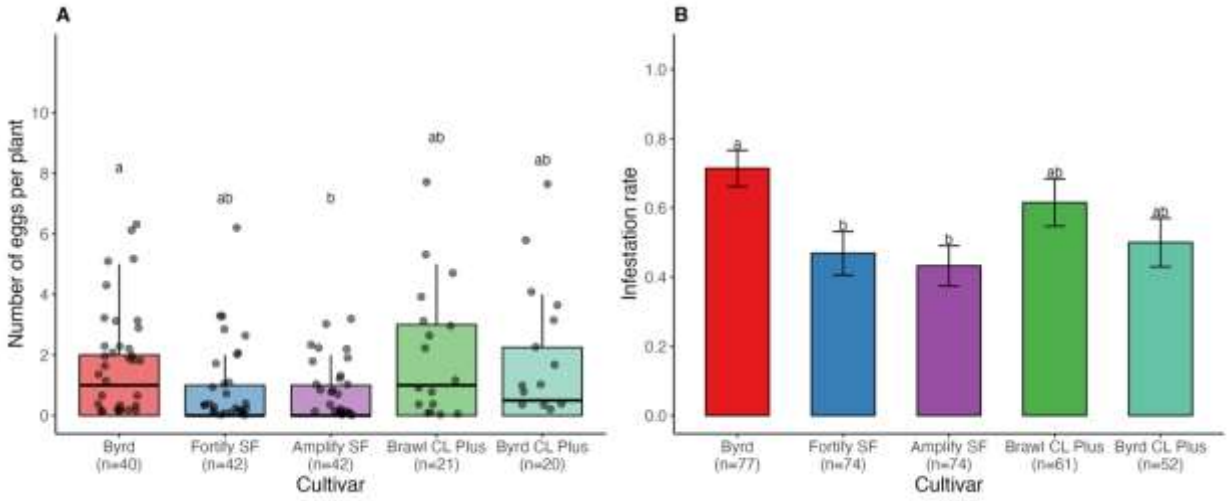

Figure 3.1: Number of wheat stem sawfly eggs per plant (**A**), and infestation rates (**B**) across five wheat cultivars in a greenhouse study conducted in 2023. (A) Data points represent individual plant observations. Statistical analysis was performed using ANOVA, followed by Tukey's HSD test ($p < 0.05$). Letters above boxplots indicate significant differences among cultivars. (**B**) Bars represent the proportion of infested plants per cultivar, with error bars indicating standard error. Statistical analysis was conducted using ANOVA followed by Tukey's HSD test ($p < 0.05$). Letters above bars denote significant differences among cultivars.

Field study

In 2024 and 2025, infestations occurred under natural field conditions, with wheat plants directly exposed to emerging WSS within wheat stubble. In 2024, Byrd had significantly more eggs oviposited per plant than Brawl CL Plus and Byrd CL Plus, while Fortify SF and Amplify SF did not differ significantly from Byrd (χ^2 (4) = 47.42, $p < 0.001$; **Figure 3.2A**). In 2025, oviposition was again significantly higher in Byrd compared to all other cultivars, including the CL lines: Fortify SF, Amplify SF, Brawl CL Plus, and Byrd CL Plus (χ^2 (4) = 58.10, $p < 0.001$; **Figure 3.2B**).

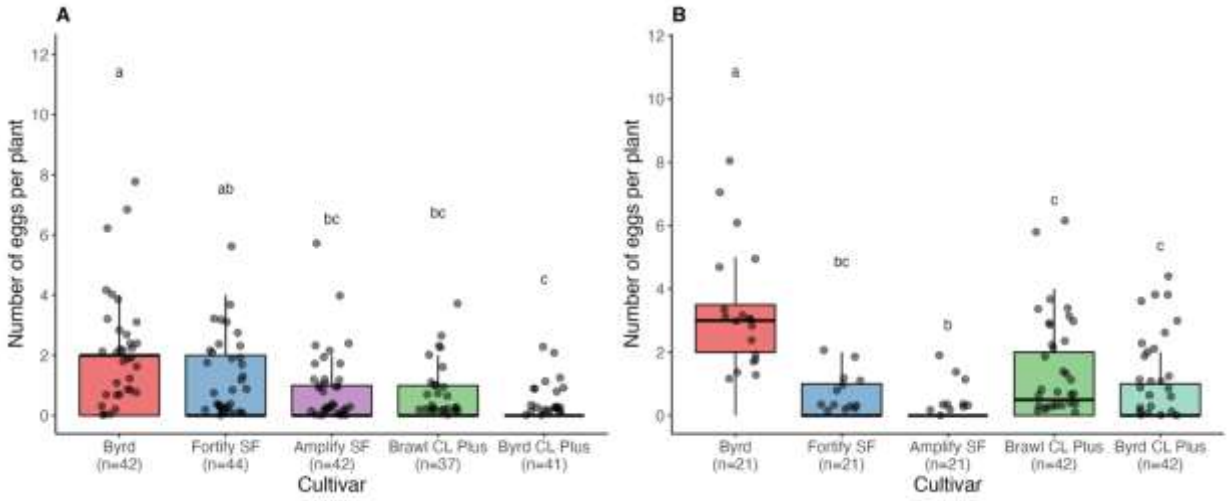

Figure 3.2: Number of wheat stem sawfly eggs per plant across five wheat cultivars in field studies. (**A**) 2024 and (**B**) 2025. Data points represent individual plant observations. Statistical analysis was performed using ANOVA followed by Tukey's HSD test ($p < 0.05$). Letters above boxplots indicate significant differences among cultivars.

For infestation rate, in 2024, Byrd CL Plus exhibited a significantly lower infestation rate than both Byrd and Amplify SF (χ^2 (4) = 25.02, $p < 0.001$; **Figure 3.3A**). In 2025, the same pattern was observed: Byrd had the highest infestation rate, significantly greater than all other cultivars, including both WSS-resistant and CL lines: Fortify SF, Amplify SF, Brawl CL Plus and Byrd CL Plus (χ^2 (4) = 49.01, $p < 0.001$; **Figure 3.3B**).

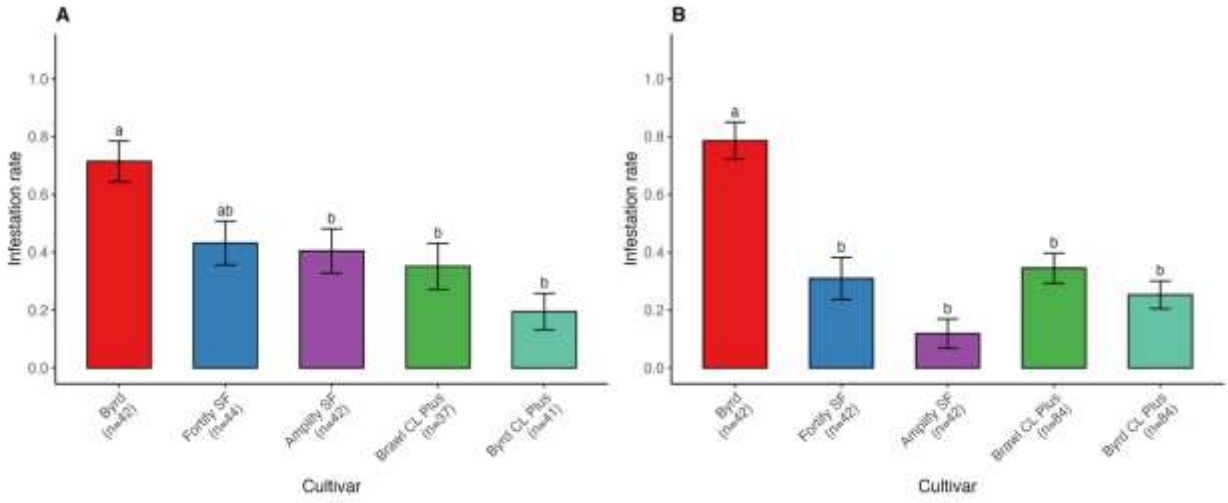

Figure 3.3: Infestation rate of wheat stem sawfly across five wheat cultivars in field studies. (**A**) 2024 and (**B**) 2025. Bars represent the proportion of infested plants per cultivar, with error bars indicating standard error. Statistical analysis was conducted using ANOVA followed by Tukey's HSD test ($p < 0.05$). Letters above bars denote significant differences among cultivars.

Due to climate-driven differences in WSS emergence timing between 2024 and 2025, plants in 2025 were at an earlier developmental stage compared to those in 2024 by the optimal time for infestation. This created an opportunity to compare the performance of semi-solid stem cultivars at different physiological stages. We observed a significant difference in egg oviposition across the two years, especially notable given that overall infestation rates were higher in 2025, when conditions were more favorable for WSS activity (Table 3.1)

Table 3.1: Wheat stem sawfly oviposition across five wheat cultivars in 2024 and 2025, correlated to Zadoks growth stage at sampling. Table includes number of plants sampled, and total eggs collected. The slope (β) represents the change in egg count per unit increase in Zadoks growth stage, indicating how oviposition varies with plant phenology. A positive β suggests increased egg laying at later stages. Statistical significance was tested using a generalized linear model ($p < 0.05$ = *, $p < 0.01$ = **).

Cultivar	2024			2025			Slope (β)	*p*-value	Sig
	Zadoks	Plants	Eggs	Zadoks	Plants	Eggs			
Byrd	56	42	88	43	21	75	-0.019	0.323	
Amplify SF	57	42	31	35	21	4	0.067	0.024	*
Fortify SF	58	44	43	46	21	8	0.102	0.007	**
Brawl CL Plus	59	37	22	35	21	21	-0.006	0.794	
Byrd CL Plus	58	41	10	36	21	25	-0.024	0.352	

Herbicide spray study

In 2023 an herbicide spray study was conducted in the greenhouse to determine the effect of herbicide sprayed versus unsprayed treatments. Wheat stem sawfly laid significantly fewer

eggs on both herbicide-treated CL Plus cultivars – Brawl CL Plus H and Byrd CL Plus H – compared to the untreated susceptible cultivar Byrd (χ^2 (4) = 22.99, $p < 0.001$; **Figure 3.4A**). Infestation rate followed a similar trend: Byrd had a significantly higher rate than Brawl CL Plus H (χ^2 (4) = 19.29, $p < 0.001$; **Figure 3.4B**). The only significant difference observed between herbicide-treated and untreated plants was in the number of eggs oviposited on Brawl CL Plus. Based on this result, we expanded the herbicide effect study to field conditions under natural WSS infestation in subsequent years. No other pairwise comparisons among treatments showed statistically significant differences in 2023.

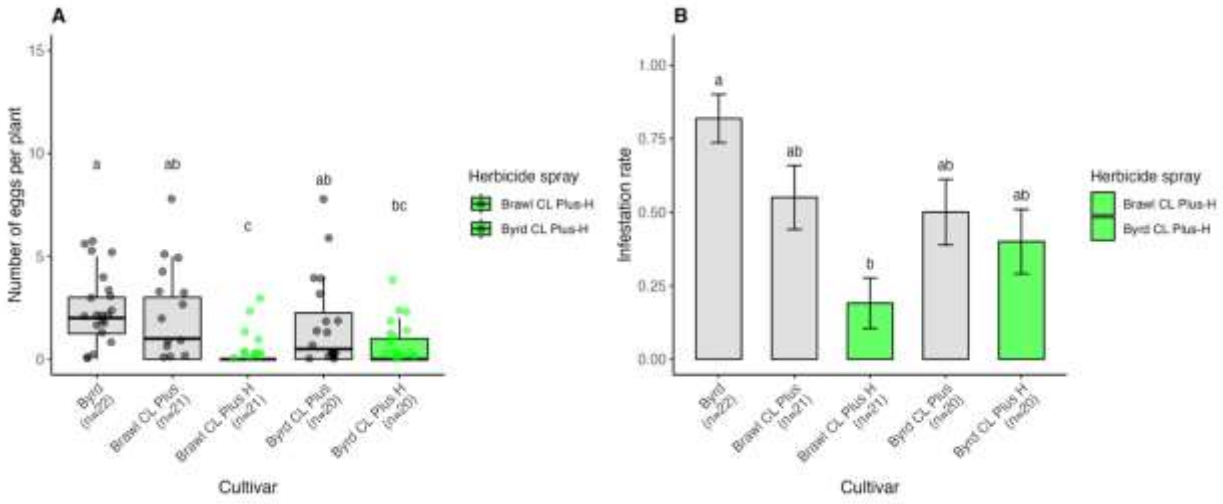

Figure 3.4: Number of wheat stem sawfly eggs per plant (**A**), and infestation rates (**B**) across Clearfield wheat cultivars with and without herbicide treatment in a greenhouse study conducted in 2023. (**A**) Data represent oviposition for WSS susceptible hollow-stem cultivar Byrd, Brawl CL Plus and Byrd CL Plus under herbicide-treated and untreated conditions. Statistical analysis was conducted using ANOVA followed by Tukey's HSD test ($p < 0.05$). Letters above boxplots indicate significant differences among treatments. (**B**) Bars represent the proportion of infested plants per cultivar, with error bars showing standard error. Data represent infestation for WSS susceptible hollow-stem cultivar Byrd, Brawl CL Plus and Byrd CL Plus under herbicide-treated and untreated conditions. Statistical analysis was conducted using ANOVA followed by Tukey's HSD test ($p < 0.05$). Letters above boxplots indicate significant differences among treatments.

In 2024 and 2025, WSS laid significantly fewer eggs on both herbicide-treated and non-treated CL Plus cultivars compared to the untreated susceptible cultivar. In 2024, Byrd had

significantly more eggs than all treatments, including both herbicide-treated and untreated plants (χ^2 (4) = 100.01, $p < 0.001$; **Figure 3.5A**). However, no differences were observed between the sprayed and non-sprayed groups within each CL Plus cultivar. In 2025, Byrd was again significantly more attacked by WSS compared to all CL Plus cultivars, regardless of herbicide treatment. As in the previous year, no significant differences were found between herbicide-treated and untreated plants within either CL Plus cultivar (χ^2 (4) =31.18, $p < 0.001$ **Figure 3.5B**).

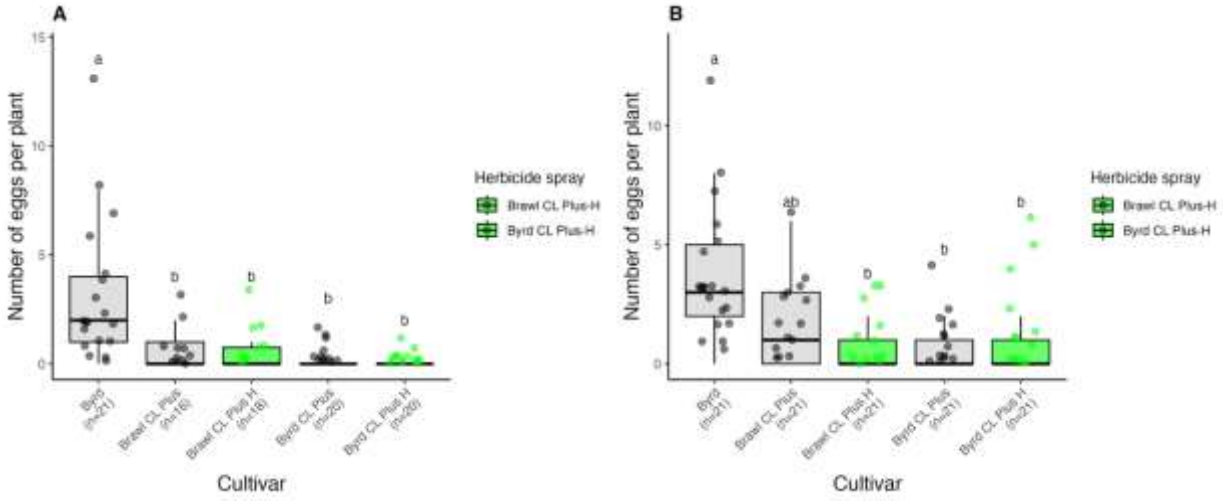

Figure 3.5: Number of wheat stem sawfly eggs per plant across Clearfield wheat cultivars with and without herbicide treatment in field studies. (**A**) 2024 and (**B**) 2025. Data represent oviposition for WSS susceptible hollow-stem cultivar Byrd, Brawl CL Plus and Byrd CL Plus under herbicide-treated and untreated conditions. Statistical analysis was conducted using ANOVA followed by Tukey's HSD test ($p < 0.05$). Letters above boxplots indicate significant differences among treatments.

In 2024, Byrd again showed a higher infestation rate than Brawl CL Plus H, Byrd CL Plus, and Byrd CL Plus H. No significant differences were observed between herbicide-treated and untreated plants within either Clearfield cultivar (χ^2 (4) = 24.18, $p < 0.001$; **Figure 3.6A**). The 2025 data followed a similar pattern: Byrd had a higher infestation rate than Brawl CL Plus H and Byrd CL Plus. As in 2024, no significant differences were found between herbicide-treated and untreated plants (χ^2 (4) = 15.45, $p = 0.004$; **Figure 3.6B**). Although herbicide

application proved to have no effect on WSS oviposition or infestation, these findings further support that CL Plus lines consistently experience reduced WSS pressure compared to the susceptible cultivar Byrd.

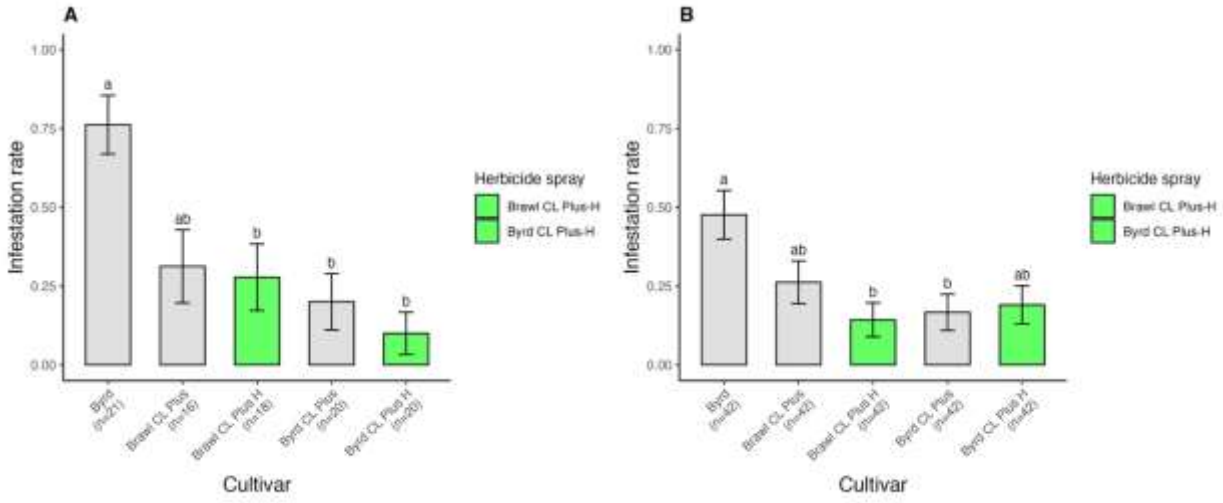

Figure 3.6: Infestation rates of wheat stem sawfly across Clearfield wheat cultivars with and without herbicide treatment in field studies. (**A**) 2024 and (**B**) 2025. Bars represent the proportion of infested plants per cultivar, with error bars showing standard error. Data represent infestation for WSS susceptible hollow-stem cultivar Byrd, Brawl CL Plus and Byrd CL Plus under herbicide-treated and untreated conditions. Statistical analysis was conducted using ANOVA followed by Tukey's HSD test ($p < 0.05$). Letters above boxplots indicate significant differences among treatments.

Stem Cutting

In 2025, severe WSS damage led to plant lodging, providing an opportunity to evaluate differences in cutting rates among cultivars. The susceptible cultivar Byrd had a significantly higher cutting rate than both CL Plus cultivars: Brawl CL Plus and Byrd CL Plus (χ^2 (4) = 25.74, $p < 0.001$; **Figure 3.7**); demonstrating the ability of these CL Plus lines to withstand the most detrimental consequence of WSS infestation.

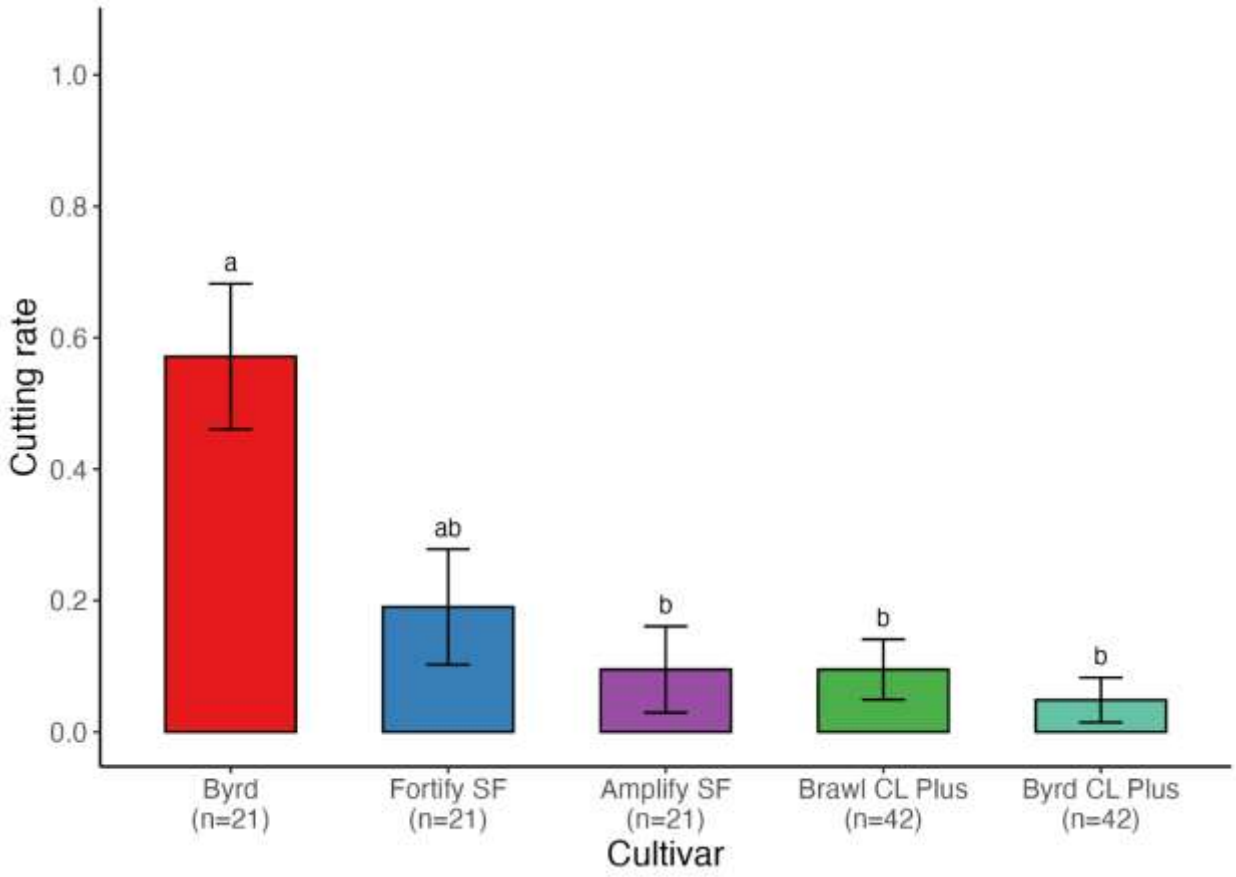

Figure 3.7: Cutting damage caused by wheat stem sawfly across five wheat cultivars in the 2025 field study. Bars represent the mean proportion of plants exhibiting stem cutting (cutting rate) per cultivar, with error bars indicating standard error. Higher values indicate greater susceptibility to WSS cutting. Statistical analysis was conducted using ANOVA followed by Tukey's HSD test ($p < 0.05$). Letters above bars indicate significant differences among cultivars.

Volatile analysis

To assess overall patterns in VOC emission profiles among wheat cultivars, a heatmap was generated using GC/MS peak areas across all compounds and treatments (**Figure 3.8**). Hierarchical clustering of VOCs and cultivars revealed distinct grouping patterns. For the most part, WSS infested and non-infested of the same cultivar cluster together. Interestingly, this was not the case for Byrd CL Plus, which was grouped with the resistant cultivar Amplify SF under

WSS infestation. In addition, Byrd CL Plus, when infested, showed down regulation for the majority of the compounds.

The infestation status clearly influenced clustering across cultivar conditions. For example, Byrd showed a marked upregulation in most compounds when infested with WSS. Some compounds exhibited elevated abundance across multiple cultivars, while others followed cultivar or infestation-specific patterns. Nearly all compounds showed some degree of variation depending on cultivar and WSS infestation. In fact, several compounds – such as (E)-2-octenal, n-hexadecanoic acid, acetophenone, hexanal, α-pinene, 6-methyl-5-hepten-2-one, and heptanal – were highly variable. Notably, 6-methyl-5-hepten-2-one has been consistently associated with WSS repellency and, more recently, to the attraction of braconid parasitoids (Strand et al. 2025).

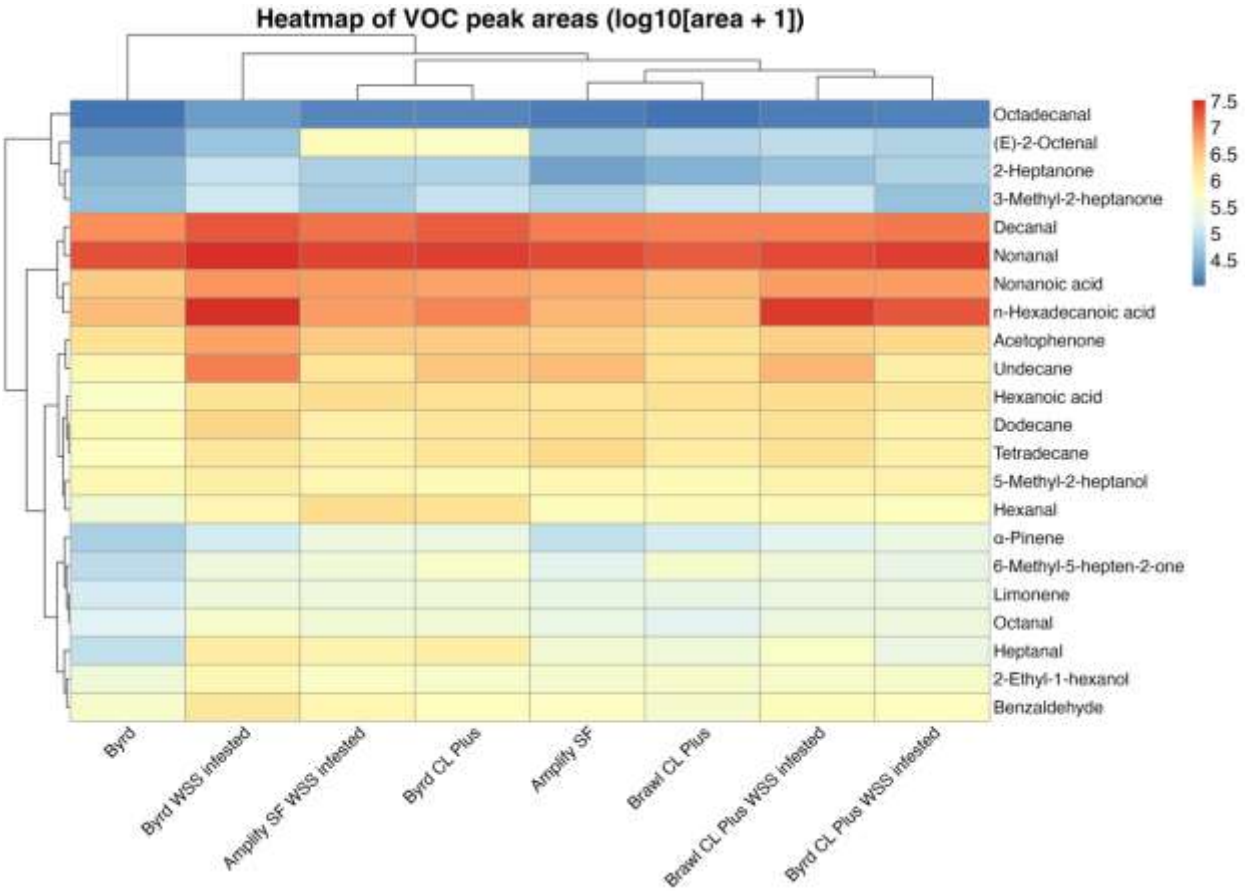

Figure 3.8: Heatmap showing the volatile organic compound (VOC) profiles based on GC-MS peak area values (log_{10}-transformed as log10(area + 1)) across eight different sample conditions: four wheat cultivars (Byrd, Amplify SF, Brawl CL Plus, Byrd CL Plus) in both non-infested and WSS-infested conditions. Compounds (rows) and cultivars (columns) were hierarchically clustered using Euclidean distance and complete linkage, allowing visualization of co-occurrence patterns and similarity in VOC profiles. Warmer colors (red) indicate higher VOC abundance, while cooler colors (blue) indicate lower abundance. The dendrograms group similar VOCs and cultivar profiles, enabling interpretation of cultivar-specific or infestation-related chemical shifts.

To explore VOC composition at a broader chemical group level, compounds were classified into functional categories, and the total area per group was aggregated per cultivar. Fold-Change values were calculated relative to Byrd as the reference cultivar under both non-infested and WSS-infested conditions (**Figure 3.9**; **Supplemental Table A1.1**).

In the non-infested panel, most cultivars exhibited elevated levels of several chemical groups compared to Byrd. Byrd CL Plus showed higher Fold-Change across almost all chemical groups: aldehyde, carboxylic acid, fatty acid, ketone, and terpene (**Supplemental Table A1.1**). Amplify SF and Brawl CL Plus also exhibited overall elevated levels, though to a lesser extent. In contrast, the infested panel revealed a strong upregulation of chemical groups in WSS-infested Byrd, while Amplify SF and Brawl CL Plus showed little to no major change (**Supplemental Table A1.2**). Byrd CL Plus, for the most part, showed a mild downregulation across chemical groups.

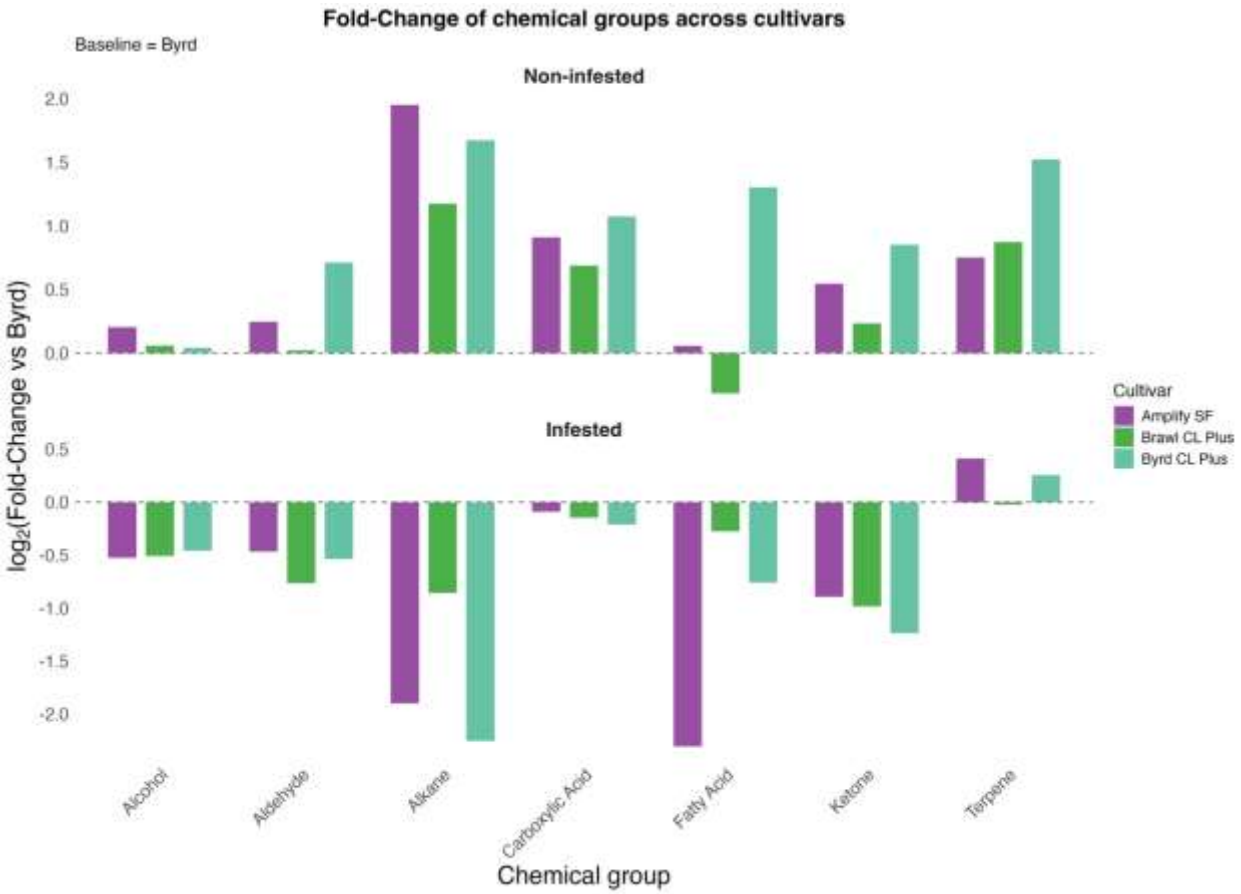

Figure 3.9: Bar plot showing the $\log_2$ fold-change in total peak area of VOC chemical groups across wheat cultivars, relative to Byrd, which serves as the baseline cultivar ($\log_2$ fold-change = 0). Separate panels are shown for non-infested (top) and WSS-infested (bottom) conditions. Each bar represents a cultivar: Amplify SF, Brawl CL Plus, and Byrd CL Plus. Positive values indicate higher abundance compared to Byrd, while negative values indicate lower abundance. A dashed horizontal line marks the baseline ($\log_2$ FC = 0). Chemical groups were derived by grouping compounds according to their functional classification, and total signal area was aggregated prior to transformation. This figure illustrates how VOC group composition shifts by cultivar and infestation status.

Discussion

Greenhouse and field analysis

Over the course of three years of integrated greenhouse and field research, our findings provide strong and consistent evidence that CL Plus cultivars are less preferred by WSS for oviposition and have lower infestation and stem cutting rates compared to the susceptible

hollow-stem cultivar Byrd. This trend emerged most clearly under natural field conditions and persisted across variable environmental contexts and plant developmental stages. Under controlled greenhouse conditions, where WSS females were given no choice and were artificially introduced into cages, we observed limited differences in host preference. In the 2023 study, oviposition rates did not differ significantly among cultivars, including CL Plus lines. This suggests that when females are restricted to a single host, they readily oviposit across cultivars without strong discrimination. These results are consistent with previous no-choice studies, where behavioral constraints often mask host preferences that would otherwise emerge under natural conditions (Weaver et al. 2009, Buteler and Weaver 2012). This finding also suggests a lack of a strong constitutive defense by the tested CL cultivars (Kaplan et al. 2008). Additionally, the high infestation observed in semi-solid stem cultivars – exceeding 40% (see **Figure 3.1**) – reinforces prior work questioning the consistency of protection provided by semi-solid phenotypes (Subedi et al. 2021, Hager et al. 2025). It is also worth noting that all WSS females were introduced simultaneously and most died within the first 24 hours, which may have shortened their oviposition behavior and affected total egg counts.

However, a contrasting pattern emerged under natural field conditions. Field trials conducted in 2024 and 2025 revealed clear differences in host preference when WSS adults were allowed to select among cultivars without restriction. In both years, the susceptible cultivar Byrd received significantly more eggs than the CL Plus cultivars Brawl CL Plus and Byrd CL Plus, and infestation rates mirrored this trend. These differences were especially pronounced in 2025, when weather conditions favored higher WSS activity, amplifying overall infestation levels and making cultivar differences even more apparent. These results are consistent with past studies showing that WSS females exhibit host discrimination when given a choice, and that traits

beyond stem solidity can influence oviposition behavior (Holmes and Peterson 1960, Piesik et al. 2008, Weaver et al. 2009, Lavergne et al. 2020). The consistent underperformance of hollow-stem cultivars across both seasons highlights its ongoing vulnerability and underscores the urgent need for more resistant alternatives.

Importantly, across both years, CL Plus lines maintained lower egg counts and infestation rates despite genetic background similarities with Byrd (as in Byrd vs. Byrd CL Plus). This supports the hypothesis that resistance in CL Plus lines may be linked to physiological or biochemical changes triggered by ALS-related mutations. In rice, such mutations disrupt feedback inhibition by branched-chain amino acids, resulting in elevated levels of valine, leucine, and isoleucine (Endo et al. 2013, Jimenez et al. 2016, Vázquez-García et al. 2022). A similar accumulation could be occuring in CL Plus wheat, potentially activating defense-related pathways (Ruan et al. 2019, Fu et al. 2022). However, this hypothesis requires further investigation.

Given the biochemical nature of resistance hypothesized in CL Plus lines, we also tested whether herbicide application could influence WSS behavior, either by modifying plant chemistry or inducing stress responses. Specifically, we evaluated the effect of imazamox herbicide on oviposition and infestation in herbicide-treated versus untreated CL cultivars. In 2023 greenhouse trials, herbicide-treated CL Plus lines experienced significantly less infestation than the susceptible Byrd; however, besides oviposition on Brawl CL Plus, no significant differences were observed between treated and untreated versions of the same cultivar.
This pattern remained consistent in field trials conducted in 2024 and 2025. Although CL Plus cultivars continued to receive fewer eggs and showed lower infestation rates compared to Byrd, herbicide treatment had no measurable effect within cultivars. These results suggest that the

reduced WSS pressure observed in CL Plus lines is not attributable to herbicide metabolism but is more likely driven by inherent plant traits; possibly linked to the ALS mutation or associated downstream metabolic changes, as previously proposed in rice (Endo et al. 2013). Similar conclusions were drawn by Kraus and Stout (Kraus and Stout 2019), who found that herbicide application did not significantly alter rice stem borer negative effect on rice, reinforcing the idea that imidazolinone-based resistance traits may not induce herbivore resistance as a secondary effect.

Beyond oviposition and infestation rates, our 2025 field trials also provided a unique opportunity to assess the most economically damaging consequence of WSS infestation: stem cutting. Favorable environmental conditions led to high WSS pressure, allowing us to evaluate whether resistance in CL Plus cultivars carries through to later stages of pest development. Cutting rates were significantly higher in Byrd than in either of the CL Plus cultivars, which exhibited cutting rates comparable to the semi-solid stem cultivars (in fact, smaller in raw numbers). This suggests that the resistance observed in CL Plus lines possibly extend beyond antixenosis, and affect host suitability for larval development.

Volatile analysis

We present the most comprehensive thermal desorption TD-GC/MS-based emission inventory for wheat to date. While TD-GC/MS has been previously employed in the context of broad VOC surveys (Winer et al. 1992) and abiotic stress responses (Hartikainen et al. 2012), its application to plant–insect interactions remain virtually unexplored. To our knowledge, this is the first study to apply this high-resolution analytical approach to biotic stress in wheat, as we focus on herbivory by the WSS.

Clustering analysis revealed that non-infested Byrd CL Plus grouped closely with WSS-infested Amplify SF, a cultivar known for both mechanical and chemical resistance to wheat stem sawfly (Hager et al. 2025). This clustering suggests that Byrd CL Plus shares key features of its VOC profile with a resistant cultivar, even in the absence of herbivory, pointing to the presence of constitutive chemical defenses. In contrast, the susceptible Byrd cultivar exhibited a markedly different pattern: only after WSS infestation did it show a strong induction of several VOCs.

The VOCs revealed distinct differences in both constitutive and inducible volatile emissions among wheat cultivars with varying resistance to WSS. Byrd CL Plus exhibited a markedly richer constitutive VOC profile compared to the susceptible Byrd, particularly in compounds previously associated with insect deterrence and defense signaling.

Among these, green leaf volatiles such as (E)-2-octenal and heptanal were especially abundant in Byrd CL Plus. These compounds are well-established mediators of plant–insect interactions, functioning as both direct deterrents and airborne defense signals (Arimura et al. 2009, War et al. 2012, Havlikova et al. 2020, Hong et al. 2023). In our study, Byrd CL Plus showed 12-fold higher constitutive levels of heptanal than Byrd, and 3-fold higher than both Brawl CL Plus and Amplify SF. (E)-2-Octenal emissions were even more impactful – approx. 25-fold higher in Byrd CL Plus than in Byrd, and over approx. 8-fold higher than in Brawl CL Plus and Amplify SF. In addition, Amplify SF when infested by WSS showed levels of (E)-2-Octenal 32-fold higher than non-infested Byrd. Recent findings in *Nicotiana benthamiana* link (E)-2-octenal to priming of the jasmonic acid (JA) and ethylene (ET) defense pathways; though their work focused on viral infection, the same signaling architecture underlies responses to

insect herbivory, positioning (E)-2-octenal as a potential broad-spectrum priming compound (Hong et al. 2023).

Another key compound was 6-methyl-5-hepten-2-one, a volatile found at approximately 5-fold higher levels in both Clearfield cultivars (Byrd CL Plus and Brawl CL Plus) compared to Byrd. This compound has been repeatedly associated with WSS repellency (Piesik et al. 2008, Weaver et al. 2009, Buteler and Weaver 2012), and more recently, with attraction of WSS parasitoids such as *Bracon cephi* and *B. lissogaster* (Strand et al. 2025). Beyond its ecological role, 6-methyl-5-hepten-2-one also acts as a signaling molecule within the plant. It has been shown to induce JA pathway genes, including lipoxygenase (LOX) and allene oxide synthase (AOS), which enhance both direct resistance mechanisms and indirect defense strategies through recruitment of natural enemies (Zhao et al. 2009, Divekar et al. 2022).

Another important VOC group – terpenes – is known to be involved in insect-plant communication. For example, limonene and α-pinene have been observed to be attractive at low concentrations but deterrent at higher levels, with potential roles in indirect defense via natural enemy recruitment (Yi et al. 2019, Bhowmik et al. 2022). In our study, α-pinene was detected at constitutively higher levels in Byrd CL Plus (4-fold higher than in Byrd) and slightly increased further following WSS infestation. In rice systems, α-pinene has been shown to deter stem borers and modulate insect chemosensory genes (Yi et al. 2019), which makes a compelling argument to investigate its functionality in wheat, particularly in the context of the stem-boring WSS. Its role in wheat remains largely unexplored, making it a worthwhile direction for future research. Fatty acid-derived compounds also emerged as key contributors to wheat defense. n-Hexadecanoic acid (palmitic acid) displayed both constitutive and inducible expression patterns: it was naturally elevated in Byrd CL Plus and strongly induced – by up to 8-fold – in Byrd and

Brawl CL Plus following WSS infestation. Similar responses have been observed in wild tomatoes, where this compound contributes to both baseline resistance and JA-linked defense signaling against herbivores (Kumaraswamy et al. 2025). Another important fatty acid, nonanoic acid, was also strongly induced by WSS feeding, particularly in Byrd CL Plus and Brawl CL Plus. In other systems, nonanoic acid was related to avoidance behavior and has been shown to reduce herbivore oviposition by activating antennal ionotropic receptors (Guo et al. 2023). Several additional VOCs detected in wheat – including tetradecane, decanal, nonanal, and dodecane – have been implicated in complex plant–insect dynamics across multiple crop systems (Li et al. 2022, Yin et al. 2022). In *Cucurbita maxima*, for instance, tetradecane and other alkanes act as herbivore attractants, while decanal facilitates host location (Bhowmik et al. 2022). In rice, prior exposure to herbivore-induced volatiles such as acetophenone and dodecane has been shown to enhance JA-related gene expression and increase the attraction of parasitoids (Yao et al. 2023).

In summary, our findings suggest that VOC-mediated defense in CL Plus wheat is both genotype-dependent and herbivore-responsive, with Byrd CL Plus exhibiting strong constitutive emissions of key defense volatiles. The chemical profile appears more complex than previously reported for the wheat–WSS system, and the dynamic changes observed between resistant cultivars and infested versus non-infested plants underscore the need to study a broader suite of compounds to fully understand plant defense outcomes. In particular, compounds such as 6-methyl-5-hepten-2-one, α-pinene, and n-Hexadecanoic acid emerge as promising targets for future research due to their potential roles in direct defense and the recruitment of natural enemies.

Conclusion

These results support the idea that CL Plus cultivars may exhibit a combination of antixenosis and antibiosis mechanisms – an integrated defense strategy previously observed in other wheat genotypes and plant-insect systems (Biyiklioglu et al. 2018, Achhami et al. 2020). Lower egg deposition reduces initial infestation pressure, while downstream physiological or structural defenses may impair larval development, ultimately reducing lodging and yield losses. The consistent performance of CL Plus cultivars across years, environments, and WSS pressure levels points toward a resistance mechanism not explained by traditional morphological traits. Instead, these patterns suggest that physiological or biochemical differences (potentially related to plant signaling or metabolism) may underlie the reduced WSS success observed in these lines. Further, this is supported by our TD-GC/MS analysis that exhibits higher plant defense volatile profile for the CL Plus cultivars, specifically Byrd CL Plus. This interpretation aligns with previous studies documenting cultivar-specific variation in WSS host preference and larval development (Beres et al. 2007, Weaver et al. 2009, Buteler and Weaver 2012, Achhami et al. 2020), reinforcing the need to explore chemical ecology as a key driver of resistance in CL Plus wheat.

Further research should investigate whether CL Plus cultivars accumulate branched-chain amino acids as observed in ALS-mutated rice by Endo et al. (Endo et al. 2013). If confirmed, this could suggest a link between ALS-mediated amino acid accumulation and the activation of induced defense pathways, particularly those involving JA signaling, as proposed by Fu et al. (Fu et al. 2022). Such a mechanism would offer a compelling biochemical explanation for the observed resistance. In addition, behavioral assays should be conducted using individual or synthetic blends of VOCs identified in our profiling work, especially those compounds

associated with jasmonate-regulated linked pathways. These studies could help determine whether specific volatiles contribute to deterrence of oviposition or reduced larval success, offering a more detailed understanding of how chemical cues mediate WSS–host plant interactions in CL Plus wheat.

References

Achhami BB, Reddy GVP, Sherman JD, et al. 2020. Antixenosis, antibiosis, and potential yield compensatory response in barley cultivars exposed to wheat stem sawfly (Hymenoptera: Cephidae) under field conditions. Journal of Insect Science. 20(5):1–14. https://doi.org/10.1093/jisesa/ieaa091

Ainslie CN. 1920. The western grass-stem sawfly. Entomological Assistant, Cereal and Forage Insect Investigations. 841.

Aljbory Z, Chen M. 2018. Indirect plant defense against insect herbivores: a review. Insect Science. 25(1):2–23. https://doi.org/10.1111/1744-7917.12436

Arimura G -i., Matsui K, Takabayashi J. 2009. Chemical and molecular ecology of herbivore-induced plant volatiles: Proximate factors and their ultimate functions. Plant and Cell Physiology. 50(5):911–923. https://doi.org/10.1093/pcp/pcp030

Atkins MD. 1980. Introduction to insect behavior. New York: Macmillan.

BASF. 2010. Clearfield® production system for wheat: Innovation in herbicide-resistant crops. https://agriculture.basf.us/crop-protection/products/herbicides/clearfield.html.

Bathini A, Mendu L, Singh NP, et al. 2023. A “solid” solution for wheat stem sawfly (Hymenoptera: Cephidae) resistance: genetics, breeding and development of solid stem wheat. Crop Science. 63(4):1934–1948. https://doi.org/10.1002/csc2.20998

Beres B, Dosdall L, Weaver D, et al. 2011. Biology and integrated management of wheat stem sawfly and the need for continuing research. The Canadian Entomologist. 143(2):105–125. https://doi.org/10.4039/n10-056

Beres BL, Cárcamo HA, Byers JR, et al. 2013. Host plant interactions between wheat germplasm source and wheat stem sawfly *Cephus cinctus* Norton (Hymenoptera: Cephidae) I. Commercial cultivars. Canadian Journal of Plant Science. 93(4):607–617. https://doi.org/10.4141/CJPS2012-088

Beres BL, Cárcamo HA, Cárcamo HA, et al. 2007. Effect of wheat stem sawfly damage on yield and quality of selected canadian spring wheat. Journal of Economic Entomology. 100(1):79–87.

Beres BL, McKenzie RH, Cárcamo HA, et al. 2012. Influence of seeding rate, nitrogen management, and micronutrient blend applications on pith expression in solid-stemmed spring wheat. Crop Science. 52(3):1316–1329. https://doi.org/10.2135/cropsci2011.05.0239

Bhowmik B, Chakraborti U, Mandal A, et al. 2022. Attraction of *Aulacophora foveicollis* Lucas (Coleoptera: Chrysomelidae) to Host Plant *Cucurbita maxima* Duchesne (Cucurbitaceae) Volatiles. Agronomy. 12(11):2640. https://doi.org/10.3390/agronomy12112640

Biyiklioglu S, Alptekin B, Akpinar BA, et al. 2018. A large-scale multiomics analysis of wheat stem solidness and the wheat stem sawfly feeding response, and syntenic associations in barley, *Brachypodium*, and rice. Functional and Integrative Genomics. 18(3):241–259. https://doi.org/10.1007/s10142-017-0585-5

Buteler M, Weaver DK. 2012. Host selection by the wheat stem sawfly in winter wheat and the role of semiochemicals mediating oviposition preference. Entomologia Experimentalis et Applicata. 143(2):138–147. https://doi.org/10.1111/j.1570-7458.2012.01237.x

Buteler M, Weaver DK, Bruckner PL, et al. 2010. Using agronomic traits and semiochemical production in winter wheat cultivars to identify suitable trap crops for the wheat stem sawfly. Canadian Entomologist. 142(3):222–233. https://doi.org/10.4039/n09-072

Cárcamo HA, Beres BL, Larson TR, et al. 2016. Effect of wheat cultivars and blends on the oviposition and larval mortality of *Cephus cinctus* (Hymenoptera: Cephidae) and parasitism by *Bracon cephi* (Hymenoptera: Braconidae). Environmental Entomology. 45(2):397–403. https://doi.org/10.1093/ee/nvv231

Cha DH, Linn CE, Teal PEA, et al. 2011. Eavesdropping on plant volatiles by a specialist moth: Significance of ratio and concentration. Marion-Poll F, editor. PLoS ONE. 6(2):e17033. https://doi.org/10.1371/journal.pone.0017033

Dicke M. 2000. Chemical ecology of host-plant selection by herbivorous arthropods: a multitrophic perspective. Biochemical Systematics and Ecology. 28:601–617.

Divekar PA, Narayana S, Divekar BA, et al. 2022. Plant secondary metabolites as defense tools against herbivores for sustainable crop protection. International Journal of Molecular Sciences. 23(5):2690. https://doi.org/10.3390/ijms23052690

Dudareva N, Negre F, Nagegowda DA, et al. 2006. Plant volatiles: Recent advances and future perspectives. Critical Reviews in Plant Sciences. 25(5):417–440. https://doi.org/10.1080/07352680600899973

Endo M, Shimizu T, Fujimori T, et al. 2013. Herbicide-resistant mutations in acetolactate synthase can reduce feedback inhibition and lead to accumulation of branched-chain amino acids. Food and Nutrition Sciences. 04(05):522–528. https://doi.org/10.4236/fns.2013.45067

Fu W, Jin G, Jiménez-Alemán GH, et al. 2022. The jasmonic acid-amino acid conjugates JA-Val and JA-Leu are involved in rice resistance to herbivores. Plant Cell and Environment. 45(1):262–272. https://doi.org/10.1111/pce.14202

Gols R. 2014. Direct and indirect chemical defences against insects in a multitrophic framework. Plant Cell & Environment. 37(8):1741–1752. https://doi.org/10.1111/pce.12318

Gripenberg S, Mayhew PJ, Parnell M, et al. 2010. A meta-analysis of preference–performance relationships in phytophagous insects. Ecology Letters. 13(3):383–393. https://doi.org/10.1111/j.1461-0248.2009.01433.x

Guo J-M, Wei Z-Q, Hou J-H, et al. 2023. Ionotropic receptor IR75q.2 mediates avoidance reaction to nonanoic acid in the fall armyworm *Spodoptera frugiperda* (Lepidoptera, Noctuidae). J. Agric. Food Chem. 71(51):20602–20612. https://doi.org/10.1021/acs.jafc.3c05704

Hager MS, Cook JP, Bothner B, et al. 2025. Untargeted metabolomic and transcriptomic analysis in spring and durum wheat reveals potential mechanisms associated with the early stem solidness phenotype and resistance to wheat stem sawfly. Frontiers in Plant Science. 16:1497732. https://doi.org/10.3389/fpls.2025.1497732

Hager MS, Hofland ML, Varella AC, et al. 2024. Untargeted metabolomics profiling of oat (*Avena sativa L.*) and wheat (*Triticum aestivum L.*) infested with wheat stem sawfly (*Cephus*

cinctus Norton) reveals differences associated with plant defense and insect nutrition. Frontiers in Plant Science. 15:1327390. https://doi.org/10.3389/fpls.2024.1327390

Hartikainen K, Kivimäenpää M, Nerg A-M, et al. 2012. Significance of leaf structure and emission of volatile organic compounds in ozone tolerance of oat and wheat. Botany. 90(2):121–135. https://doi.org/10.1139/b11-090

Havlikova M, Bosakova T, Petschenka G, et al. 2020. Analysis of defensive secretion of a milkweed bug *Lygaeus equestris* by 1D GC-MS and GC×GC-MS: sex differences and host-plant effect. Scientific Reports. 10(1):3092. https://doi.org/10.1038/s41598-020-60056-9

Heil M. 2008. Indirect defence via tritrophic interactions. New Phytologist. 178(1):41–61. https://doi.org/10.1111/j.1469-8137.2007.02330.x

Hilker M, Kobs C, Varama M, et al. 2002. Insect egg deposition induces *Pinus sylvestris* to attract egg parasitoids. Journal of Experimental Biology. 205(4):455–461. https://doi.org/10.1242/jeb.205.4.455

Hilker M, Stein C, Schröder R, et al. 2005. Insect egg deposition induces defence responses in *Pinus sylvestris*: Characterisation of the elicitor. Journal of Experimental Biology. 208(10):1849–1854. https://doi.org/10.1242/jeb.01578

Holmes ND. 1977. The effect of the wheat stem sawfly, *Cephus cinctus* (Hymenoptera: Cephidae), on the yield and quality of wheat. The Canadian Entomologist. 109(12):1591–1598. https://doi.org/10.4039/Ent1091591-12

Holmes ND, Peterson LK. 1960. The influence of the host on oviposition by the wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae). Canadian Journal of Plant Science. 40(1):29–46. https://doi.org/10.4141/cjps60-004

Hong Y, Zheng Q, Cheng L, et al. 2023. Identification and characterization of TMV-induced volatile signals in *Nicotiana benthamiana*: evidence for JA/ET defense pathway priming in congeneric neighbors via airborne (E)-2-octenal. Functional & Integrative Genomics. 23(3):272. https://doi.org/10.1007/s10142-023-01203-z

Jabran K, Mahmood K, Melander B, et al. 2017. Weed dynamics and management in wheat. In: Advances in Agronomy. Vol. 145. Elsevier. p. 97–166. https://doi.org/10.1016/bs.agron.2017.05.002

Jiménez F, Fernández P, Rojano-Delgado AM, et al. 2015. Resistance to imazamox in Clearfield soft wheat (*Triticum aestivum L.*). Crop Protection. 78:15–19. https://doi.org/10.1016/j.cropro.2015.08.004

Jimenez F, Rojano-Delgado AM, Fernández PT, et al. 2016. Physiological, biochemical and molecular characterization of an induced mutation conferring imidazolinone resistance in wheat. Physiologia Plantarum. 158(1):2–10. https://doi.org/10.1111/ppl.12445

Kaplan I, Halitschke R, Kessler A, et al. 2008. Constitutive and induced defenses to herbivory in above - and bellowground plant tissues. Ecology. 89(2):392–406. https://doi.org/10.1890/07-0471.1

Kraus EC, Stout MJ. 2019. Direct and indirect effects of herbicides on insect herbivores in rice, *Oryza sativa*. Scientific Reports. 9(1). https://doi.org/10.1038/s41598-019-43361-w

Kumaraswamy S, Yogendra K, Sotelo-Cardona P, et al. 2025. Non-targeted metabolomics reveals fatty acid and associated pathways driving resistance to whitefly and tomato leafminer in wild tomato accessions. Scientific Reports. 15(1):3754. https://doi.org/10.1038/s41598-025-86191-9

Lavergne FD, Broeckling CD, Brown KJ, et al. 2020. Differential stem proteomics and metabolomics profiles for four wheat cultivars in response to the insect pest wheat stem sawfly. Journal of Proteome Research. 19(3):1037–1051. https://doi.org/10.1021/acs.jproteome.9b00561

Li M, Xia S, Zhang T, et al. 2022. Volatiles from cotton plants infested by *Agrotis segetum* (Lep.: Noctuidae) attract the larval parasitoid *Microplitis mediator* (Hym.: Braconidae). Plants. 11(7):863. https://doi.org/10.3390/plants11070863

Morrill WL, Gabor JW, Hockett EA, et al. 1992. Wheat stem sawfly (Hymenoptera: Cephidae) resistance in winter wheat. Journal of Economic Entomology. 85(5):2008–2011. https://doi.org/10.1093/jee/85.5.2008

Nakka S, Jugulam M, Peterson D, et al. 2019. Herbicide resistance: Development of wheat production systems and current status of resistant weeds in wheat cropping systems. Crop Journal. 7(6):750–760. https://doi.org/10.1016/j.cj.2019.09.004

Nilsen KT, Clarke JM, Beres BL, et al. 2016. Sowing density and cultivar effects on pith expression in solid-stemmed durum wheat. Agronomy Journal. 108(1):219–228. https://doi.org/10.2134/agronj2015.0298

Nilsen KT, N'Diaye A, MacLachlan PR, et al. 2017. High density mapping and haplotype analysis of the major stem-solidness locus SSt1 in durum and common wheat. PLoS ONE. 12(4). https://doi.org/10.1371/journal.pone.0175285

Peck GE. 2004. Potential semiochemicals of wheat (*Triticum aestivum L.*) induced by oviposition and feeding of the wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae). Montana State University.

Peirce ES, Cockrell DM, Ode PJ, et al. 2022. Triticale as a potential trap crop for the wheat stem sawfly (Hymenoptera: Cephidae) in winter wheat. Frontiers in Agronomy. 4:779013. https://doi.org/10.3389/fagro.2022.779013

Peirce ES, Evers B, Winn ZJ, et al. 2024. Identifying novel sources of resistance to wheat stem sawfly in five wild wheat species. Pest Management Science. https://doi.org/10.1002/ps.8008

Pérez-Hedo M, Alonso-Valiente M, Vacas S, et al. 2021. Eliciting tomato plant defenses by exposure to herbivore induced plant volatiles. Entomologia Generalis. 41(3):209–218. https://doi.org/10.1127/entomologia/2021/1196

Piesik D, Weaver DK, Runyon JB, et al. 2008. Behavioural responses of wheat stem sawflies to wheat volatiles. Agricultural and Forest Entomology. 10(3):245–253. https://doi.org/10.1111/j.1461-9563.2008.00380.x

Price PW, Bouton CE, Gross P, et al. 1980. Interactions among three trophic levels: Influence of plants on interactions between insect herbivores and natural enemies. Annual Review of Ecology and Systematics. 11(1):41–65. https://doi.org/10.1146/annurev.es.11.110180.000353

R Core Team. 2025. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.

Rojano-Delgado AM, Priego-Capote F, Luque De Castro MD, et al. 2015. Mechanism of imazamox resistance of the Clearfield® wheat cultivar for better weed control. Agronomy for Sustainable Development. 35(2):639–648. https://doi.org/10.1007/s13593-014-0232-7

Ruan J, Zhou Y, Zhou M, et al. 2019. Jasmonic acid signaling pathway in plants. International Journal of Molecular Sciences. 20(10):2479. https://doi.org/10.3390/ijms20102479

Rudgers JA, Whitney KD. 2006. Interactions between insect herbivores and a plant architectural dimorphism. Journal of Ecology. 94(6):1249–1260. https://doi.org/10.1111/j.1365-2745.2006.01161.x

Schoonhoven LM, Van Loon JJA, Dicke M. 2005. Insect-plant biology. 2nd ed. Oxford, UK: Oxford University Press. https://books.google.com/books?id=zcQRDAAAQBAJ.

Shen C, Yu S, Tan X, et al. 2024. Infestation of rice striped stem borer (*Chilo suppressalis*) larvae induces emission of volatile organic compounds in rice and repels female adult oviposition. International Journal of Molecular Sciences. 25(16):8827. https://doi.org/10.3390/ijms25168827

Soni N, Westra EP, Allegretta G, et al. 2022. Survey of ACCase and ALS resistance in winter annual grasses identifies target-site and nontarget-site imazamox resistance in *Secale cereale*. Pest Management Science. 78(12):5080–5089. https://doi.org/10.1002/ps.7154

Strand JR, Perez Moya OG, Peterson RKD, et al. 2025. Sensory and behavioral responses of braconid parasitoids to changes in volatile emissions induced by wheat stem sawfly

(Hymenoptera: Cephidae) larval feeding in winter wheat and smooth brome. Del Pozo-Valdivia A, editor. Journal of Insect Science. 25(2). https://doi.org/10.1093/jisesa/ieaf016

Subedi M, Cárcamo HA, Knodel JJ, et al. 2021. Stability analysis of stem solidness, grain yield, and grain protein concentration in spring wheat. Canadian Journal of Plant Science. 101(4):456–475. https://doi.org/10.1139/cjps-2020-0089

Szczepaniec A, Glover KD, Berzonsky W. 2015. Impact of solid and hollow varieties of winter and spring wheat on severity of wheat stem sawfly (Hymenoptera: Cephidae) infestations and yield and quality of grain. Journal of Economic Entomology. 108(5):2316–2323. https://doi.org/10.1093/jee/tov207

Varella AC, Weaver DK, Peterson RKD, et al. 2017. Host plant quantitative trait loci affect specific behavioral sequences in oviposition by a stem-mining insect. Theoretical and Applied Genetics. 130(1):187–197. https://doi.org/10.1007/s00122-016-2805-0

Varella AC, Weaver DK, Sherman JD, et al. 2015. Association analysis of stem solidness and wheat stem sawfly resistance in a panel of North American spring wheat germplasm. Crop Science. 55(5):2046–2055. https://doi.org/10.2135/cropsci2014.12.0852

Vázquez-García JG, De Portugal J, Torra J, et al. 2022. Comparison between the mechanisms of Clearfield ® wheat and *Lolium rigidum* multiple resistant to acetyl CoA carboxylase and acetolactate synthase inhibitors. Environmental Pollution. 306:119438. https://doi.org/10.1016/j.envpol.2022.119438

Vet LEM, Dicke M. 1992. Ecology of infochemical use by natural enemies in a tritrophic context. Annual Review of Entomology. 37:141–172.

Vieira HV, Bradford BZ, Osterholzer A, et al. 2025. A new growing degree-day phenology model for wheat stem sawfly (Hymenoptera: Cephidae) in Colorado wheat fields. Fand BB, editor. PLoS ONE. 20(4):e0320497. https://doi.org/10.1371/journal.pone.0320497

Wallace LE, McNeal FH, Berg MA. 1973. Minimum stem solidness required in wheat for resistance to the wheat stem sawfly. Journal of Economic Entomology. 66(5):1121–1124. https://doi.org/10.1093/jee/66.5.1121

War AR, Paulraj MG, Ahmad T, et al. 2012. Mechanisms of plant defense against insect herbivores. Plant Signaling & Behavior. 7(10):1306–1320. https://doi.org/10.4161/psb.21663

Weaver DK, Buteler M, Hofland ML, et al. 2009. Cultivar preferences of ovipositing wheat stem sawflies as influenced by the amount of volatile attractant. Journal of Economic Entomology. 102(3):1009–1017. https://doi.org/10.1603/029.102.0320

Weiss MJ, Morrill WL. 1992. Wheat stem sawfly (Hymenoptera: Cephidae) revisited. American Entomology. 38(4):241–245. https://doi.org/10.1093/ae/38.4.241

Winer AM, Arey J, Atkinson R, et al. 1992. Emission rates of organics from vegetation in California's Central Valley. Atmospheric Environment. Part A. General Topics. 26(14):2647–2659. https://doi.org/10.1016/0960-1686(92)90116-3

Wong M -L., Bruckner PL, Berg JE, et al. 2022 Nov 23. Evaluation of wheat stem sawfly resistant solid stem *Qss.msub-3BL* alleles in hard red winter wheat. Crop Science. https://doi.org/10.1002/csc2.20866

Yao C, Du L, Liu Q, et al. 2023. Stemborer-induced rice plant volatiles boost direct and indirect resistance in neighboring plants. New Phytologist. 237(6):2375–2387. https://doi.org/10.1111/nph.18548

Yi X, Shi S, Wang P, et al. 2019. Characterizing potential repelling volatiles for “push-pull” strategy against stem borer: a case study in *Chilo auricilius*. BMC Genomics. 20(1):751. https://doi.org/10.1186/s12864-019-6112-4

Yin H, Li W, Xu M, et al. 2022. The role of tetradecane in the identification of host plants by the mirid bugs *Apolygus lucorum* and *Adelphocoris suturalis* and potential application in pest management. Frontiers in Physiology. 13:1061817. https://doi.org/10.3389/fphys.2022.1061817

Zadoks JC, Chang TT, Konzak CF. 1974. A decimal code for the growth stages of cereals. Weed Research. 14(6):415–421. https://doi.org/10.1111/j.1365-3180.1974.tb01084.x

Zhao LY, Chen JL, Cheng DF, et al. 2009. Biochemical and molecular characterizations of *Sitobion avenae*-induced wheat defense responses. Crop Protection. 28(5):435–442. https://doi.org/10.1016/j.cropro.2009.01.005

CHAPTER 4: EVALUATING CROP ROTATION EFFECTS ON WHEAT STEM SAWFLY INFESTATION, DAMAGE AND LARVAL SURVIVAL

Introduction

The wheat stem sawfly (WSS – *Cephus cinctus*) is a significant pest of wheat and causes severe damage where its population is established (Weiss and Morrill 1992, Beres et al. 2011). Adults live for approximately one week, during which females lay eggs inside elongating wheat stems (Wallace and McNeal 1966). Larvae hatch within the stem, and it is this stage that causes the majority of plant injury and crop damage (Holmes 1979, Morrill et al. 1994). Feeding begins inside the stem, often just below one or more nodes in the subnodal region (Platt and Farstad 1946). Larval tunneling can reduce photosynthetic capacity (Weiss and Morrill 1992, Macedo et al. 2007), lower grain weight (Holmes 1977, Morrill et al. 1994), and weaken stems to the point of lodging before harvest (Ainslie 1920, Morrill and Kushnak 1999, Nansen et al. 2005). Such damage not only decreases yield but also complicates mechanical harvesting (Holmes 1977). Larvae spend most of their life cycle protected inside the stem, making them difficult to control with pesticides or other direct interventions (Beres et al. 2011, Peirce et al. 2024).

Early cultural control measures such as burning, tillage, and ploughing proved largely ineffective and often caused serious soil degradation (Criddle 1922, 1923). Crop rotation is a historically important and widely adopted cultural practice; revolutionary in its simplicity, beneficial to both crops and the environment, and relatively easy to implement (Bullock 1992). Among the many advantages of crop rotation are improved soil health and structure, enhanced nitrogen supply, a more balanced nutrient profile, reduced economic risk from reliance on a single crop or agricultural activity, increased environmental resilience through crop diversity, and better control of weeds, diseases, and insect pests (Berzsenyi et al. 2000, Sieling and Christen

2015, Hasanuzzaman 2019). Crop rotations enhance pest control by disrupting the life cycles of insects, weeds, and diseases, making them a key component of integrated pest management (Francis and Clegg 1990). Insect pests can be particularly susceptible to crop rotation (Onstad 2007), especially non-generalist pests like the WSS (Ainslie 1920, Criddle 1923, Holmes 1979, Cockrell et al. 2017). In the Northern Great Plains, the effectiveness of crop diversification in reducing WSS pressure is still unclear (Fischer 2019). Some studies indicate that increasing the diversity of host plants (such as native grasses for WSS) in agricultural landscapes may actually lead to increased herbivory, without a corresponding rise in parasitoid benefits (Rand et al. 2014). Theoretically, rotating to non-host or non-susceptible crops should reduce WSS population pressure (Ainslie 1920, Criddle 1922, Sing 2002). However, in many dryland areas, rotations are often limited (e.g., wheat–wheat or wheat–fallow) due to economic constraints and lower profitability of alternative crops (Weiss and Morrill 1992, Meers 2005), but in Colorado, some growers we work with are planting more profitable crops such as corn and millet. These fields offer an opportunity to examine how these alternative rotations might influence WSS populations.

Although the benefits of crop rotation are well established, the closest insight we have regarding wheat rotation in the Great Plains suggests that production was optimized through the inclusion of alternative Fabaceae and Brassicaceae crops, where wheat following these crops yielded on average 21% more than wheat after wheat, with improved nitrogen removal efficiency and soil water retention (Miller et al. 2002, Brandt et al. 2008). However, these studies were agronomic in nature and did not specifically address WSS dynamics. Therefore, in this study, we evaluated whether the use of different crop rotations can influence WSS populations in Colorado, where this pest is a major concern.

This study aimed to evaluate how grower-implemented crop rotation systems influence the abundance and damage caused by WSS, while accounting for cultivar resistance as a key mitigating factor. We hypothesize that rotations incorporating non-host crops suppress WSS populations and reduce stem cutting in subsequent wheat crops, with these effects further shaped by the level of resistance in the wheat cultivar.

Methods

Field sites

To determine whether crop rotation influences WSS infestation and damage, we sampled 24 sites across North and Central-Eastern Colorado over a three-year period (**Table 4.1).** For all participating growers, we were able to gather crop history data going back at least four years, which allowed us to standardize our evaluation to a four-year rotation framework.

Rotations

The sampled sites represented a range of rotation intensities, from simple wheat-fallow (FWFW) to more diverse rotation like corn-millet-fallow-wheat (CMFW). Fallow is an essential part of the agricultural system in Colorado due the limitations to water access (Armenta et al. 2014). All sites were part of our Colorado statewide WSS survey and were managed under dryland, no-till conditions, except for J1 and J2 fields (Table 4.1), where FWFW rotations involved minimal tillage. For each year, sampling was conducted at previously established sites where grower contacts facilitated the collection of crop rotation histories and wheat cultivar information. In 2023, however, detailed records were not available. At each site, WSS-related data were collected, including presence, larval mortality, and plant cutting.

Samples

In 2024, we sampled one site with fallow–wheat–corn–wheat (FWCW) rotation, one with WCFW, one with WMFW, and three with FWFW. In 2025, we expanded the dataset to include records from the previous five crop seasons by resampling the same region as in 2024, demonstrating the potential for continued monitoring. That year, rotations included five FWFW, one corn–corn–fallow–wheat (CCFW), and one sorghum–millet–fallow–wheat (SMFW). Cultivars were recorded for years 2024 and 2025, to ensure correct interpretation of results (**Table 4.2**). Fields were selected within the same County that had similar reports of heavy WSS infestation. (**Figure 4.1**). Sampling in 2024 and 2025 were collected during the winter for 2024 (November), and collected right after harvest in 2025 (August). Samples consisted of 16 subsamples per site: half positioned 10 m from the field edge and half positioned 30 m from the edge. At each subsample, all stubble within a 0.3 m row was collected. This approach allows for consistent evaluation of crop rotations under similar WSS pressure in subsequent years by selecting adjacent fields when possible, or otherwise the closest available site. Data recorded included Zadoks stage, stem solidness, larval presence, frass, plant cutting, and larval status (alive or dead). For infestation assessment, wheat stubble was considered infested if larvae and/or frass were present. For larval survival calculations, stubble was considered positive if diapausing larvae were present or if there were signs indicating larval presence (cutting and/or frass). For cutting calculations, only infested stubble (i.e., stubble with larvae and/or frass) was used.

Table 4.1: Summary of field sites surveyed from 2023 to 2025, including site codes, field names, grower information, county location, tillage practice, geographic coordinates, type of crop rotation, and cultivar grown.

Year	Code	Site/Field	Grower	County	Tillage	Latitude	Longitude	Rotation*	Cultivar
2024	AL1	A1	Alan Linnebur	Adams/Washington	NO	39.7407438	-103.77805	FWCW	Windom SF
2024	AL2	A2	Alan Linnebur	Adams/Washington	NO	39.6929495	-103.79838	WCFW	Snowmass 2.0
2024	AL6	A6	Alan Linnebur	Adams/Washington	NO	39.8695392	-104.24459	WMFW	Kivari/Crescent AX
2024	JC1	J1	Jerry Craig	Adams/Washington	YES	39.73982	-104.11542	FWFW	Amplify SF
2024	JC2	J2	Jerry Craig	Adams/Washington	YES	39.74831	-104.0779	FWFW	Amplify SF
2024	JC3	J3	Jerry Craig	Adams/Washington	YES	39.82857	-103.70606	FWFW	Amplify SF
2025	AL2.2	Monahan 18 N	Alan Linnebur	Adams/Washington	NO	39.71059	-103.79713	WFWFW	Snowmass/Windom
2025	AL3.2	Monahan 20	Alan Linnebur	Adams/Washington	NO	39.69133	-103.7947	WCCFW	Snowmass 2.0
2025	AL7.2	Linnebur Grain	Alan Linnebur	Adams/Washington	NO	39.75534	-104.07994	WFWFW	Kivari AX
2025	CC1	Jolly 5	Alan Linnebur	Adams/Washington	NO	39.73705	-103.79217	MFWFW	Kivari AX
2025	CC2	CC2	Gary Meier	Adams/Washington	NO	39.74263	-103.76531	WSMFW	Kivari AX
2025	JC1.2	J1	Jerry Craig	Adams/Washington	YES	39.7971	-103.69744	WFWFW	Amplify SF
2025	JC2.2	J2	Jerry Craig	Adams/Washington	YES	39.77056	-104.07978	WFWFW	Amplify SF

*Rotation legend: C – corn; F – fallow; M – millet; S – sorghum; W – wheat

Table 4.2: Summary of field sites surveyed from 2023 to 2025, including site codes, field names, grower information, county location, tillage practice, geographic coordinates, type of crop rotation, and cultivar grown.

Cultivar	Type	Maturity	Height	Solidness	Pith Rating*	WSS resistance
Snowmass 2.0	Hard White	Medium-early	Medium-short	Hollow	-	NO
Crescent AX	Hard Red	Early	Medium	Hollow	-	NO
Kivari AX	Hard Red	Medium-early	Medium	Hollow	-	NO
Amplify SF	Hard Red	Medium	Medium	Semi-solid	17	YES
Windom SF	Hard White	Medium-early	Medium-short	Semi-solid	16	YES

*Pith rating goes from 5 (completely hollow) to 25 (completely solid).

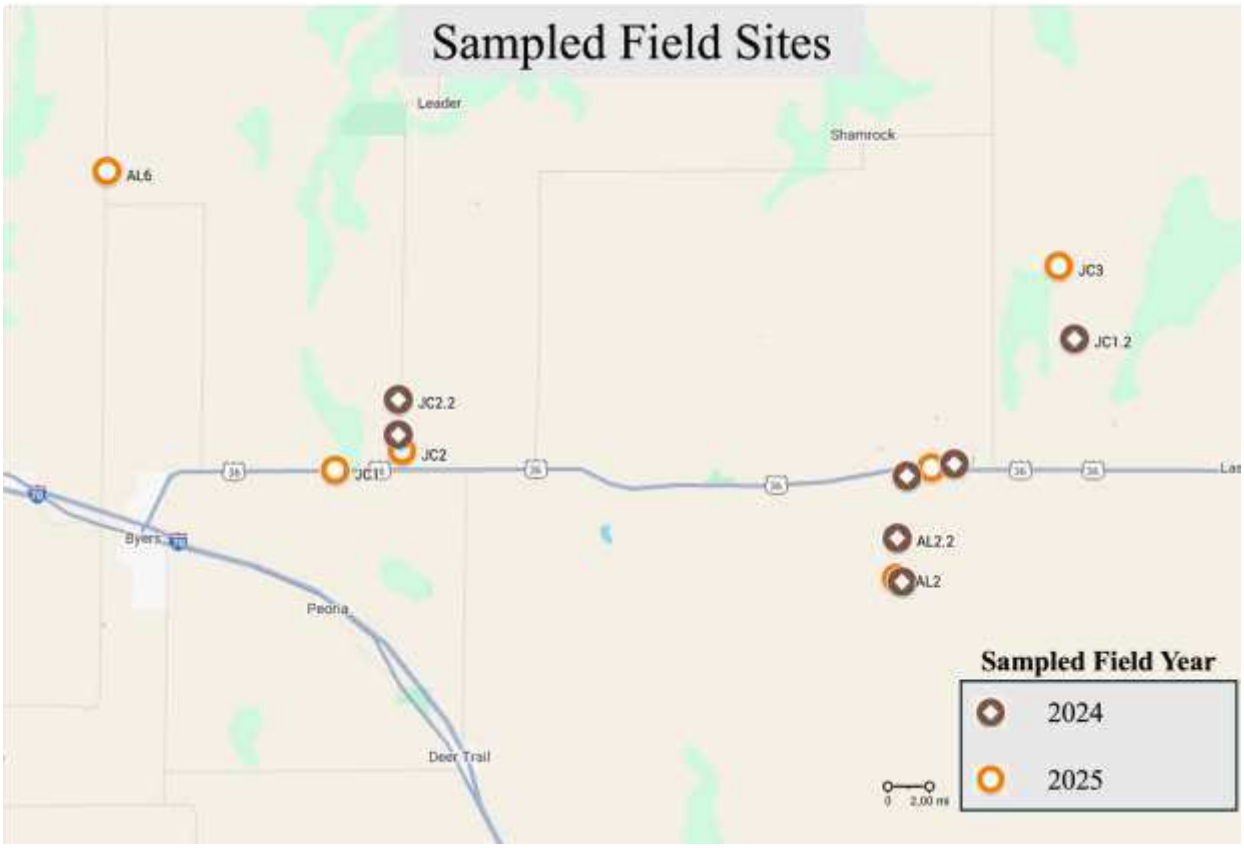

Figure 4.1: Geographic distribution of wheat field sites sampled in 2024 and 2025 in eastern Colorado.

Statistical methods

All analyses were conducted using R version 4.4.3 (R Core Team 2025). Due to differences in field rotations (different rotations and number of fields), we conducted a more detailed analysis to assess differences across rotations.

Crop rotation effects on WSS population and damage

To test whether crop rotations differed in infestation, cutting, and larval survival, we modeled binary outcomes at the individual stub level. Fixed effects included rotation (and location, when relevant), with random intercepts for the nested sampling structure (field, location within field, and bag within location). Models were estimated by maximum likelihood (Laplace approximation) using the bobyqa optimizer. We compared five candidate models (with different interaction terms and random effects structures) using AIC and likelihood-ratio tests to identify the model structure best supported by the sampling design. The selected model included rotation as a fixed effect and nested random effects for bag, location, and field: glmer(infested ~ rotation + (1 | field:location/bag), family = binomial). This model provided improved fit (lower AIC) and higher variance explained (R^2).

Cultivar-specific analyses

In 2025, within the FWFW rotation, three cultivar types were available: a susceptible hollow-stem cultivar (Kivari AX), a resistant semi-solid cultivar (Amplify SF), and a blend of hollow- and semi-solid cultivars (Snowmass 2.0/Windom SF). From those, we had two fields with the Amplify SF, two fields with the hollow stem Kivari AX, and one field for the blend of Snowmass 2.0/Windom SF. A similar GLMM structure was applied to test cultivar effects within rotation.

Inference

The aggregate effect of rotation were tested using Type II Wald χ^2 tests (car::Anova). Model-based estimated marginal means (EMMs) were computed on the response scale with emmeans. Pairwise contrasts were Šidák-adjusted (adjust = "sidak") because sample sizes were unbalanced and the assumption of homogeneity of variances was not met, making Tukey's method unsuitable. Compact letter displays (CLDs) were generated with multcomp::cld. Figures display predicted infestation proportions with 95% CIs, with x-axis labels indicating the total number of stubs evaluated per rotation.

Results

Effect of crop rotations on WSS infestation, larval survival and stem cutting

In 2024, WSS infestation levels varied significantly across crop rotations (**Figure 4.2A;** $\chi^2 = 16.50$, $p < 0.001$). The FWCW rotation showed the highest mean infestation proportion (0.542 ± 0.071), significantly higher than FWFW (0.268 ± 0.033) and WCFW (0.306 ± 0.050). Infestation in WMFW (0.442 ± 0.066) did not differ significantly from any other rotation except FWCW, which remained the only statistically distinct group. Larval survival also differed significantly by rotation (**Figure 4.2B;** $\chi^2 = 22.78$, $p < 0.001$). WMFW had the highest survival rate (0.899 ± 0.043), significantly greater than FWFW (0.371 ± 0.066), WCFW (0.637 ± 0.081), and FWCW (0.605 ± 0.095). Cutting followed a similar pattern (**Figure 4.2C**). FWFW had the lowest proportion (0.179 ± 0.030), significantly lower than FWCW (0.468 ± 0.086) and WMFW (0.420 ± 0.079). WCFW (0.249 ± 0.053) had an intermediate cutting rate, not statistically different from either FWFW or the higher-cutting rotations.

In 2025, infestation levels, cutting proportion and larval survival varied across crop rotations but were not statistically significant (**Figure 4.3**). The CCFW rotation had the highest

mean infestation proportion (0.469 ± 0.189), followed by SMFW (0.267 ± 0.149) and FWFW (0.152 ± 0.044) (**Figure 4.3A;** $\chi^2 = 3.93$, $p = 0.140$). Larval survival did not differ significantly across rotations (**Figure 4.3B;** $\chi^2 = 0.39$, $p = 0.823$). Mean survival was highest in CCFW (0.691 ± 0.167), followed by SMFW (0.584 ± 0.196) and FWFW (0.563 ± 0.102). Cutting followed a similar trend (**Figure 4.3C;** $\chi^2 = 4.21$, $p = 0.122$). CCFW showed the highest cutting proportion (0.362 ± 0.193), followed by SMFW (0.166 ± 0.116), while FWFW again had the lowest value (0.084 ± 0.030).

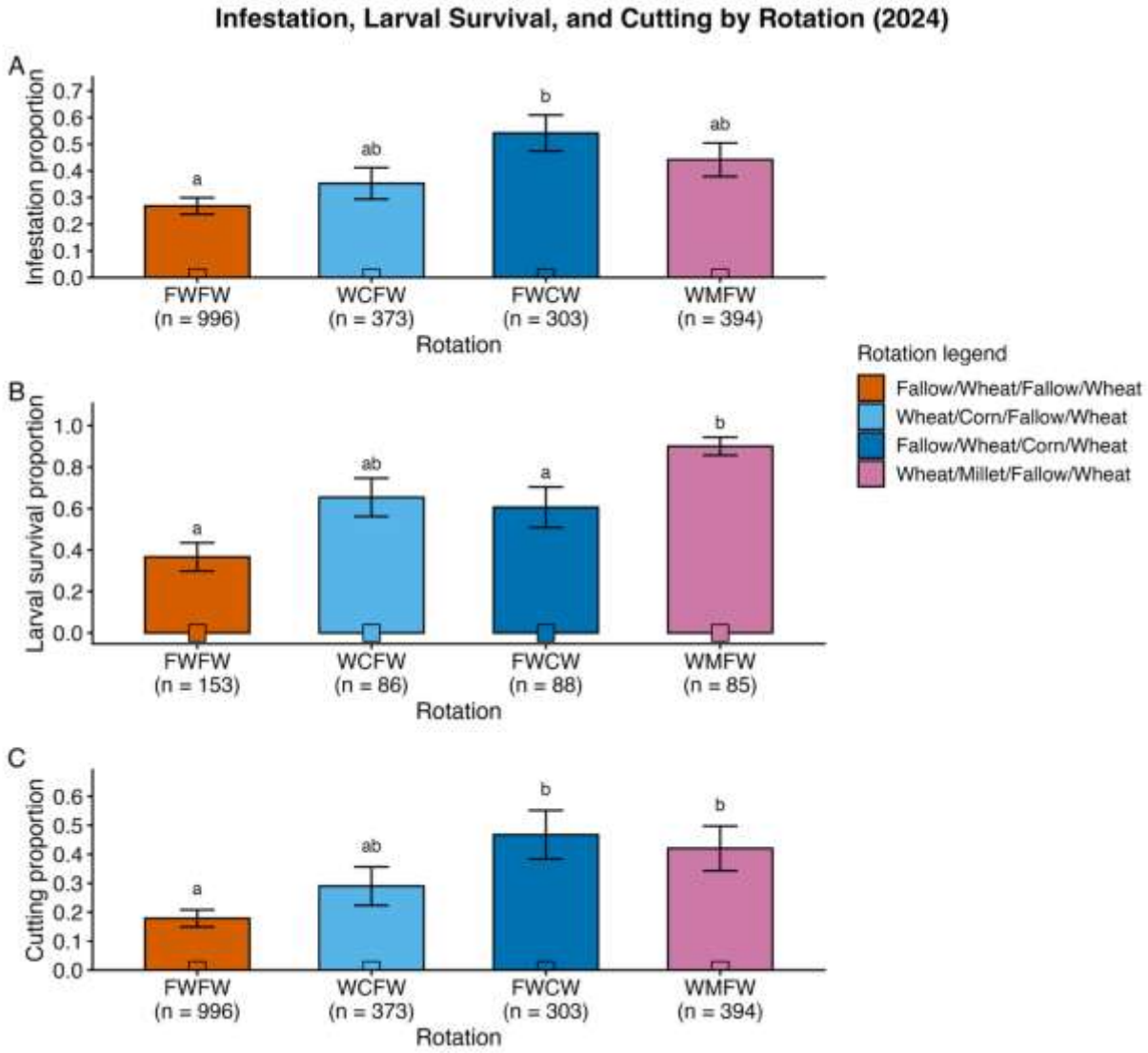

Figure 4.2: Estimated (**A**) infestation proportion, (**B**) cutting proportion, and (**C**) larval survival proportion by crop rotation in 2024. Bars represent marginal means from binomial generalized linear mixed models (GLMMs) with a logit link. Chi-square (χ^2) and p-values reflect Type II Wald χ^2 tests for the fixed effect of rotation. Letters above bars indicate statistically distinct groups based on post-hoc pairwise comparisons with Šidák correction ($\alpha = 0.05$). Sample sizes (n) shown below each bar reflect the number of stems evaluated in each analysis (larval survival n includes only stems for which larval status was scored). The rotation legend provides full names for each cropping sequence. The cultivar legend lists all cultivars used within each rotation, noting that different fields under the same rotation may have included different wheat cultivars. Cultivar bar colors correspond to their associated rotation.

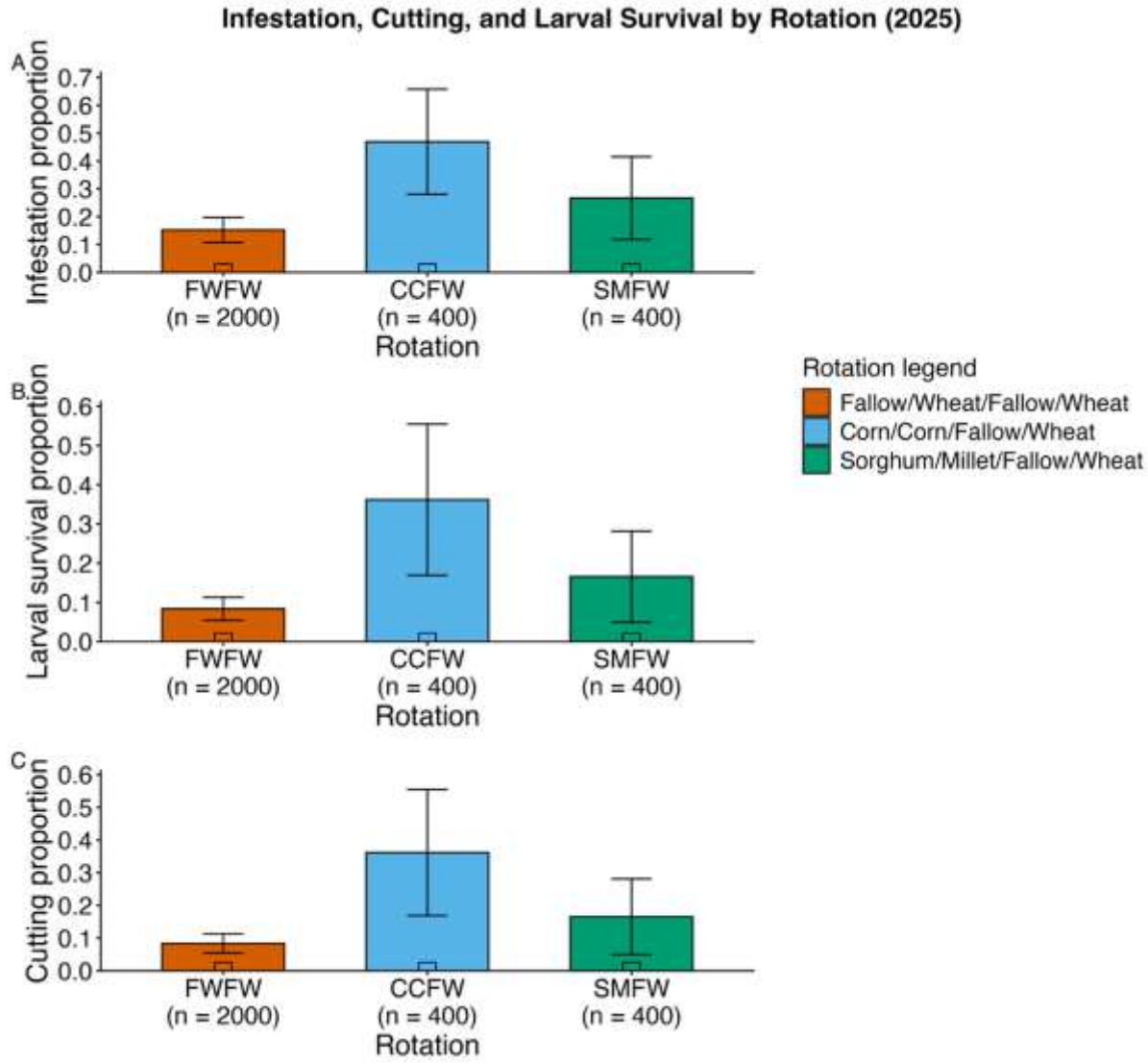

Figure 4.3: Estimated (**A**) infestation proportion, (**B**) cutting proportion, and (**C**) larval survival proportion by crop rotation in 2025. Bars represent marginal means from binomial generalized linear mixed models (GLMMs) with a logit link. Chi-square (χ^2) and p-values reflect Type II Wald χ^2 tests for the fixed effect of rotation. Letters above bars indicate statistically distinct

groups based on post-hoc pairwise comparisons with Šidák correction ($\alpha = 0.05$). Sample sizes (n) shown below each bar reflect the number of stems evaluated in each analysis (larval survival n includes only stems for which larval status was scored). The rotation legend provides full names for each cropping sequence. The cultivar legend lists all cultivars used within each rotation, noting that different fields under the same rotation may have included different wheat cultivars. Cultivar bar colors correspond to their associated rotation.

Effect of cultivar within same rotation on WSS infestation, larval survival and stem cutting

Due to the use of different cultivars in some rotations, and the inability to account for them in the statistical modeling, we analyzed the differences in infestation, cutting, and larval survival within the same rotation (FWFW) that had those different cultivars. In 2025, cultivar differences within the FWFW rotation were more pronounced for cutting and larval survival than for infestation (**Figure 4.4**). Infestation means varied – Kivari (0.181 ± 0.087) and the Snowmass 2.0/Windom SF blend (0.276 ± 0.165) were higher than Amplify SF (0.089 ± 0.048) – but these differences were not statistically significant (**Figure 4.4A;** $\chi^2 = 2.03$, $p = 0.363$). Larval survival followed a similar trend: Kivari (0.814 ± 0.067) and the blend (0.688 ± 0.076) had significantly greater survival than Amplify SF (0.205 ± 0.060; **Figure 4.4B;** $\chi^2 = 27.24$, $p < 0.001$). Cutting proportions differed significantly in the ANOVA; however, post-hoc comparisons did not reach the $\alpha = 0.05$ threshold for statistical significance. Kivari (0.145 ± 0.069) and the blend (0.185 ± 0.117) both showed higher average than Amplify SF (0.028 ± 0.016; **Figure 4.4C;** $\chi^2 = 6.52$, $p = 0.038$). Among FWFW cultivars, Amplify SF consistently suppressed insect performance across all metrics.

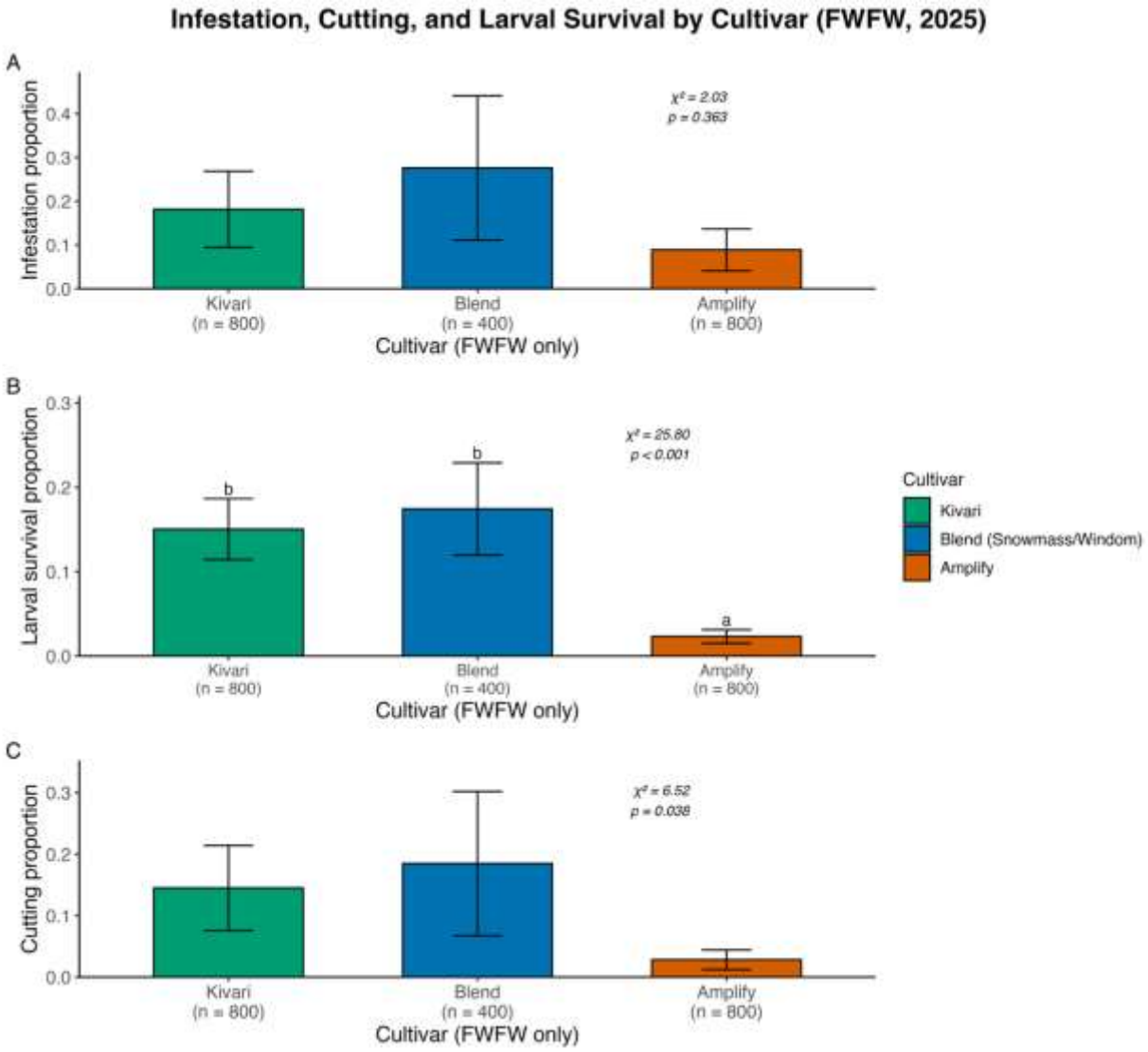

Figure 4.4: Estimated (**A**) infestation proportion, (**B**) cutting proportion, and (**C**) larval survival proportion by cultivar within the WCFW rotation in 2025. Bars represent marginal means from binomial generalized linear mixed models (GLMMs) with a logit link. Chi-square (χ^2) and p-values reflect Type II Wald tests for the fixed effect of cultivar. Letters above bars denote groupings from Šidák-adjusted pairwise comparisons ($\alpha = 0.05$), where different letters indicate statistically significant differences. Sample sizes (n) below each bar represent the number of stems analyzed per outcome (for larval survival, n includes only stems with scored larval status).

Discussion

The WSS is a persistent insect pest capable of causing substantial economic losses in wheat production. Its range continues to expand into southern areas of the Northern Hemisphere (Olfert et al. 2019). Over a century of research has shown that no single tactic is sufficient to manage WSS; instead, an integrative approach is necessary to mitigate its impact (Weiss and Morrill 1992, Beres et al. 2011). Among cultural control practices, many have proven to be either inconsistent or dependent on external factors such as weather and population pressure (Weaver et al. 2005). Although crop rotation has been studied in wheat systems (Miller et al. 2002, Jalli et al. 2021), its effects on WSS (larval infestation and consequently cutting) have received limited empirical attention. Historical references to crop rotation as a potential control strategy exist (Criddle 1923, Munro 1945, Farstad and Platt 1946, Wallace and McNeal 1966), but no studies have systematically evaluated its role in WSS suppression. This study presents the first method-driven, multi-year attempt to evaluate the short-term effects of crop rotation on WSS populations. We analyzed two years of field data from Colorado wheat growers practicing diverse rotations in WSS-infested regions. Specifically, we assessed how rotation patterns influenced WSS infestation, larval survivability and cutting (i.e., plant lodging due to larval feeding). All fields had been under their respective rotation systems for at least four years, providing a suitable context to investigate possible rotation effects. Broadly, our data indicates that crop rotation alone does not significantly affect WSS infestation levels or associated damage to wheat (**Figures 4.3** and **4.4**). However, this general trend warrants closer examination in light of the underlying conditions and potential limitations influencing these outcomes.

Across the 2024 and 2025 seasons, most sampled fields followed the wheat-fallow (FWFW) rotation, a system still widely used by Colorado wheat growers. While all fields came

from the same general region, rotations and cultivars varied notably between years (**Table 4.1**). Consistently, FWFW paired with Amplify SF showed reduced WSS infestation, cutting, and larval survival. Other rotations, such as FWCW and WMFW, performed similarly across these metrics despite using different cultivars. Interestingly, FWCW was planted with the semi-solid Windom SF, while WMFW used a blend of hollow-stemmed, more susceptible varieties. One possible explanation is that WMFW, with millet and fallow preceding wheat, provided a longer break than FWCW, which had only one corn season between wheat crops.

In 2024, the WCFW rotation – despite using a WSS-susceptible cultivar – performed statistically similar to FWFW and FWCW, both of which used resistant cultivars. In 2025, FWFW again showed numerically lower infestation and cutting rates, but statistical comparisons among FWFW, CCFW, and SMFW revealed no significant differences (**Figure 4.4**), largely due to sample imbalance: five FWFW fields versus only one each for CCFW and SMFW.
While these results could indicate some protection from extended breaks between wheat crops, the small sample sizes and short study duration limit the strength of any conclusions. Additional years and more balanced sampling are needed to clarify the effect of rotation alone.

Importantly, the 2025 FWFW fields allowed for a focused assessment of cultivar effects. With five fields under FWFW – two planted to hollow-stemmed Kivari AX, two to semi-solid Amplify SF, and one to a Snowmass 2.0/Windom SF blend – we were able to isolate cultivar impacts within a consistent rotation. While infestation and cutting remained uniformly low, larval survival varied sharply by cultivar (Figure 4.5C): only 20% in Amplify SF, compared to over 81% in Kivari AX and 69% in the Snowmass/Windom blend. These findings underscore the critical role of cultivar resistance in limiting WSS larval success.

Given these findings, it would be valuable to further explore how Amplify SF performs across different rotation systems. This could help determine whether cultivar resistance alone can offset the risks associated with shorter or more susceptible rotations, and whether pairing Amplify SF with more diverse rotations could further enhance WSS management. These results reinforce the need for cautious interpretation. While trends suggest that FWFW – particularly when paired with semi-solid cultivars – can limit WSS success, firm conclusions about the role of rotation type require more balanced and replicated sampling across all systems. These limitations and their implications are addressed later in the discussion.

Crop rotation has long been an effective and economically viable strategy for managing a variety of insect pests (Ouda et al. 2018). A well-documented example is corn rootworm (Diabrotica spp.), which lays its eggs in soil; by rotating out of corn with a non-host such as soybeans, growers disrupt the pest's lifecycle and reduce subsequent infestations (Hill and Mayo 1980, Gray et al. 2009, Carrière et al. 2020). Similarly, pests like the cereal leaf beetle and Colorado potato beetle have been suppressed through strategic rotations that limit host crop availability and increase spatial separation between planting years (Sexson and Wyman 2005). Even marginal benefits from rotation can have meaningful impacts. For instance, in potato systems, rotation with non-Solanaceous crops such as cereals and legumes, significantly reduced insecticide use against tuber flea beetle populations (Kabaluk and Vernon 2000). This highlights the potential value of even modest effects from rotation-based management, offering a reason to continue exploring whether similar benefits might eventually be realized in WSS systems, especially under the right ecological and agronomic conditions.

Interpretation of our results should be approached with caution due to several limitations. First, although fields were relatively close to each other, WSS pressure can be highly variable,

even within a single field (Holmes 1982, McCullough et al. 2020). While our statistical models accounted for this spatial clustering using nested effects, a truly randomized and spatially dispersed landscape design, accounting for regional dispersal dynamics, would offer a stronger foundation for future conclusions (Peirce et al. 2021, Hausmann et al. 2024). Additionally, some data collection inconsistencies across years limit comparison. For instance, variation in collection timing: in 2024, samples were taken near the end of winter, whereas in 2025, they were collected post-harvest. This difference may affect interpretation due to potential winter mortality of WSS (Morrill et al. 1994, Perez-Mendoza and Weaver 2006). Finally, due to inconsistencies and conflicting findings in previous studies on how soil nutrients and conditions influence WSS infestation and cutting behavior (Luginbill and McNeal 1954, O'Keeffe et al. 1960, Pauw and Read 1982, Delaney et al. 2010), we did not include soil-related variables in our analysis. However, collecting and analyzing such data within the crop rotations examined here could offer new insights.

Field-level management practices further contribute to variability in WSS outcomes. For instance, one grower practices minimal tillage and this might have negative effects on WSS (Runyon 2001, Weaver et al. 2005, Jalli et al. 2021). However, while reduced tillage may support reduction of WSS population, it contrasts with maintaining wheat stubble to support *Bracon spp*. parasitoid habitat, another key aspect of integrated WSS management. Certain tillage practices can destroy this overwintering habitat, potentially undermining natural biological control (Morrill et al. 1994, 1998, Runyon et al. 2002, Rand et al. 2011). Ultimately, the biology of WSS presents significant challenges for relying on crop rotation alone, particularly when applied at the individual field level. Coordinated rotations across neighboring farms, potentially in combination with other integrated strategies, may be necessary to improve efficacy.

Conclusion

This study contributes to a broader understanding of WSS management by moving beyond the traditional "host vs. non-host" framework. While FWFW showed some promise, our results emphasize that cultivar resistance, particularly the use of semi-solid stem wheat like Amplify SF, is currently the most reliable tool for reducing WSS infestation and survivorship. Rotations with longer intervals between wheat crops may still offer benefits, but demonstrating this will require additional years of observation, with consistent cultivar use and standardized field conditions. Moreover, collaborative efforts across neighboring farms may be necessary to amplify the benefits of rotation-based strategies in WSS-prone regions. This research provides early evidence that crop rotation alone may not significantly reduce WSS populations in high-pressure regions. Instead, cultivar resistance, particularly semi-solid stem wheat varieties, emerges as the most reliable strategy observed to date. Agreeing with various research studies (Kemp 1934, Szczepaniec et al. 2015, Bathini et al. 2023, Peirce et al. 2024). Even in the presence of multiple rotations, WSS persisted unless resistant cultivars were in place. Future work should focus on building long-term datasets to track multi-year trends in WSS pressure under varying rotation regimes. Continued sampling of the same fields will allow the construction of more thorough analysis and enable time-lag analyses of rotation effects. Additionally, studies should aim to isolate variables such as cultivar type, WSS exposure uniformity, and environmental conditions.

Acknowledgements

We would like to express our sincere appreciation to the wheat growers of Colorado who provided field access and generously contributed their time and knowledge to this project. Their

willingness to share detailed information about their fields and management histories was invaluable. We also extend our gratitude to the Colorado Wheat Research Foundation for their support and for serving as a vital bridge between Colorado State University and the local wheat-growing community.

References

Armenta R, Leikam D, Burgener P, et al. 2014. Wheat production and pest management for the Great Plains region. Fort Collins, CO: Colorado State University Extension.

Bathini A, Mendu L, Singh NP, et al. 2023. A “solid” solution for wheat stem sawfly (Hymenoptera: Cephidae) resistance: genetics, breeding and development of solid stem wheat. Crop Science. 63(4):1934–1948. https://doi.org/10.1002/csc2.20998

Beres B, Dosdall L, Weaver D, et al. 2011. Biology and integrated management of wheat stem sawfly and the need for continuing research. The Canadian Entomologist. 143(2):105–125. https://doi.org/10.4039/n10-056

Berzsenyi Z, Győrffy B, Lap D. 2000. Effect of crop rotation and fertilisation on maize and wheat yields and yield stability in a long-term experiment. European Journal of Agronomy. 13(2).

Brandt SA, Kutcher HR, Smith EG. 2008. Agronomic implications of more intensive canola rotations. In: Proceedings of FARMTECH 2008. p. 136.

Bullock DG. 1992. Crop rotation. Critical Reviews in Plant Sciences. 11(4):309–326. https://doi.org/10.1080/07352689209382349

Carrière Y, Brown Z, Aglasan S, et al. 2020. Crop rotation mitigates impacts of corn rootworm resistance to transgenic *Bt* corn. Proceedings of the National Academy of Sciences. 117(31):18385–18392. https://doi.org/10.1073/pnas.2003604117

Cockrell DM, Griffin-Nolan RJ, Rand TA, et al. 2017. Host plants of the wheat stem sawfly (Hymenoptera: Cephidae). Environmental Entomology. 46(4):847–854. https://doi.org/10.1093/ee/nvx104

Criddle N. 1922. The western wheat stem sawfly in Canada. The Report of the Entomological Society of Ontario. 36:18–21.

Criddle N. 1923. Popular and practical entomology: the life habits of *Cephus cinctus* Nort. in Manitoba. The Canadian Entomologist. 55(1). https://doi.org/10.4039/Ent551-1

Delaney KJ, Weaver DK, Peterson RKD. 2010. Photosynthesis and yield reductions from wheat stem sawfly (Hymenoptera: Cephidae): interactions with wheat solidness, water stress, and phosphorus deficiency. Journal of Economic Entomology. 103(2):516–524. https://doi.org/10.1603/EC09229

Farstad CW, Platt AW. 1946. The reaction of barley varieties to wheat stem sawfly attack. Scientific Agriculture. 26(5).

Fischer BV. 2019. The effects of increasing crop diversity on populations of wheat stem sawfly (*Cephus cinctus*) and associated braconid parasitoids [Master's thesis]. Montana State University.

Francis CA, Clegg MD. 1990. Crop Rotations in sustainable production systems. In: Sustainable Agricultural Systems. 1st ed. CRC Press. p. 16.

Gray ME, Sappington TW, Miller NJ, et al. 2009. Adaptation and invasiveness of western corn rootworm: Intensifying research on a worsening pest. Annual Review of Entomology. 54:303–321. https://doi.org/10.1146/annurev.ento.54.110807.090434

Hasanuzzaman M, editor. 2019. Agronomic crops: Management practices. Singapore: Springer Singapore. https://doi.org/10.1007/978-981-32-9783-8

Hausmann J, Heimbach U, Gabriel D, et al. 2024. Effects of regional crop rotations on autumn insect pests in winter oilseed rape. Pest Management Science. 80(5):2371–2382. https://doi.org/10.1002/ps.7716

Hill RE, Mayo ZB. 1980. Distribution and abundance of corn rootworm species as influenced by topography and crop rotation in eastern Nebraska. Environmental Entomology. 9(1):122–127. https://doi.org/10.1093/ee/9.1.122

Holmes N. 1979. The wheat stem sawfly. 26th Annual Meetings of the Entomological Society of Alberta and Entomological Society of Saskatchewan. 26.

Holmes N. 1982. Population dynamics of the wheat stem sawfly, *Cephus cinctus* (Hymenoptera:Cephidae), in wheat. The Canadian Entomologist. 114(9):775–788.

Holmes ND. 1977. The effect of the wheat stem sawfly, *Cephus cinctus* (Hymenoptera: Cephidae), on the yield and quality of wheat. The Canadian Entomologist. 109(12):1591–1598. https://doi.org/10.4039/Ent1091591-12

Jalli M, Huusela E, Jalli H, et al. 2021. Effects of crop rotation on spring wheat yield and pest occurrence in different tillage systems: A multi-year experiment in finnish growing conditions. Frontiers in Sustainable Food Systems. 5:647335. https://doi.org/10.3389/fsufs.2021.647335

Kabaluk JT, Vernon RS. 2000. Effect of crop rotation on populations of *Epitrix tuberis* (Coleoptera: Chrysomelidae) in potato. Journal of Economic Entomology. 93(2):315–322. https://doi.org/10.1603/0022-0493-93.2.315

Kemp HJ. 1934. Studies of solid stem wheat varieties in relation to wheat stem sawfly control. Scientific Agriculture. 15(1):30–38. https://doi.org/10.4141/sa-1934-0076

Luginbill P, McNeal FH. 1954. Effect of fertilizers on the resistance of certain winter and spring wheat varieties to the wheat stem sawfly. Agronomy Journal. 46(12):570–573. https://doi.org/10.2134/agronj1954.00021962004600120010x

Macedo TB, Weaver DK, Peterson RKD. 2007. Photosynthesis in wheat at the grain filling stage is altered by larval wheat stem sawfly (Hymenoptera: Cephidae) injury and reduced water availability. Journal of Entomological Science. 42(2):228–238. https://doi.org/10.18474/0749-8004-42.2.228

McCullough CT, Hein GL, Bradshaw JD. 2020. Phenology and dispersal of the wheat stem sawfly (Hymenoptera: Cephidae) into winter wheat fields in Nebraska. Journal of Economic Entomology. 113(4):1831–1838. https://doi.org/10.1093/jee/toaa093

Meers SB. 2005. Impact of harvest operations on parasitism of the wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae). Montana State University. https://scholarworks.montana.edu/xmlui/handle/1/1853.

Miller PR, Waddington J, McDonald CL, et al. 2002. Cropping sequence affects wheat productivity on the semiarid northern Great Plains. Canadian Journal of Plant Science. 82(2):307–318. https://doi.org/10.4141/P01-116

Morrill WL, Kushnak GD. 1999. Planting date influence on the wheat stem sawfly (Hymenoptera: Cephidae) in spring wheat. Journal of Agriculture and Urban Entomology. 16(2):123–128.

Morrill WL, Kushnak GD, Bruckner PL. 1994. Wheat stem sawfly (Hymenoptera: Cephidae) damage, rates of parasitism, and overwinter survival in resistant wheat lines. Journal of Economic Entomology. 87:1373–1376.

Morrill WL, Kushnak GD, Gabor JW. 1998. Parasitism of the wheat stem sawfly (Hymenoptera: Cephidae) in Montana. Biological Control. 12(3):159–163. https://doi.org/10.1006/bcon.1998.0629

Munro JA. 1945. Wheat stem sawfly and harvest loss. Bi-Monthly Bulletin. North Dakota Agricultural Experiment Station. 1(4):12–16.

Nansen C, Payton ME, Runyon JB, et al. 2005. Preharvest sampling plan for larvae of the wheat stem sawfly, *Cephus cinctus* (Hymenoptera: Cephidae), in winter wheat fields. The Canadian Entomologist. 137(5):602–614. https://doi.org/10.4039/n04-087

O'Keeffe LE, Callenbach JA, Lebsock KL. 1960. Effect of culm solidness on the survival of the wheat stem sawfly. Journal of Economic Entomology. 53(2):244–246. https://doi.org/10.1093/jee/53.2.244

Olfert O, Weiss RM, Catton H, et al. 2019. Bioclimatic assessment of abiotic factors affecting relative abundance and distribution of wheat stem sawfly (Hymenoptera: Cephidae) in western Canada. Canadian Entomologist. 151(1):16–33. https://doi.org/10.4039/tce.2018.46

Onstad DW. 2007. Resistance to crop rotation. In: Onstad DW, editor. Insect Resistance Management. Amsterdam: Academic Press. p. 153–183. https://doi.org/10.1016/B978-0-12-373858-5.X5001-1

Ouda S, Zohry AE-H, Noreldin T. 2018. Crop rotation: An approach to secure future food. Cham: Springer International Publishing. https://doi.org/10.1007/978-3-030-05351-2

Pauw RMD, Read DWL. 1982. The effect of nitrogen and phosphorous on the expression of stem solidness in Canuck wheat at four locations in southwestern Saskatchewan. Can. J. Plant Sci. 62(3):593–598. https://doi.org/10.4141/cjps82-089

Peirce ES, Evers B, Winn ZJ, et al. 2024. Identifying novel sources of resistance to wheat stem sawfly in five wild wheat species. Pest Management Science. https://doi.org/10.1002/ps.8008

Peirce ES, Rand TA, Cockrell DM, et al. 2021. Effects of landscape composition on wheat stem sawfly (Hymenoptera: Cephidae) and its associated braconid parasitoids. Journal of Economic Entomology. 114(1):72–81. https://doi.org/10.1093/jee/toaa287

Perez-Mendoza J, Weaver DK. 2006. Temperature and relative humidity effects on postdiapause larval development and adult emergence in three populations of wheat stem sawfly (Hymenoptera: Cephidae). Environmental Entomology. 35(5):1222–1231. https://doi.org/10.1093/ee/35.5.1222

Platt AW, Farstad CW. 1946. The reaction of wheat varieties to wheat stem sawfly attack. Scientific Agriculture. 26(6):231–247. https://doi.org/10.4141/sa-1946-0028

R Core Team. 2025. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.

Rand TA, Waters DK, Blodgett SL, et al. 2014. Increased area of a highly suitable host crop increases herbivore pressure in intensified agricultural landscapes. Agriculture, Ecosystems and Environment. 186:135–143. https://doi.org/10.1016/j.agee.2014.01.022

Rand TA, Waters DK, Shanower TG. 2011. Unexpectedly high levels of parasitism of wheat stem sawfly larvae in postcutting diapause chambers. Canadian Entomologist. 143(5):455–459. https://doi.org/10.4039/n11-023

Runyon JB. 2001. Wheat stem sawfly parasitism in varying field sizes and tillage systems in dryland wheat in Montana. Montana State University.

Runyon JB, Morrill WL, Weaver DK, et al. 2002. Parasitism of the wheat stem sawfly (Hymenoptera: Cephidae) by *Bracon cephi* and *B. lissogaster* (Hymenoptera: Braconidae) in wheat fields bordering tilled and untilled fallow in Montana. Journal of Economic Entomology. 95(6):1130–1134. https://doi.org/10.1603/0022-0493-95.6.1130

Sexson DL, Wyman JA. 2005. Effect of crop rotation distance on populations of Colorado potato beetle (Coleoptera: Chrysomelidae): Development of areawide Colorado potato beetle pest management strategies. ec. 98(3):716–724. https://doi.org/10.1603/0022-0493-98.3.716

Sieling K, Christen O. 2015. Crop rotation effects on yield of oilseed rape, wheat and barley and residual effects on the subsequent wheat. Archives of Agronomy and Soil Science. 61(11):1531–1549. https://doi.org/10.1080/03650340.2015.1017569

Sing SE. 2002. Spatial and biotic interactions of the wheat stem sawfly with wild oat and Montana dryland spring wheat [PhD dissertation]. Montana State University - Bozeman.

Szczepaniec A, Glover KD, Berzonsky W. 2015. Impact of solid and hollow varieties of winter and spring wheat on severity of wheat stem sawfly (Hymenoptera: Cephidae) infestations and yield and quality of grain. Journal of Economic Entomology. 108(5):2316–2323. https://doi.org/10.1093/jee/tov207

Wallace L, McNeal F. 1966. Stem sawflies of economic importance in grain crops in the United States. Entomology Research Division.

Weaver DK, Sing SE, Runyon JB, et al. 2005. Potential impact of cultural practices on wheat stem sawfly (Hymenoptera: Cephidae) and associated parasitoids. Journal of Agricultural Urban Entomology. 21(4):271–287.

Weiss MJ, Morrill WL. 1992. Wheat stem sawfly (Hymenoptera: Cephidae) revisited. American Entomology. 38(4):241–245. https://doi.org/10.1093/ae/38.4.241

CHAPTER 5: ASSESSMENT OF PHYLOGEOGRAPHY AND HOST-ASSOCIATE DIFFERENTIATION IN *Bracon spp.* ACROSS THE GREAT PLAINS

Introduction

The wheat stem sawfly (WSS – *Cephus cinctus* Norton, Hymenoptera: Cephidae) is a destructive pest in North America (Ainslie 1920, Holmes 1979, Weiss and Morrill 1992). The larvae cause significant damage by boring into or cutting wheat stems, leading to substantial yield losses, resulting in an estimated $350 million of damage annually in the Great Plains (Beres et al. 2011), and after more than a century as a persistent problem, researchers are now tackling WSS with a comprehensive, integrative approach (Cárcamo, et al. 2011, Beres et al. 2011, Peirce et al. 2024). Historically, WSS fed on native grasses and wheat (Ainslie 1920, Criddle 1923). It has adapted over time, shifting from native grasses to spring wheat and eventually to winter wheat (Morrill et al. 1992, 1994, Cockrell et al. 2017). Since then, conventional management strategies of this insect in cultivated wheat have proven insufficient (Peirce et al. 2024), and as WSS populations are increasing, and starting to outbreak in southern regions (Irell and Peairs 2014, Cockrell et al. 2021), the need for effective control measures becomes more critical.

The use of biological control is an alternative that demonstrated high efficacy, particularly in areas where the parasitic wasps *Bracon cephi* Gahan (Gahan 1918) and *Bracon lissogaster* Muesebeck (Muesebeck 1953) (Hymenoptera: Braconidae) has been established (Rand et al. 2011). These host-specific bivoltine ectoparasitoids are idiobionts and they immobilize their host upon attack and consume it (Nelson and Farstad 1953, Runyon et al. 2001, 2002, Weaver et al. 2005). Both species share similar life cycle (Nelson and Farstad 1953, Runyon et al. 2001, Beres et al. 2011, Cárcamo et al. 2012). Because WSS spend their whole immature life protected inside the stem, it makes this highly specialized parasitoid a valuable

control agent. Although *Bracon spp*. populations are well stablished in northern parts of the U.S. and Canada (Weaver et al. 2005, Rand et al. 2011), researchers have yet to see significant numbers in regions with cultivated wheat in the Central Great Plains, especially in Colorado (Peirce et al. 2021). The outbreak of WSS in cultivated winter wheat in this region is relatively recent, thus, an understanding of the differences amongst *Bracon spp*. populations could help facilitate their adaptation to WSS in infested wheat plants.

This raises the broader question of how geographical, environmental, and ecosystemic factors may influence genetic variability. The uneven genetic distribution within a population is related to extreme temperatures, precipitation and latitude (French et al. 2023). With the increasingly concerning changes in climate, we should expect changes in population genetics, and of course further implications in pest ecology and related interactions (Rosenzweig et al. 2001). Early work from Lou et al. (Lou et al. 1998) noticed segregation groups within WSS, then later, Lesieur et al. (Lesieur et al. 2016) observed different haplotypes within WSS populations in the Great Plains, which led to the characterization of three different genetic clusters of this species. As a host-specific parasitoid, *B. cephi* and *B. lissogaster* are closely coevolved with their host, WSS, and are described from observations in North American wild grasses (Ainslie 1920, Criddle 1923, Nelson and Farstad 1953, Peters et al. 2017). Therefore, it is imperative to collect population genetic data on these parasitoid species and evaluate if this geographic genetic cluster formation in WSS is driving a similar pattern for its closely related parasitoids.

However, while geographic factors can shape genetic structure, they are not the only possible explanation. Another mechanism is Host-Associated Differentiation (HAD), the genetic divergence of closely related species driven by adaptation to different hosts (Stireman III et al. 2005, 2006, Scheffer and Hawthorne 2007). In the case of *Bracon spp*., HAD could arise if

populations adapt to WSS associated with different host plants, such as wild grasses versus cultivated winter wheat.

Various studies suggest that parasitoid population divergence can be strongly driven by ecological phenomena (Althoff and Thompson 2001, Stireman III et al. 2006). They can form close, specialized, and specific relationships with their hosts (Godfray and Müller 1998). HAD in parasitoids associated with herbivore and their hosts show different outcomes, indicating that for some species we can observe genetic differentiation according to host use, but not always (Stireman III et al. 2005, 2006, Lozier et al. 2009, Bilodeau et al. 2013). To fully assess the potential role of HAD in *Bracon spp*., analyses should be conducted in Colorado, where WSS outbreaks in winter wheat are more recent. Addressing this question requires not only ecological observations but also genetic approaches capable of detecting subtle patterns of population structure.

Within this framework, our study first conducted a greenhouse experiment that tested (1) whether parasitism rates of WSS in wheat varied when exposed to *Bracon spp*. collected from North Dakota, Nebraska, and Colorado. We then used molecular barcoding (COI) to (2) examine genetic variation among *Bracon spp*. across different regions of the Great Plains, as well as in their host, WSS, in the Central Great Plains (Colorado and Nebraska). Here, we tested two related hypotheses: first, that phylogeographic structure drives genetic differentiation among geographically distinct populations of the WSS parasitoid *B. cephi*; and second, that HAD contributes to genetic divergence in *B. lissogaster* populations parasitizing WSS in wild grasses versus cultivated winter wheat in Colorado. To complete the host-parasitoid context, we also analyzed WSS genetic variation from Colorado and Nebraska. Together, these results clarify

patterns of genetic variation in the WSS-*Bracon spp*. relationship and offer insights that may improve biological control strategies, particularly in novel outbreak regions such as Colorado.

Methods

Greenhouse experiment

Plant growth and care

This experiment was conducted aiming the evaluation of *Bracon spp*. parasitism from populations from three different regions: Colorado, Nebraska, and North Dakota. To account for temperature-driven variability in WSS emergence (Vieira et al. 2025) and their preference for wheat at the stem elongation stage (Holmes and Peterson 1960, Beres et al. 2011) we staggered germination across three planting dates, each spaced one week apart. Hard red winter wheat (hollow-stem cultivar 'Byrd', provided by CSU breeding department) was used. Seeds were germinated on blotter paper moistened with 5 ml of a 10 ppm aqueous solution of Dividend XL RTA seed treatment (Syngenta, Greensboro, NC) to prevent fungal growth. For each planting, 15 seeds were placed into Ziploc bags containing blue germination paper and 5 mm of water, with a total of 400 seeds germinated across 25 bags. Seeds were held at 25 °C in the laboratory (Insectary, Colorado State University) for five days to sprout, then transferred to a cold room (2°C) for an eight-week vernalization. This process was repeated for each germination date, spaced one week apart.

Following cold treatment, a single wheat seedling was planted per conetainer (Stuewe & Sons SC10U UV-stabilized cones: 3.8 cm diameter × 20.9 cm depth, 164 ml volume). Containers were fitted with compacted cotton at the base to prevent soil loss and retain moisture, then filled with a soil mixture prepared in bulk by combining seven parts Lambert 20B/30V germination

blend (Québec, Canada) with two parts of perlite, mixed thoroughly in greenhouse containers. Seedlings were planted at 2.5-3.8 cm depth, covered with moistened soil, and watered thoroughly. Conetainers (20 per tray) were set in trays lined with mesh screens to prevent insect drowning (when introduced) and arranged in the greenhouse under 25 °C/18 °C day/night temperatures and a 16:8 h light:dark photoperiod. Plants were bottom watered as needed and fertilized with Peters Excel 15-15-15 General Purpose Fertilizer (J.R. Peters, Allentown, PA) regularly. Secondary tillers were removed 10 days prior to WSS infestation to encourage uniform primary stem growth. Aphids, mites, and thrips were managed using biological control agents (lady beetles and green lacewings), and powdery mildew was controlled with horticultural oil (1.5 tsp/pint distilled water) when necessary. These measures did not interfere with oviposition by WSS females.

WSS and parasitoids collection and care

Stubble collections for WSS and parasitoids were made from three regions (Colorado, Nebraska, and North Dakota) between February and March of each year. In Colorado and Nebraska, parasitoid stubble collection was done directly from fields from wheat farmers with reported parasitoid activity, while material from North Dakota was provided by Dr. Tatyana Rand (USDA Montana). Wheat stubs with diapausing WSS was collected in Colorado, from wheat fields with reported high WSS activity. All stubble was stored in refrigerated conditions until use.

For parasitoid emergence and care, stubble containing parasitoids was removed from refrigerated storage and placed in cardboard cannisters fitted with a funnel leading to a vial at the outlet. This design directed emerging parasitoids toward the light and into the collection vial. Cannisters were maintained at 25 °C under continuous light and checked daily. Emerging parasitoids were collected immediately and transferred into plastic cups provisioned with a honey drip to increase their longevity (Reis et al. 2019, Rand and Waters 2020) and moistened

cotton. Separate cups were maintained for each emergence date and population source, with detailed records of emergence date, location, sex, and number of individuals.

Greenhouse experiment conduction

The experiment was done in mesh cages housed at the CSU Insectary. Three treatments were established using *Bracon spp*. from Colorado, Nebraska, and North Dakota, with all wheat plants infested using WSS collected in Colorado. Coordinating the experiment required careful synchronization of WSS emergence, plant growth stage, and parasitoid emergence. Each treatment cage contained 20 wheat plants and was replicated three times. To infest plants with WSS, refrigerated stubble containing adults was removed from storage based on emergence tests and placed into cages once wheat plants had reached at least Zadoks stage 32, ensuring oviposition on suitable stems. After several weeks, when WSS larvae had developed to an advanced stage (approximately one month after infestation), parasitoids were introduced into cages to assess parasitism rates. Seven adults (five females and two males) were released per cage. Parasitism was evaluated 14 days after release, when *Bracon spp*. development could be detected. Wheat tillers were dissected, and data were recorded as presence/absence of parasitoid larvae or cocoon formation. Wheat stem sawfly infested plants were considered by presence of live larvae, presence of cut stem (stub), or presence of WSS frass. Consequently, parasitism success was calculated by the presence of larvae or cocoon of parasitoids inside the dissected stems.

Statistical analysis

To assess parasitism success of *Bracon spp*. populations, a series of generalized linear models (GLMs) were fitted to data collected from 141 experimental units (plants). Each plant was assigned one of the three treatments (CO – Colorado; NE – Nebraska; and ND – North Dakota). In addition, developmental stage (Zadoks scale) and stem diameter were recorded. The

presence of host larvae, parasitoids, presence of frass, and stub formation were also recorded. We retained only plants where WSS infestation was successful, which reduced the dataset to 69 observations.

Analysis was done in R version 4.4.3 (R Core Team 2025). Models were fitted using Poisson and Binomial distributions, as appropriate. Model assumptions were checked using the DHARMa package, testing for overdispersion, zero-inflation, and residual uniformity. When overdispersion or poor fit was detected, alternative models were tested. Model comparison was performed using AIC values via the AICctab function from the bbmle package. The best-fitting model for parasitism data was a Binomial generalized linear model (GLM) including Treatment, Stem diameter, and their interaction: glm(Para ~ Trt * Sd, family = poisson(link = "log"). Pairwise comparisons between treatments were conducted using estimated marginal means (emmeans), with Tukey-adjusted p-values.

Molecular analysis

Population genetic analysis

DNA extraction, PCR amplification and sequencing DNA was extracted from individual specimens using the HotSHOT method (Truett et al., 2000), yielding a final volume of 80 µL. Extracted DNA was used as a template for PCR amplification of the COI gene region, with primers C1-J-2195 and TL2-N-3014 (820 bp) (Simon et al. 1994) for *Bracon spp*., and C1-J-2183 and TL2-N-3014 (762 bp) for WSS. PCR reactions were carried out in 25 µL volumes containing 4 µL DNA template, 12.5 µL DreamTaq PCR Master Mix (Thermo Scientific™), 0.25 µL of each primer (10 µM), and 8 µL nuclease-free water. Thermal cycling conditions consisted of an initial denaturation at 95 °C for 5 min; 40 cycles of 95 °C for 30 s, 47 °C for 30 s, and 72 °C for 45 s; followed by a final extension at 72 °C for 10 min. To verify successful amplification, 5 µL of each PCR product was electrophoresed on a 1% agarose gel stained with

GelRed® (Biotium). PCR products were purified using the Wizard PCR Preps DNA Purification System (Promega, Madison, WI, USA) and sequenced bidirectionally (GENEWIZ, South Plainfield, NJ, USA).

We extracted DNA from 34 WSS specimens: 23 from Colorado and 11 from Nebraska. In addition, 54 *B. cephi* specimens: 18 from Colorado, 14 from Nebraska, and 22 from North Dakota. Finally, 15 *B. lissogaster* specimen: All from Colorado: 9 from specimens collected in winter wheat and 6 collected in wild grasses (grasses non identified) (**Table 5.1**).

Table 5.1: Sampling locations, years, host plants, and coordinates for WSS (*Cephus cinctus*), *Bracon cephi*, and *Bracon lissogaster* populations used in this study.

WSS - *Cephus cinctus*				
Location	**Year**	**Host**	**Code**	**Coordinates**
Colorado	2023	Winter wheat	Mertens farm	40.58024, -103.90011
Nebraska	2025	Winter wheat	Sidney	41.14010, -103.00017
Bracon cephi				
Location	**Year**	**Host**	**Code**	**Coordinates**
Colorado	2022	Winter wheat	Mertens farm	40.58024, -103.90011
	2023	Winter wheat	Mertens farm	40.56643, -103.88266
Nebraska	2022	Winter wheat	Prescott farm	41.16065, -103.49634
	2023	Winter wheat	Cullens farm	42.291273, -103.140925
North Dakota	2022	Spring wheat	21W05	48.69174, -103.565794
	2023	Spring wheat	20W37	48.869999, -103.355470
		Spring wheat	Lavern	48.684399, -103.840465
Bracon lissogaster				
Location	**Year**	**Host**	**Code**	**Coordinates**
Colorado	2017	Wild grasses	Mertens farm	NA
	2023	Winter wheat	Mertens farm	NA

Genetic analysis

Sequence chromatograms were inspected and edited in Geneious version 2023.0.4 to ensure agreement between forward and reverse reads. Low-quality sequences were no included in the analysis. DNA sequences were compared against GenBank references using BLASTn

searches. The COI phylogenetic trees and haplotype networks were constructed using Population Analysis with Reticulate Trees (PopART) version 1.7. Method used for tree construction was Maximum Likelihood. Genetic diversity parameters were determined using DnaSP 5.10.01 (Librado and Rozas 2009) and included the number of haplotypes (h), haplotype diversity (Hd) and nucleotide diversity (Pi). Tajima's *D* (Tajima 1989) and Fu's *Fs* (Fu 1997) values were estimated to test for changes in population size of each species using DnaSP (version 5.10.01). One thousand simulations under a model of selective neutrality were used to generate Tajima's *D* and Fu's *Fs* values.

To test for genetic structure, we conducted Analyses of Molecular Variance (AMOVA) in R version 4.4.3, using the packages 'ape' version 5.8-1 and 'pegas' version 1.3. Pairwise genetic distances were calculated with the dist.dna function in 'ape' under the raw proportion of differences. AMOVA models were implemented with the amova function in 'pegas', with population assignments defined by collection region (Colorado, Nebraska, North Dakota) for WSS and *B. cephi*, and by host association (winter wheat vs wild grasses) for *B. lissogaster*. Statistical significance was assessed with 1,000 permutations, and results were summarized as variance components, Φ-statistics, and permutation-based *P*-values.

Results

Greenhouse experiment

Parasitism rates by *Bracon spp*. from Colorado, North Dakota, and Nebraska did not differ significantly among treatments ($\chi^2 = 3.67$, $p = 0.160$; **Figure 5.1**). North Dakota populations showed the highest mean parasitism rate, followed by Colorado, with Nebraska showing the lowest. In terms of parasitism outcomes (**Figure 5.2**), North Dakota parasitoids

achieved the most successful events. Colorado parasitoids had a more even distribution between success and failure, while Nebraska parasitoids had relatively few successful parasitism events. Notably, Nebraska treatment cages had fewer WSS infested plants, which may have limited opportunities for parasitism.

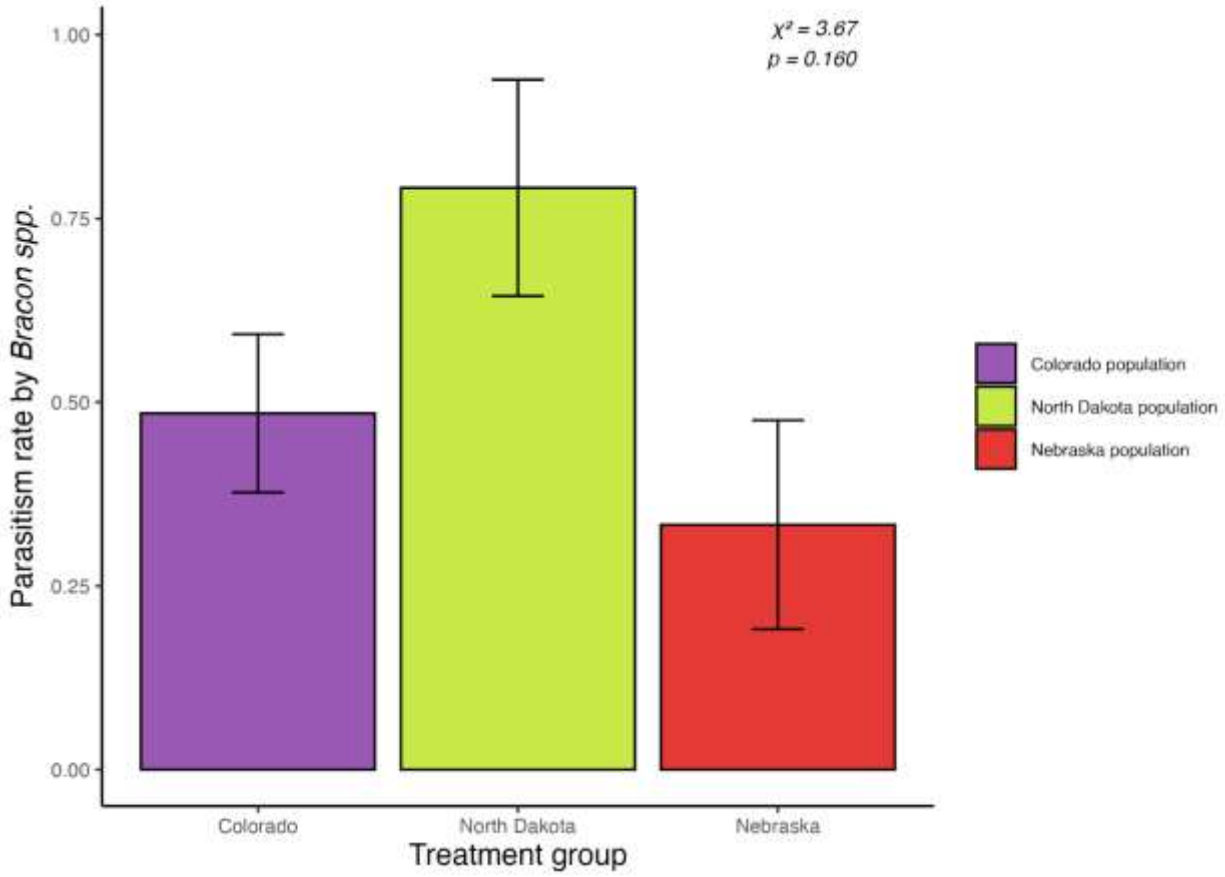

Figure 5.1: Parasitism rate (± SE) of *Bracon spp*. populations from Colorado, North Dakota, and Nebraska on WSS in Colorado wheat. Differences were not statistically significant.

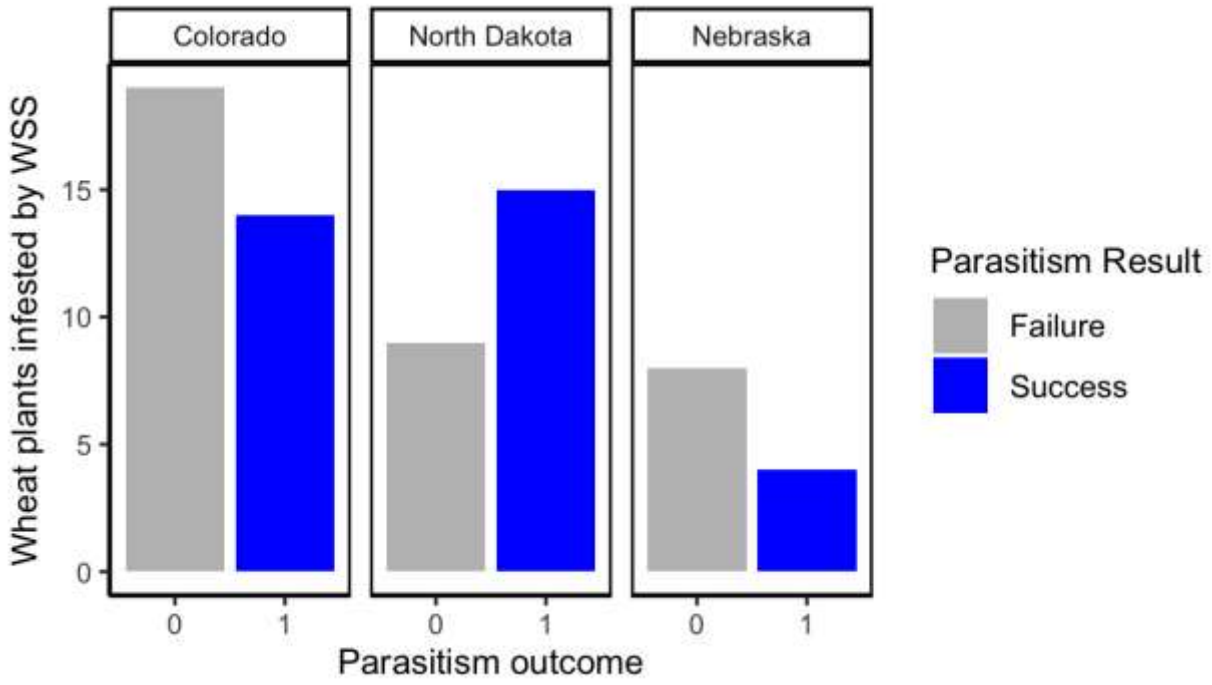

Figure 5.2: Parasitism by *Bracon spp*. outcomes (success vs. failure) across wheat plants infested by WSS in Colorado. North Dakota parasitoids exhibited more successful parasitism events compared with Colorado and Nebraska, which showed balanced or low success rates, respectively.

Molecular analysis

The COI was successfully amplified for all the different species: WSS, *B. cephi* and *B. lissogaster*. All new haplotypes discovered in this study will be deposited in GenBank. Maximum likelihood phylogenetic trees were generated for both WSS and *Bracon spp*. The WSS phylogenetic tree included haplotypes previously described by Lesieur et al. (Lesieur et al. 2016), along with those from our samples. Among these were three new haplotypes identified in our study: two from Colorado (H03 and H08), and one from Nebraska (H07) (**Figure 5.3**). The *Bracon spp*. phylogenetic tree incorporated haplotypes described Rand et al. (Rand et al. 2024), together with those from our samples, including a new *B. lissogaster* haplotype found in wild grasses in Colorado (H03), collected by Peirce et al (Peirce et al. 2021) (**Figure 5.4**).

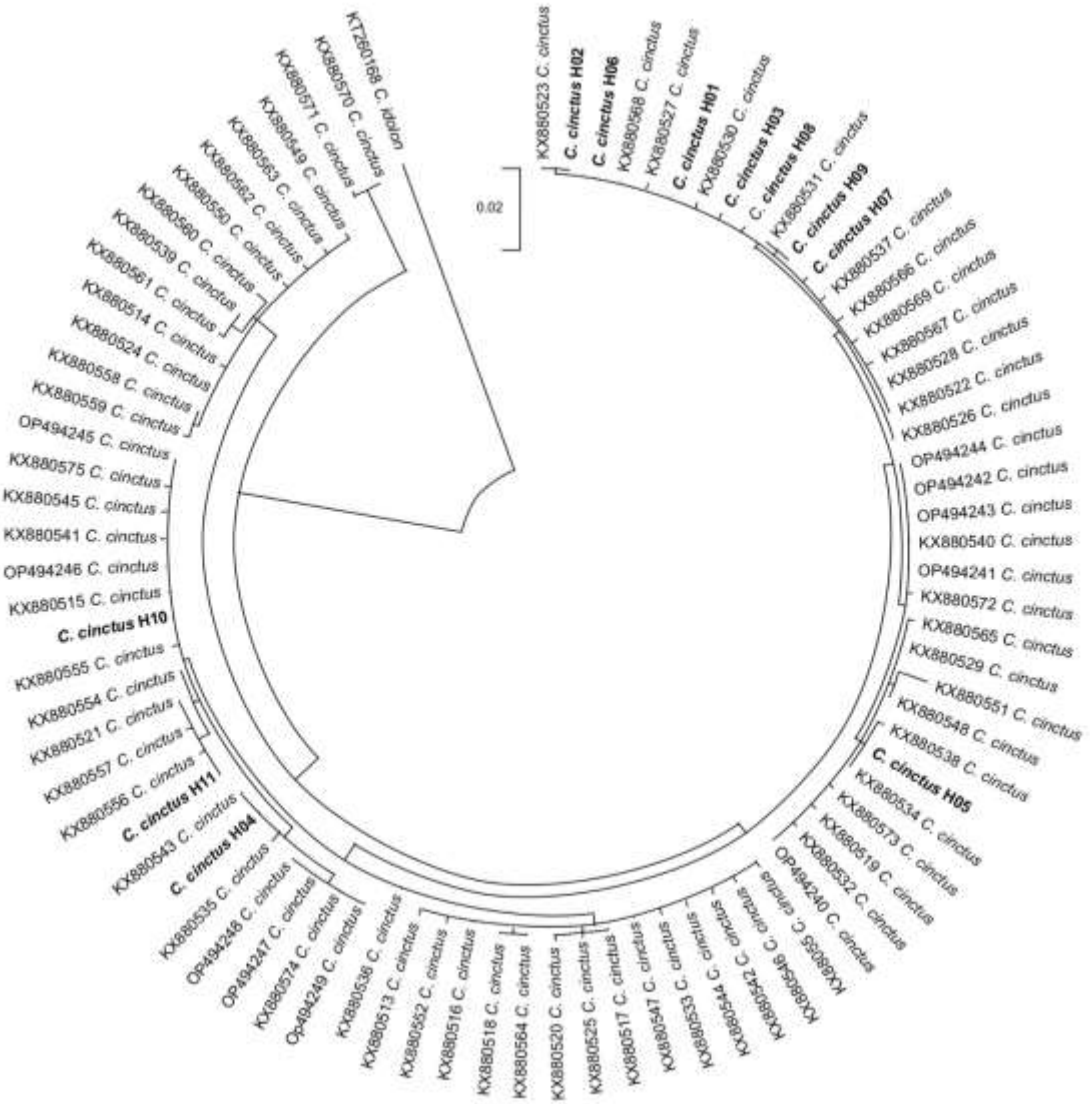

Figure 5.3: Maximum likelihood phylogenetic tree of WSS – *Cephus cinctus* – haplotypes based on mitochondrial COI sequences, including previously reported haplotypes from Lesieur et al. (2016) and newly identified haplotypes (H03, H07, H08) from this study. The tree is rooted using *Bracon idolon* as an outgroup. Branch lengths represent the number of nucleotide substitutions per site, with the scale bar (0.02) indicating a 2% sequence divergence.

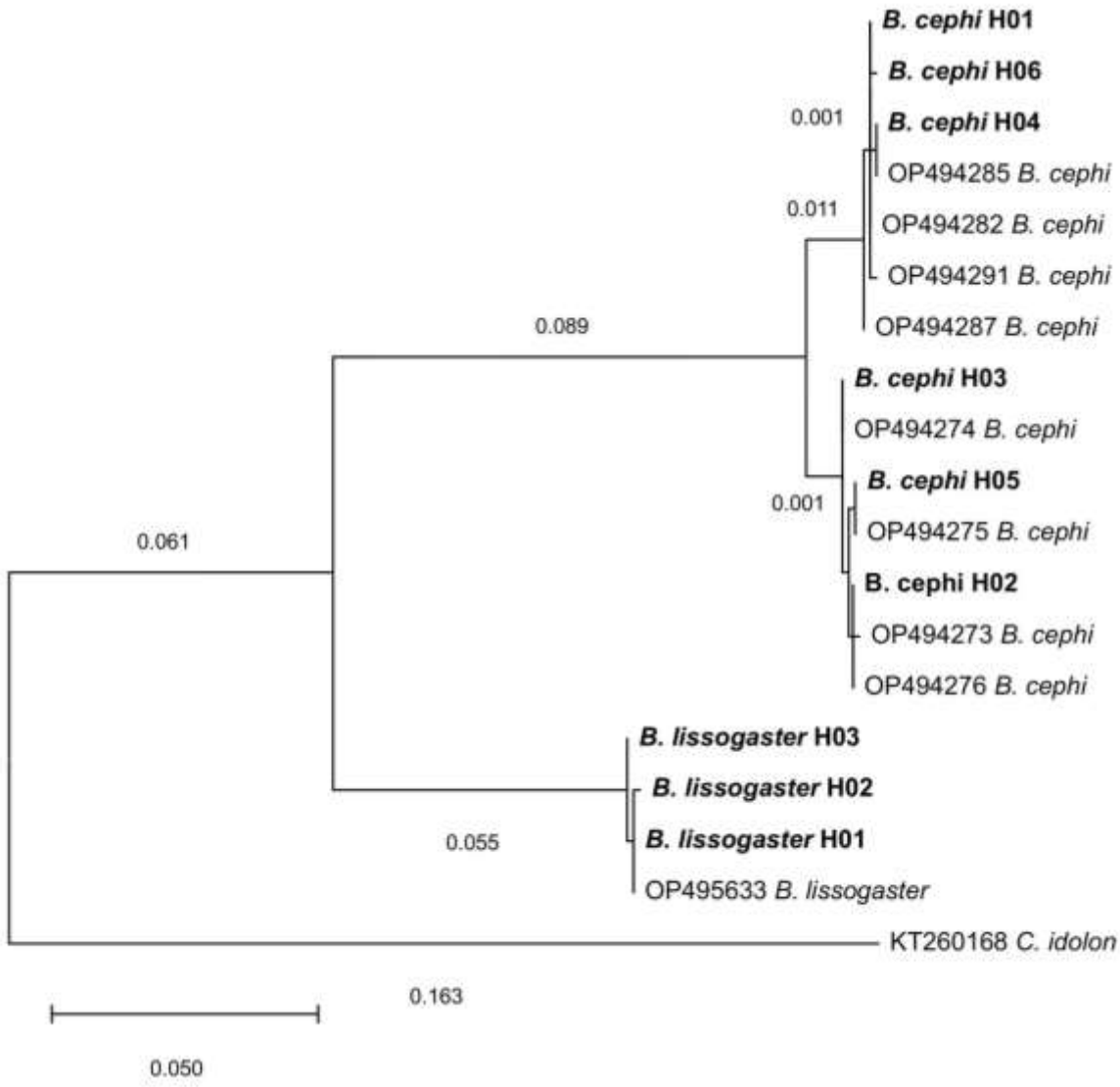

Figure 5.4: Maximum likelihood phylogenetic tree based on mitochondrial COI sequences of *Bracon spp*., including previously reported haplotypes from Rand et al. (2024) and the newly identified *Bracon lissogaster* haplotype (H03) from this study. The tree is rooted using *Cotesia idolon* as an outgroup. Branch lengths represent the number of nucleotide substitutions per site. The scale bar (0.050) corresponds to a 5% sequence divergence, with longer branches indicating greater genetic distances.

Sequence variation

For WSS, the final COI alignment was 751 bp and included 34 individuals (23 from Colorado, 11 from Nebraska). Eleven haplotypes were identified: seven in Colorado and four in Nebraska. Haplotype diversity was slightly higher in Colorado (Hd = 0.708) than in Nebraska

(Hd = 0.600), while nucleotide diversity was low in both regions (π = 0.00336 and 0.00608, respectively). Across all samples, diversity was Hd = 0.679 and π = 0.00438. Neutrality tests suggested population expansion or selection pressures in Colorado (Tajima's D = –1.86, P < 0.05), while Nebraska did not deviate from neutrality (**Table 5.2**).

For *B. cephi*, the alignment was 741 bp and included 54 individuals from Colorado (n = 18), Nebraska (n = 14), and North Dakota (n = 22). Nineteen polymorphic sites defined six haplotypes. Populations from Colorado, Nebraska, and North Dakota contained three, four, and five haplotypes, respectively. Haplotype diversity was highest in North Dakota (Hd = 0.788) and lowest in Nebraska (Hd = 0.692) and Colorado (Hd = 0.699). Nucleotide diversity was also low (π = 0.00571 – 0.01012). For all samples combined, diversity was Hd = 0.720 and π = 0.00873. Neutrality tests indicated significant positive values in Colorado (**Table 5.2**).

For *B. lissogaster*, 15 individuals were analyzed (9 from winter wheat, 6 from wild grasses). The alignment was 741 bp and contained only two polymorphic sites, producing three haplotypes. Two haplotypes were found in winter wheat samples, while all three occurred in wild grasses. Haplotype diversity was higher in wild grasses (Hd = 0.600) than in winter wheat (Hd = 0.389), but nucleotide diversity was very low in both (π = 0.00090 and 0.00053). Neutrality tests were non-significant (**Table 5.2**).

Table 5.2: Indices of genetic diversity for WSS – *Cephus cinctus, Bracon cephi*, and *Bracon lissogaster* – populations based on mitochondrial COI sequences, including results from Tajima's *D* and Fu's *Fs* neutrality tests.

WSS - *Cephus cinctus*											
Gene	**Population**	**n**	**h**	**S**	**k**	**Hd**	**Pi**	***D***	***Fs***	***D* sig**	***Fs* sig**
COI	**Colorado**	23	7	17	2.387	0.708	0.00336	-1.85903	-0.384	*P < 0.05	P > 0.1
	Nebraska	11	5	11	4.327	0.6	0.00608	0.655	2.851	P > 0.1	P > 0.1
	All Samples	34	11	19	3.116	0.679	0.00438	-1.234546	-1	P > 0.1	P > 0.1
Bracon cephi											
Gene	**Population**	**n**	**h**	**S**	**k**	**Hd**	**Pi**	***D***	***Fs***	***D* sig**	***Fs* sig**
COI	**Colorado**	18	3	15	7.013	0.699	0.00934	2.30595*	9.546**	*P < 0.05	**P < 0.02
	Nebraska	14	4	15	4.286	0.692	0.00571	-0.37267	3.595	P > 0.1	P > 0.1
	North Dakota	22	5	19	7.602	0.788	0.01012	1.6957	5.17	P > 0.1	P > 0.1
	All Samples	54	6	19	6.556	0.72	0.00873	1.79179	7.134	0.10 > P > 0.05	P > 0.1
Bracon lissogaster											
Gene	**Population**	**n**	**h**	**S**	**k**	**Hd**	**Pi**	***D***	***Fs***	***D* sig**	***Fs* sig**
COI	**Winter wheat**	9	2	1	0.38889	0.389	0.00053	0.15647	0.477	P > 0.1	P > 0.1
	Wild grasses	6	3	2	0.66667	0.6	0.0009	-1.13197	-0.858	P > 0.1	P > 0.1
	All Samples	14	3	2	0.47619	0.448	0.000645245	-0.59491	-0.577	P > 0.1	P>0.1

n: Number of individuals analyzed; *h*: Number of haplotypes; *S*: Number of segregating sites; *k*: Average number of nucleotide differences; *Hd*: Haplotype diversity; π (Pi): Nucleotide diversity; *D*: Tajima's D statistic; *Fs*: Fu's Fs statistic; *D sig* / *Fs sig*: Significance levels of neutrality tests (*P* values); $P < 0.05$ and $P < 0.02$ denote significant deviations from neutrality.

Haplotype network analysis

Haplotype networks constructed from COI sequences supported the indices of genetic diversity among WSS, *B. cephi*, and *B. lissogaster* populations (**Figure 5.5**). For WSS, samples collected in Colorado (2023) and Nebraska (2025) yielded eleven haplotypes (**Figure 5.5A**). The network was centered on a dominant haplotype (H01) shared between regions, with multiple low-frequency haplotypes radiating from it. Colorado harbored a greater number of unique haplotypes when compared to Nebraska. This topology is consistent with a recent population

expansion, with partial regional differentiation. For *B. cephi*, six haplotypes were identified across Colorado, Nebraska, and North Dakota (**Figure 5.5B**). The network was dominated by one widespread haplotype – H03 – which is evenly shared across regions. H01 and H02 were also shared across regions, while H04 and H06 were restricted to North Dakota, and H05 was restricted to Nebraska. In *B. lissogaster*, three haplotypes were detected from parasitoids collected on winter wheat and wild grasses (**Figure 5.5C**). The dominant haplotype H01 was present on both hosts, as well as H02. In contrast, H03 were restricted to a wild grasses.

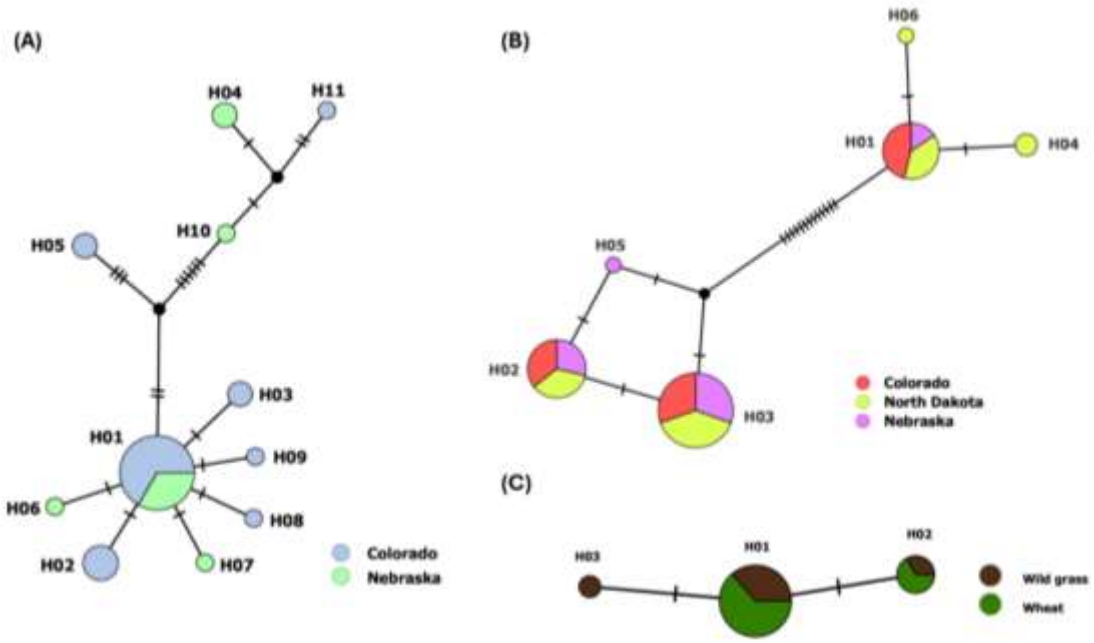

Figure 5.5: Haplotype networks for COI partial DNA sequences of (**A**) WSS (*Cephus cinctus*) sampled in Colorado and Nebraska in 2023 and 2025, respectively. (**B**) *Bracon cephi* sampled in 2022 and 2023 in Colorado, North Dakota and Nebraska, (**C**) *Bracon lissogaster* sampled from wild grasses in 2017 and winter wheat in 2023. Networks were created in PopART using the Templeton, Crandall, and Sing (TCS) method. Smaller circles indicate fewer individuals per haplotype. Black circle represent missing intermediate haplotype. Hash marks on the lines connecting haplotypes represent base-pair differences.

Population structure

Analyses of Molecular Variance (AMOVA) revealed contrasting patterns among species. In WSS, 12.6% of the genetic variance was explained by differences between Colorado and

Nebraska (ΦST = 0.126), with the remaining 87.4% within populations. This differentiation was marginally non-significant ($P = 0.066$), suggesting borderline geographic structure. In *B. cephi*, approximately 4% of the variance was attributed to differences among states (ΦST = 0.0043, $P = 0.349$ with approximately 96% of the variation occurring within populations, indicating little evidence of regional differentiation. For *B. lissogaster*, no host-associated genetic differentiation was detected between winter wheat and wild grass populations (ΦST = –0.051, $P = 0.600$).

Discussion

Our study found no significant differences in parasitism ability among *Bracon spp*. populations across regions. In greenhouse experiments, parasitoids from Colorado, North Dakota, and Nebraska showed statistically similar parasitism rates, suggesting they are equally capable of recognizing and parasitizing WSS larvae in Colorado winter wheat. Yet despite this biological potential, large-scale parasitism of WSS in wheat fields remains low or absent in Colorado (Peirce et al. 2021).

To better understand this wheat-WSS-*Bracon spp*. dynamics, we examined genetic variation in both the host and parasitoid. Our results for WSS are consistent with previous findings that southern populations (Colorado, Nebraska) represent local adaptation to wheat rather than recent introductions from northern populations (Lesieur et al. 2016). Nebraska populations showed stable diversity patterns, while Colorado populations exhibited signatures of recent expansion, in agreement with the species' more recent emergence as a pest in this region (Irell and Peairs 2014). In contrast, the parasitoid *B. cephi* displayed no strong geographic structure among the three states, suggesting a broadly homogeneous population. Nonetheless, significant neutrality test values in Colorado may indicate internal subdivision or balancing

processes, potentially reflecting asynchronous dynamics with its expanding host. Finally, our analysis of *B. lissogaster* for potential HAD between populations from wild grasses and winter wheat yielded non-significant results, though sample sizes were limited.

Recent WSS expansion in Colorado

Mitochondrial COI data from WSS populations in Colorado and Nebraska closely mirrored the patterns described by Lesieur et al. (Lesieur et al. 2016) who identified distinct genetic clusters across North America and linked the "southern cluster" to a recent demographic expansion. Our haplotype network results align with this interpretation: we detected seven haplotypes in Colorado compared to four in Nebraska, indicating greater genetic diversity in the expanding Colorado populations. AMOVA revealed moderate genetic differentiation between Colorado and Nebraska ($\Phi ST = 0.126$, $P = 0.066$), suggesting some population structure, though not statistically significant. This finding complements neutrality tests, which showed a significantly negative Tajima's *D* in Colorado, supporting recent expansion, while Nebraska populations showed no departure from neutrality.

These results support the view that Colorado WSS populations are not only part of the broader southern expansion but also represent a distinct, dynamic component of it. We identified three novel haplotypes (H03, H07, H08), further indicating ongoing diversification. In contrast, the parasitoid B. *cephi* showed no geographic structure, highlighting a potential mismatch in population dynamics between host and parasitoid that may affect biological control outcomes.

Phylogenetic and haplotype patterns in Bracon spp.

The haplotype networks revealed that *B. cephi* populations are genetically intermixed across Colorado, Nebraska, and North Dakota (Figure 5.5B). All major haplotypes were shared among regions, with no evidence of geographic structuring. This pattern supports the field data,

which showed no statistical differences in parasitism rates among parasitoid populations, and suggests that *B. cephi* broadly distributed and capable of exploiting WSS hosts across central and northern Great Plains. AMOVA results further support this interpretation: only about 4% of the genetic variation was attributed to differences among states (ΦST = 0.0043, $P = 0.349$), with the vast majority of variation (96%) occurring within populations.

Despite this shared haplotype distribution, neutrality tests identified important population-level differences, as AMOVA showed. Colorado *B. cephi* displayed a significantly positive Tajima's *D*, and a strongly significant Fu's *Fs*. Positive Tajima's *D* values indicate an excess of intermediate-frequency alleles, often interpreted as evidence of population subdivision, balancing selection, or demographic contraction. The high Fu's *Fs* further suggests a deficit of recent mutations, reinforcing the signal of population fragmentation or structure. In contrast, *B. cephi* populations from Nebraska and North Dakota showed non-significant Tajima's *D* and Fu's *Fs* values, consistent with demographic equilibrium. While *B. cephi* populations are generally well-mixed across the regions, these contrasting signals suggests that *B. cephi* in Colorado may harbor subtle internal structure and could be undergoing local subdivision or experiencing asynchronous dynamics relative to its rapidly expanding host, the WSS.

Our dataset also included *B. lissogaster* individuals from both winter wheat (n=9) and wild grasses (n=6), allowing a preliminary test for potential host-associated differentiation (HAD). Haplotype diversity was higher in wild grass populations, and Tajima's D was slightly negative, but neither Tajima's *D* nor Fu's *Fs* reached statistical significance. These findings align with Rand et al. (Rand et al. 2024), who reported no evidence of HAD in braconid parasitoids in Montana. AMOVA results further supported this conclusion: no genetic differentiation was detected between wheat and wild grass populations (ΦST = –0.051, $P = 0.600$), indicating no

measurable host-associated structure. While the trend toward higher diversity in wild grasses is noteworthy, these patterns remain speculative due to the limited sample size, and further sampling is needed to draw robust conclusions.

Biological contrasts between B. cephi and B. lissogaster

The coexistence of *B. cephi* and *B. lissogaster* in our dataset, highlight the importance of considering how species-specific traits shape ecological roles. In our Colorado winter wheat samples, about 30% of the parasitoids gathered were *B. lissogaster*, whereas *B. cephi* dominated in Nebraska and North Dakota. Although both species share broadly similar biology and attack WSS, their roles in agroecosystems appear to diverge due to life-history differences. In the northern Great Plains, *B. cephi* is generally more abundant in spring wheat, while *B. lissogaster* is more frequently associated with native grasses and winter wheat (Meers 2005). *B. lissogaster* develops more rapidly (6-8 days) and lays one to four eggs per host, a gregarious behavior that can further shorten development under crowded conditions (Somsen and Luginbill 1956, Meers 2005, Reis 2018). In contrast, *B. cephi* has a slower developmental period of 10 days (Nelson and Farstad 1953, Holmes et al. 1963). Differences in development time, the ability to occasionally produce a second generation, and landscape context likely influence which species is more prevalent in different agroecological settings (Somsen and Luginbill 1956, Holmes et al. 1963, Meers 2005, Rand et al. 2006, Cavallini et al. 2022, 2023). Additionally, abiotic factors may also contribute to their contrasting distributions, as the impacts of weather and climate on *Bracon spp*. remain poorly studied (Hance et al. 2007, Sjolie 2022). Moreover, understanding how these biological and environmental factors interact will be especially important in Colorado. Such knowledge could help identify which parasitoid is best suited for long-term biological control in this region.

Implications for integrated pest management

WSS has been a major pest of wheat in the northern Great Plains for over a century (Ainslie 1920, Holmes 1977, 1979, Portman et al. 2018) and successfully adapted to spring wheat by the mid-1980s (Morrill and Kushnak 1996). For just as long, *Bracon spp*. parasitoids have been linked to WSS and regarded as an important tool to suppress its population (Criddle 1922, Nelson and Farstad 1953, Somsen and Luginbill 1956, Holmes et al. 1963, Beres, Dosdall, et al. 2011). In Colorado, however, recognition of WSS as a serious winter wheat pest is relatively recent (Irell and Peairs 2014), reflecting its rapid expansion into new wheat systems in the region. Additionally, Peirce et al. (Peirce et al. 2021) surveyed approximately 30,000 winter wheat stems over three years, and no evidence of parasitism was found, echoing the low parasitism issue observed in Colorado.

This study highlights the complexity of the wheat-WSS-parasitoid system, emphasizing that tritrophic interactions are often more variable than bitrophic ones (Stireman III and Singer 2003, Stireman III et al. 2006). By integrating molecular and field results, we provide insights into sustainable management of WSS. Our experiments confirmed that *Bracon spp*. can parasitize Colorado WSS populations, even though overall parasitism of WSS in winter wheat remains low. This suggests that the lack of parasitism in Colorado is not due to host incompatibility or geographic genetic differentiation but may instead reflect subdivision between wild grasses and winter wheat, reduced haplotype diversity in Colorado due to loss of natural habitat, asynchronous dynamics with WSS, environmental constraints, or simply an evolutionary lag following WSS rapid expansion. For *B. cephi*, the significantly positive Tajima's *D* and Fu's *F*s in Colorado indicate population instability, consistent with subdivision or lagging behind host expansion. Such instability suggests that *B. cephi* may require targeted support to establish

resilient populations in newly invaded areas (ongoing work from Bradshaw et al. (Bradshaw et al. 2025)). In contrast, *B. lissogaster* presented one more haplotype in wild grasses than in winter wheat and accounted for close to 30% of parasitoids in Colorado winter wheat, traits that may allow it to persist where *B. cephi* is less competitive. This highlights the potential of *B. lissogaster* as a complementary biological control agent in Colorado winter wheat. Ultimately, long-term monitoring and landscape-level planning will be critical to determine whether *Bracon spp*. populations can adapt quickly enough to keep pace with the rapid expansion of WSS.

Future research direction

Neutrality tests such as Tajima's *D* and Fu's *Fs* are highly sensitive to n, since segregating sites and average pairwise differences become strongly correlated in small datasets (Tajima 1989). Although our sample sizes fall within the range Tajima explicitly tested (n=20), higher n increases power to detect deviations from neutrality. Fu (Fu 1997) also demonstrated that *Fs* tests have limited power when sample sizes are low, as variance increases with few segregating sites. Statistical reliability improves substantially with larger samples, which would enable stronger inference about demographic expansion, population structure, or selection.

Future work should therefore prioritize increasing sample size across both hosts and parasitoids. Collecting additional *B. cephi* from wild grasses would help test whether the observed trend in Colorado reflect HAD or other forms of population subdivision. Similarly, expanding *B. lissogaster* collections from both winter wheat and wild grasses would also clarify the preliminary patterns we observed, particularly the higher haplotype diversity in wild grasses. However, these collections remain challenging due to low population densities.

Complementary studies of *Bracon spp*. phenology are equally important. Comparative monitoring of WSS and *Bracon spp*. across Colorado could help determine whether mismatched

life cycles explain the parasitoid's reduced effectiveness. Such data would clarify which species could be best suited to Colorado's agroecosystem conditions, and whether targeted conservation or introductions could improve biological control. Finally, expanding the geographic scope to include states such as Kansas, where WSS has recently been reported, would provide a more complete picture of host-parasitoid dynamics across the expanding range of this pest.

References

Althoff DM, Thompson JN. 2001. Geographic structure in the searching behaviour of a specialist parasitoid: combining molecular and behavioural approaches. Journal of Evolutionary Biology. 14(3):406–417. https://doi.org/10.1046/j.1420-9101.2001.00286.x

Beres B, Cárcamo H, Weaver D, et al. 2011. Integrating the building blocks of agronomy and biocontrol into an IPM strategy for wheat stem sawfly. Prairie Soils & Crops Journal. 4.

Beres B, Dosdall L, Weaver D, et al. 2011. Biology and integrated management of wheat stem sawfly and the need for continuing research. The Canadian Entomologist. 143(2):105–125. https://doi.org/10.4039/n10-056

Bilodeau E, Simon JC, Guay JF, et al. 2013. Does variation in host plant association and symbiont infection of pea aphid populations induce genetic and behaviour differentiation of its main parasitoid, *Aphidius ervi*? Evolutionary Ecology. 27(1):165–184. https://doi.org/10.1007/s10682-012-9577-z

Bradshaw JD, Rand TA, Peirce ES, et al. 2025. 'Beneficial Bug Baler': A novel technique for mass relocation of *Bracon* in support of *Cephus cinctus* biocontrol in wheat, *Triticum aestivum*, 2024. Crow W, editor. Arthropod Management Tests. 50(1). https://doi.org/10.1093/amt/tsaf072

Cárcamo HA, Weaver D, Meers S, et al. 2012. First record of *Bracon lissogaster* (Hymenoptera: Braconidae) in Canada - a potentially important parasitoid of *Cephus cinctus* (Hymenoptera: Cephidae) in the prairies. Biocontrol Science and Technology. 22(3):367–369. https://doi.org/10.1080/09583157.2012.658355

Cavallini L, Peterson RKD, Weaver DK. 2022. Dietary sugars and amino acids increase longevity and enhance reproductive parameters of *Bracon cephi* and *B. lissogaster*, two parasitoids that specialise on wheat stem sawfly. Physiological Entomology. https://doi.org/10.1111/phen.12399

Cavallini L, Peterson RKD, Weaver DK. 2023. Cowpea extrafloral nectar has potential to provide ecosystem services lost in agricultural intensification and support native parasitoids that suppress the wheat stem sawfly. Dara S, editor. Journal of Economic Entomology. https://doi.org/10.1093/jee/toad083

Cockrell DM, Griffin-Nolan RJ, Rand TA, et al. 2017. Host plants of the wheat stem sawfly (Hymenoptera: Cephidae). Environmental Entomology. 46(4):847–854. https://doi.org/10.1093/ee/nvx104

Cockrell DM, Randolph T, Peirce E, et al. 2021. Survey of wheat stem sawfly (Hymenoptera: Cephidae) infesting wheat in eastern Colorado. Journal of Economic Entomology. 114(2):998–1004. https://doi.org/10.1093/jee/toab015

Criddle N. 1922. The western wheat-stem sawfly and its control. Ottawa Report No.: No. 6. https://www.cabdirect.org/cabdirect/abstract/19220501075.

Criddle N. 1923. Popular and practical entomology: the life habits of *Cephus cinctus* Nort. in Manitoba. The Canadian Entomologist. 55(1). https://doi.org/10.4039/Ent551-1

French CM, Bertola LD, Carnaval AC, et al. 2023. Global determinants of insect mitochondrial genetic diversity. Nature Communications. 14(1):5276. https://doi.org/10.1038/s41467-023-40936-0

Fu Y-X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics. 147(2):915–925. https://doi.org/10.1093/genetics/147.2.915

Gahan A. 1918. Description of a new Hymenopterous parasite (Braconidae). Proceedings of the Entomological Society. 20(1):18–19.

Godfray HCJ, Müller CB. 1998. Host-parasitoid dynamics. In: Dempster JP, McLean IFG, editors. Insect Populations In theory and in practice: 19th Symposium of the Royal Entomological Society 10–11 September 1997 at the University of Newcastle. Dordrecht: Springer Netherlands. p. 135–165. https://doi.org/10.1007/978-94-011-4914-3_6

Hance T, Van Baaren J, Vernon P, et al. 2007. Impact of extreme temperatures on parasitoids in a climate change perspective. Annual Review of Entomology. 52(1):107–126. https://doi.org/10.1146/annurev.ento.52.110405.091333

Holmes N. 1979. The wheat stem sawfly. 26th Annual Meetings of the Entomological Society of Alberta and Entomological Society of Saskatchewan. 26.

Holmes N, Nelson W, Peterson L, et al. 1963. Causes of variations in effectiveness of *Bracon cephi* (Gahan) (Hymenoptera: Braconidae) as a parasite of the wheat stem sawfly. The Canadian Entomologist. 95(2):113–126.

Holmes ND. 1977. The effect of the wheat stem sawfly, *Cephus cinctus* (Hymenoptera: Cephidae), on the yield and quality of wheat. The Canadian Entomologist. 109(12):1591–1598. https://doi.org/10.4039/Ent1091591-12

Holmes ND, Peterson LK. 1960. The influence of the host on oviposition by the wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae). Canadian Journal of Plant Science. 40(1):29–46. https://doi.org/10.4141/cjps60-004

Irell B, Peairs F. 2014. Wheat stem sawfly: A new pest of Colorado wheat. Colorado State University Extension. [accessed 2024 Apr 18]. https://extension.colostate.edu/topic-areas/insects/wheat-stem-sawfly-a-new-pest-of-colorado-wheat-5-612/.

Lesieur V, Martin JF, Weaver DK, et al. 2016. Phylogeography of the wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae): implications for pest management. PLoS ONE. 11(12). https://doi.org/10.1371/journal.pone.0168370

Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics. 25(11):1451–1452. https://doi.org/10.1093/bioinformatics/btp187

Lou KF, Weiss MJ, Bruckner PL, et al. 1998. RAPD variation within and among geographic populations of wheat stem sawfly (*Cephus cinctus* Norton). The American Genetic Association. 89:329–335. https://doi.org/10.1093/jhered/89.4.329

Lozier JD, Roderick GK, Mills NJ. 2009. Molecular markers reveal strong geographic, but not host associated, genetic differentiation in *Aphidius transcaspicus*, a parasitoid of the aphid genus *Hyalopterus*. Bulletin of Entomological Research. 99(1):83–96. https://doi.org/10.1017/S0007485308006147

Meers SB. 2005. Impact of harvest operations on parasitism of the wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae). Montana State University. https://scholarworks.montana.edu/xmlui/handle/1/1853.

Morrill WL, Gabor JW, Hockett EA, et al. 1992. Wheat stem sawfly (Hymenoptera: Cephidae) resistance in winter wheat. Journal of Economic Entomology. 85(5):2008–2011. https://doi.org/10.1093/jee/85.5.2008

Morrill WL, Kushnak GD. 1996. Wheat stem sawfly (Hymenoptera: Cephidae) adaptation to winter wheat. Environmental Entomology. 25(5):1128–1132.

Morrill WL, Kushnak GD, Bruckner PL. 1994. Wheat stem sawfly (Hymenoptera: Cephidae) damage, rates of parasitism, and overwinter survival in resistant wheat lines. Journal of Economic Entomology. 87:1373–1376.

Muesebeck CFW. 1953. Three new reared Braconidae (Hymenoptera). Proceedings of the Entomological Society of Washington. 55(3):149–151.

Nelson WA, Farstad CW. 1953. Biology of *Bracon cephi* (Gahan) (Hymenoptera: Braconidae), an important native parasite of the wheat stem sawfly, *Cephus cinctus* Nort. (Hymenoptera: Cephidae), in western Canada. The Canadian Entomologist. 85(3):103–107. https://doi.org/10.4039/Ent85103-3

Peirce ES, Evers B, Winn ZJ, et al. 2024. Identifying novel sources of resistance to wheat stem sawfly in five wild wheat species. Pest Management Science. https://doi.org/10.1002/ps.8008

Peirce ES, Rand TA, Cockrell DM, et al. 2021. Effects of landscape composition on wheat stem sawfly (Hymenoptera: Cephidae) and its associated braconid parasitoids. Journal of Economic Entomology. 114(1):72–81. https://doi.org/10.1093/jee/toaa287

Peters RS, Krogmann L, Mayer C, et al. 2017. Evolutionary history of the Hymenoptera. Current Biology. 27(7):1013–1018. https://doi.org/10.1016/j.cub.2017.01.027

Portman SL, Jaronski ST, Weaver DK, et al. 2018. Advancing biological control of the wheat stem sawfly: New strategies in a 100-yr struggle to manage a costly pest in the northern great plains. Annals of the Entomological Society of America. 111(3):85–91. https://doi.org/10.1093/aesa/say002

R Core Team. 2025. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.

Rand TA, Kula RR, Gaskin JF. 2024. Evaluating the use of common grasses by the wheat stem sawfly (Hymenoptera: Cephidae) and its native parasitoids in rangeland and Conservation Reserve Program grasslands. Liu T-X, editor. Journal of Economic Entomology. 117(3):858–864. https://doi.org/10.1093/jee/toae046

Rand TA, Tylianakis JM, Tscharntke T. 2006. Spillover edge effects: The dispersal of agriculturally subsidized insect natural enemies into adjacent natural habitats. Ecology Letters. 9(5):603–614. https://doi.org/10.1111/j.1461-0248.2006.00911.x

Rand TA, Waters DK. 2020. Aphid honeydew enhances parasitoid longevity to the same extent as a high-quality floral resource: implications for conservation biological control of the wheat

stem sawfly (Hymenoptera: Cephidae). Journal of Economic Entomology. 113(4):2022–2025. https://doi.org/10.1093/jee/toaa076

Rand TA, Waters DK, Shanower TG. 2011. Unexpectedly high levels of parasitism of wheat stem sawfly larvae in postcutting diapause chambers. Canadian Entomologist. 143(5):455–459. https://doi.org/10.4039/n11-023

Reis DA. 2018. The potential of sugar resources in the reproductive biology of wheat stem sawfly parasitoids [PhD dissertation]. Montana State University-Bozeman. https://scholarworks.montana.edu/xmlui/handle/1/14678.

Reis DA, Hofland ML, Peterson RKD, et al. 2019. Effects of sucrose supplementation and generation on life-history traits of *Bracon cephi* and *B. lissogaster*, parasitoids of the wheat stem sawfly. Physiological Entomology. 44(3–4):266–274. https://doi.org/10.1111/phen.12303

Rosenzweig C, Iglesius A, Yang XB, et al. 2001. Climate change and extreme weather events-Implications for Climate change and extreme weather events-Implications for food production, plant diseases, and pests food production, plant diseases, and pests. https://digitalcommons.unl.edu/nasapub/24.

Runyon J, Hurley R, Morrill W, et al. 2001. Distinguishing adults of *Bracon cephi* and *Bracon lissogaster* (Hymenoptera: Braconidae), parasitoids of the wheat stem sawfly (Hymenoptera: Cephidae). The Canadian Entomologist. 133(2):215–217.

Runyon JB, Morrill WL, Weaver DK, et al. 2002. Parasitism of the wheat stem sawfly (Hymenoptera: Cephidae) by *Bracon cephi* and *B. lissogaster* (Hymenoptera: Braconidae) in

wheat fields bordering tilled and untilled fallow in Montana. Journal of Economic Entomology. 95(6):1130–1134. https://doi.org/10.1603/0022-0493-95.6.1130

Scheffer SJ, Hawthorne DJ. 2007. Molecular evidence of host-associated genetic divergence in the holly leafminer *Phytomyza glabricola* (Diptera: Agromyzidae): apparent discordance among marker systems. Molecular Ecology. 16(12):2629–2639. https://doi.org/10.1111/j.1365-294X.2007.03303.x

Simon C, Frati F, Beckenbach A, et al. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America. 87(6):651–701. https://doi.org/10.1093/aesa/87.6.651

Sjolie DM. 2022. Investigating population dynamics of the wheat stem sawfly (*Cephus cinctus*) (Hymenoptera: Cephidae) in Canadian agroecosystems [Master's thesis]. University of Saskatchewan.

Somsen HW, Luginbill P. 1956. *Bracon lissogaster* Mues: A parasite of the wheat stem sawfly. U.S. Department of Agriculture (Technical bulletin (United States. Department of Agriculture)). https://books.google.com/books?id=Go3i5AKCfa8C.

Stireman III JO, Nason JD, Heard SB. 2005. Host-associated genetic differentiation in phytophagous insects: general phenomenom or isolated exceptions? Evidence from a goldenrod-insect community. Evolution. 59(12):2573–2587. https://doi.org/10.1554/05-222.1.s1

Stireman III JO, Nason JD, Heard SB, et al. 2006. Cascading host-associated genetic differentiation in parasitoids of phytophagous insects. Proceedings of the Royal Society B: Biological Sciences. 273(1586):523–530. https://doi.org/10.1098/rspb.2005.3363

Stireman III JO, Singer MS. 2003. Determinants of parasitoid-host associations: Insights from a natural tachinid-lepidopteran community. Ecology. 84(2):296–310.

Tajima F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics. 123(3):585–595. https://doi.org/10.1093/genetics/123.3.585

Vieira HV, Bradford BZ, Osterholzer A, et al. 2025. A new growing degree-day phenology model for wheat stem sawfly (Hymenoptera: Cephidae) in Colorado wheat fields. Fand BB, editor. PLoS ONE. 20(4):e0320497. https://doi.org/10.1371/journal.pone.0320497

Weaver DK, Sing SE, Runyon JB, et al. 2005. Potential impact of cultural practices on wheat stem sawfly (Hymenoptera: Cephidae) and associated parasitoids. Journal of Agricultural Urban Entomology. 21(4):271–287.

Weiss MJ, Morrill WL. 1992. Wheat stem sawfly (Hymenoptera: Cephidae) revisited. American Entomology. 38(4):241–245. https://doi.org/10.1093/ae/38.4.24

APPENDIX

A1 Chapter 3 supplemental information

Supplemental Table A1.1: Peak area measurements for each volatile compound identified by TD-GC/MS, organized by wheat cultivar and infestation status. Chemical group classification is included for each compound.

Cultivar + infestation status	Compound	Peak area	Chemical group
Amplify SF WSS infested	(E)-2-Octenal	638946.5	Aldehyde
Amplify SF WSS infested	2-Ethyl-1-hexanol	532951.5	Alcohol
Amplify SF WSS infested	2-Heptanone	62759	Ketone
Amplify SF WSS infested	3-Methyl-2-heptanone	58036	Ketone
Amplify SF WSS infested	5-Methyl-2-heptanol	854292.5	Alcohol
Amplify SF WSS infested	6-Methyl-5-hepten-2-one	309470.5	Ketone
Amplify SF WSS infested	Acetophenone	3030204	Ketone
Amplify SF WSS infested	Benzaldehyde	846896	Aldehyde
Amplify SF WSS infested	Decanal	12132901	Aldehyde
Amplify SF WSS infested	Dodecane	1077155	Alkane
Amplify SF WSS infested	Heptanal	887342.5	Aldehyde
Amplify SF WSS infested	Hexanal	2211797	Aldehyde
Amplify SF WSS infested	Hexanoic acid	2254037	Carboxylic Acid
Amplify SF WSS infested	Limonene	299904.5	Terpene
Amplify SF WSS infested	Nonanal	22725254	Aldehyde
Amplify SF WSS infested	Nonanoic acid	6410601	Carboxylic Acid
Amplify SF WSS infested	Octadecanal	14825.5	Aldehyde
Amplify SF WSS infested	Octanal	338797.5	Aldehyde
Amplify SF WSS infested	Tetradecane	1096226	Alkane
Amplify SF WSS infested	Undecane	1806073	Alkane
Amplify SF WSS infested	n-Hexadecanoic acid	6388736	Fatty Acid
Amplify SF WSS infested	α-Pinene	278483	Terpene
Amplify SF	(E)-2-Octenal	49619.5	Aldehyde
Amplify SF	2-Ethyl-1-hexanol	404209.5	Alcohol
Amplify SF	2-Heptanone	23298	Ketone
Amplify SF	3-Methyl-2-heptanone	68163	Ketone
Amplify SF	5-Methyl-2-heptanol	863932	Alcohol
Amplify SF	6-Methyl-5-hepten-2-one	201386	Ketone
Amplify SF	Acetophenone	2748768	Ketone
Amplify SF	Benzaldehyde	503410.5	Aldehyde
Amplify SF	Decanal	10450458	Aldehyde
Amplify SF	Dodecane	1860365	Alkane

Amplify SF	Heptanal	349740.5	Aldehyde
Amplify SF	Hexanal	633305.5	Aldehyde
Amplify SF	Hexanoic acid	1744948	Carboxylic Acid
Amplify SF	Limonene	235674.5	Terpene
Amplify SF	Nonanal	20849997	Aldehyde
Amplify SF	Nonanoic acid	5108718	Carboxylic Acid
Amplify SF	Octadecanal	12548	Aldehyde
Amplify SF	Octanal	248741.5	Aldehyde
Amplify SF	Tetradecane	2322974	Alkane
Amplify SF	Undecane	3818065	Alkane
Amplify SF	n-Hexadecanoic acid	4146983	Fatty Acid
Amplify SF	α-Pinene	94890	Terpene
Brawl CL Plus WSS infested	(E)-2-Octenal	90962	Aldehyde
Brawl CL Plus WSS infested	2-Ethyl-1-hexanol	438235.5	Alcohol
Brawl CL Plus WSS infested	2-Heptanone	47612.5	Ketone
Brawl CL Plus WSS infested	3-Methyl-2-heptanone	110627	Ketone
Brawl CL Plus WSS infested	5-Methyl-2-heptanol	963413	Alcohol
Brawl CL Plus WSS infested	6-Methyl-5-hepten-2-one	316700	Ketone
Brawl CL Plus WSS infested	Acetophenone	2777444	Ketone
Brawl CL Plus WSS infested	Benzaldehyde	643478.5	Aldehyde
Brawl CL Plus WSS infested	Decanal	9507987	Aldehyde
Brawl CL Plus WSS infested	Dodecane	2030298	Alkane
Brawl CL Plus WSS infested	Heptanal	494658	Aldehyde
Brawl CL Plus WSS infested	Hexanal	729189.5	Aldehyde
Brawl CL Plus WSS infested	Hexanoic acid	2172348	Carboxylic Acid
Brawl CL Plus WSS infested	Limonene	242354.5	Terpene
Brawl CL Plus WSS infested	Nonanal	20627636	Aldehyde
Brawl CL Plus WSS infested	Nonanoic acid	6174779	Carboxylic Acid
Brawl CL Plus WSS infested	Octadecanal	12278	Aldehyde
Brawl CL Plus WSS infested	Octanal	263527	Aldehyde
Brawl CL Plus WSS infested	Tetradecane	1956640	Alkane
Brawl CL Plus WSS infested	Undecane	4216785	Alkane
Brawl CL Plus WSS infested	n-Hexadecanoic acid	26108567	Fatty Acid
Brawl CL Plus WSS infested	α-Pinene	185334	Terpene
Brawl CL Plus	(E)-2-Octenal	74331	Aldehyde
Brawl CL Plus	2-Ethyl-1-hexanol	423367	Alcohol
Brawl CL Plus	2-Heptanone	33120.5	Ketone
Brawl CL Plus	3-Methyl-2-heptanone	108018	Ketone
Brawl CL Plus	5-Methyl-2-heptanol	723648.5	Alcohol
Brawl CL Plus	6-Methyl-5-hepten-2-one	409461.5	Ketone
Brawl CL Plus	Acetophenone	1901729	Ketone

Brawl CL Plus	Benzaldehyde	399988.5	Aldehyde
Brawl CL Plus	Decanal	9779539	Aldehyde
Brawl CL Plus	Dodecane	1385902	Alkane
Brawl CL Plus	Heptanal	312660.5	Aldehyde
Brawl CL Plus	Hexanal	713492	Aldehyde
Brawl CL Plus	Hexanoic acid	2119809	Carboxylic Acid
Brawl CL Plus	Limonene	224151	Terpene
Brawl CL Plus	Nonanal	16874052	Aldehyde
Brawl CL Plus	Nonanoic acid	3759041	Carboxylic Acid
Brawl CL Plus	Octadecanal	10798	Aldehyde
Brawl CL Plus	Octanal	185449.5	Aldehyde
Brawl CL Plus	Tetradecane	1221265	Alkane
Brawl CL Plus	Undecane	2063844	Alkane
Brawl CL Plus	n-Hexadecanoic acid	3207284	Fatty Acid
Brawl CL Plus	α-Pinene	135404.5	Terpene
Byrd CL Plus WSS infested	(E)-2-Octenal	67769	Aldehyde
Byrd CL Plus WSS infested	2-Ethyl-1-hexanol	429053.5	Alcohol
Byrd CL Plus WSS infested	2-Heptanone	66370	Ketone
Byrd CL Plus WSS infested	3-Methyl-2-heptanone	45297.5	Ketone
Byrd CL Plus WSS infested	5-Methyl-2-heptanol	1023529	Alcohol
Byrd CL Plus WSS infested	6-Methyl-5-hepten-2-one	226637	Ketone
Byrd CL Plus WSS infested	Acetophenone	2391441	Ketone
Byrd CL Plus WSS infested	Benzaldehyde	577764.5	Aldehyde
Byrd CL Plus WSS infested	Decanal	10818917	Aldehyde
Byrd CL Plus WSS infested	Dodecane	966229.5	Alkane
Byrd CL Plus WSS infested	Heptanal	250786.5	Aldehyde
Byrd CL Plus WSS infested	Hexanal	628462.5	Aldehyde
Byrd CL Plus WSS infested	Hexanoic acid	1648962	Carboxylic Acid
Byrd CL Plus WSS infested	Limonene	259995	Terpene
Byrd CL Plus WSS infested	Nonanal	25264901	Aldehyde
Byrd CL Plus WSS infested	Nonanoic acid	6321528	Carboxylic Acid
Byrd CL Plus WSS infested	Octadecanal	13444.5	Aldehyde
Byrd CL Plus WSS infested	Octanal	299051	Aldehyde
Byrd CL Plus WSS infested	Tetradecane	959460	Alkane
Byrd CL Plus WSS infested	Undecane	1185778	Alkane
Byrd CL Plus WSS infested	n-Hexadecanoic acid	18693917	Fatty Acid
Byrd CL Plus WSS infested	α-Pinene	258697.5	Terpene
Byrd CL Plus	(E)-2-Octenal	495146.5	Aldehyde
Byrd CL Plus	2-Ethyl-1-hexanol	427217.5	Alcohol
Byrd CL Plus	2-Heptanone	68516.5	Ketone
Byrd CL Plus	3-Methyl-2-heptanone	100130.5	Ketone

Byrd CL Plus	5-Methyl-2-heptanol	706218	Alcohol
Byrd CL Plus	6-Methyl-5-hepten-2-one	474548.5	Ketone
Byrd CL Plus	Acetophenone	3117148	Ketone
Byrd CL Plus	Benzaldehyde	624331	Aldehyde
Byrd CL Plus	Decanal	16451236	Aldehyde
Byrd CL Plus	Dodecane	1660161	Alkane
Byrd CL Plus	Heptanal	1159164	Aldehyde
Byrd CL Plus	Hexanal	1846990	Aldehyde
Byrd CL Plus	Hexanoic acid	2026949	Carboxylic Acid
Byrd CL Plus	Limonene	318361.5	Terpene
Byrd CL Plus	Nonanal	24810806	Aldehyde
Byrd CL Plus	Nonanoic acid	5648849	Carboxylic Acid
Byrd CL Plus	Octadecanal	14503	Aldehyde
Byrd CL Plus	Octanal	346253	Aldehyde
Byrd CL Plus	Tetradecane	1705218	Alkane
Byrd CL Plus	Undecane	3238715	Alkane
Byrd CL Plus	n-Hexadecanoic acid	9848316	Fatty Acid
Byrd CL Plus	α-Pinene	246176.5	Terpene
Byrd WSS infested	(E)-2-Octenal	48401.5	Aldehyde
Byrd WSS infested	2-Ethyl-1-hexanol	788556	Alcohol
Byrd WSS infested	2-Heptanone	103107	Ketone
Byrd WSS infested	3-Methyl-2-heptanone	117986	Ketone
Byrd WSS infested	5-Methyl-2-heptanol	1205554	Alcohol
Byrd WSS infested	6-Methyl-5-hepten-2-one	285782.5	Ketone
Byrd WSS infested	Acetophenone	5924464	Ketone
Byrd WSS infested	Benzaldehyde	1574947	Aldehyde
Byrd WSS infested	Decanal	17603978	Aldehyde
Byrd WSS infested	Dodecane	2572767	Alkane
Byrd WSS infested	Heptanal	1294561	Aldehyde
Byrd WSS infested	Hexanal	856683.5	Aldehyde
Byrd WSS infested	Hexanoic acid	1890759	Carboxylic Acid
Byrd WSS infested	Limonene	298662	Terpene
Byrd WSS infested	Nonanal	33134413	Aldehyde
Byrd WSS infested	Nonanoic acid	7333367	Carboxylic Acid
Byrd WSS infested	Octadecanal	22083	Aldehyde
Byrd WSS infested	Octanal	423648	Aldehyde
Byrd WSS infested	Tetradecane	1632623	Alkane
Byrd WSS infested	Undecane	10639394	Alkane
Byrd WSS infested	n-Hexadecanoic acid	31598800	Fatty Acid
Byrd WSS infested	α-Pinene	135321.5	Terpene
Byrd	(E)-2-Octenal	19906	Aldehyde

Byrd	2-Ethyl-1-hexanol	306088	Alcohol
Byrd	2-Heptanone	37240	Ketone
Byrd	3-Methyl-2-heptanone	40685	Ketone
Byrd	5-Methyl-2-heptanol	795311	Alcohol
Byrd	6-Methyl-5-hepten-2-one	90819	Ketone
Byrd	Acetophenone	1916397	Ketone
Byrd	Benzaldehyde	432539	Aldehyde
Byrd	Decanal	8006153	Aldehyde
Byrd	Dodecane	694870	Alkane
Byrd	Heptanal	92287	Aldehyde
Byrd	Hexanal	354672	Aldehyde
Byrd	Hexanoic acid	495578	Carboxylic Acid
Byrd	Limonene	134323	Terpene
Byrd	Nonanal	18839264	Aldehyde
Byrd	Nonanoic acid	3151423	Carboxylic Acid
Byrd	Octadecanal	10289	Aldehyde
Byrd	Octanal	162811	Aldehyde
Byrd	Tetradecane	618011	Alkane
Byrd	Undecane	757774	Alkane
Byrd	n-Hexadecanoic acid	3988455	Fatty Acid
Byrd	α-Pinene	61936	Terpene

Supplemental Table A1.2: Fold-Change values for each compound identified in the TD-GC/MS analysis, using the cultivar "Byrd" (non-infested by wheat stem sawfly) as the baseline. Compounds are grouped by cultivar and infestation status.

Compound	WSS Non-infested			WSS Infested			
	Amplify SF	Brawl CL Plus	Byrd CL Plus	Byrd	Amplify SF	Brawl CL Plus	Byrd CL Plus
(E)-2-Octenal	2.492691	3.7341	24.87423	2.431503	32.09819	4.569577	3.404451
2-Ethyl-1-hexanol	1.320566	1.383155	1.395734	2.57624	1.741171	1.43173	1.401733
2-Heptanone	0.625618	0.88938	1.839863	2.768716	1.685258	1.278531	1.782223
3-Methyl-2-heptanone	1.675384	2.654983	2.461116	2.899988	1.426472	2.71911	1.113371
5-Methyl-2-heptanol	1.086282	0.909894	0.887977	1.515827	1.074162	1.211366	1.286954
6-Methyl-5-hepten-2-one	2.217443	4.508544	5.225212	3.146726	3.407552	3.487156	2.49548
Acetophenone	1.434341	0.992346	1.626567	3.09146	1.581198	1.449305	1.247884
Benzaldehyde	1.16385	0.924746	1.44341	3.641168	1.957964	1.487677	1.335751
Decanal	1.305303	1.221503	2.054824	2.198806	1.515447	1.187585	1.351325
Dodecane	2.677284	1.994476	2.389167	3.702516	1.550153	2.921838	1.390518
Heptanal	3.789705	3.387915	12.56043	14.02755	9.615032	5.359997	2.717463
Hexanal	1.785609	2.011695	5.2076	2.415425	6.236176	2.055955	1.771954
Hexanoic acid	3.521035	4.277447	4.09007	3.815259	4.548299	4.383462	3.32735
Limonene	1.754536	1.668746	2.370119	2.223461	2.232711	1.804267	1.935596
Nonanal	1.106731	0.895685	1.316973	1.758796	1.206271	1.094928	1.341077
Nonanoic acid	1.621083	1.192807	1.792475	2.327002	2.034192	1.959362	2.005928
Octadecanal	1.219555	1.04947	1.409564	2.146273	1.440908	1.193313	1.306687
Octanal	1.527793	1.139048	2.126717	2.602085	2.080925	1.618607	1.836798
Tetradecane	3.75879	1.976121	2.759203	2.641737	1.773796	3.166027	1.552497
Undecane	5.038527	2.723561	4.273985	14.04033	2.383393	5.5647	1.564817
n-Hexadecanoic acid	1.039747	0.804142	2.469206	7.922566	1.601807	6.546035	4.687007
α-Pinene	1.532065	2.1862	3.974692	2.18486	4.496303	2.992347	4.176852