Literature Review (Thesis)

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# Project Overview

## Motivation

The motivation for my research is to improve yield for wheat growers by reducing wheat stem sawfly (WSS) populations. If we can show that healthier, more robust smooth brome leads to increased parasitoid populations and thus, increased WSS parasitism and potentially decreased damage by WSS, we can better educate growers on how to increase yields. In terms of scientific merit, understanding these mechanisms is key in ongoing WSS research as it explains several how and why questions associated with the ecological relationship between smooth brome and WSS parasitism.

## Research Question

What role does smooth brome (Bromus inermis) play in the ecological relationship between wheat stem sawfly (Cephus cinctus) and wheat stem sawfly parasitoids (Bracon cephi and Bracon lissogaster) in dryland wheat production landscape within Montana?

## Hypothesis

Smooth brome acts as an ecological sink for WSS, while serving as a parasitoid source because WSS larvae experience high mortality within smooth brome stems, while any survivors experience high rates of parasitism as a second source of mortality. In addition, early season parasitoids are attracted to infested smooth brome because it produces volatiles similar to those produced by winter wheat infested with larval WSS.

## Prediction

WSS larvae will experience both high levels of mortality and similar levels of parasitism within smooth brome stems when compared to the survivability and parasitism of WSS larvae in the adjacent fields. Also, the blend of volatile compounds produced by WSS infested smooth brome will qualitatively match in composition and exceed the amounts produced by infested winter wheat.

## Research Approach

My research is composed of three separate studies. First, there is a field survey to assess the survivability of WSS larvae and associated parasitoid frequency within both winter wheat and adjacent smooth brome. Second, there are controlled field infestations which will assess the survivability of WSS larvae within smooth brome stems when controlled for other density driven factors. And third, there is a greenhouse study to measure the volatile compounds produced by winter wheat and smooth brome. Using the results of the volatile collection, we can assess the parasitoid response both chemically with electroantennography and behaviorally with a Y-tube olfactory bioassay.

## Importance of Topic

The wheat stem sawfly (WSS, Cephus cinctus Norton, Hymenoptera: Cephidae), is a serious problem for wheat producers throughout the Northern Great Plains of North America ([Fulbright et al. 2017](#ref-Fulbright2017)). In Montana alone, the economic damage caused by the pest was reported to be upwards of $80 million annually between 2011-2016 ([Fulbright et al. 2017](#ref-Fulbright2017)). Current techniques in combatting the damage caused by the WSS use a variety of chemical, cultural, and biocontrol strategies with limited short-term effectiveness and long-term sustainability. Utilizing biological control via promoting braconid parasitoid habitat holds considerable value to both growers and agricultural observers. As we seek to increase agricultural yields, we must also consider the environmental impact that tactics such as pesticides and tillage have on the future success on wheat farming.

# Prior Research on Topic (Literature Review)

## History and Distribution of Wheat Stem Sawfly

The wheat stem sawfly was first described in 1872 by Edward Norton based upon a specimen found in Colorado ([Norton 1872](#ref-Norton1872)). In 1890 it was found in California and Montana ([Ainslie 1920](#ref-Ainslie1920)). Native to North America ([Lesieur et al. 2016](#ref-Lesieur2016)), the largest populations of WSS are currently found in the western Canadian prairies as well as Wyoming, Montana, Colorado, and North and South Dakota ([Wallace and McNeal 1966](#ref-Wallace1966); [Weiss and Morrill 1992](#ref-Weiss1992); [Cockrell et al. 2021](#ref-Cockrell2021)).

## Host Plants of Wheat Stem Sawfly

Wheat stem sawfly was first found the hollow-stemmed wild grasses such as Agropyron and Elymus in Colorado in 1872 (Ainslie, 1920). Soon thereafter it was found in wheat in 1895 in Manitoba, Canada. First referred to as the “western-grass stem sawfly”, WSS was found with native Montana grasses including western wheatgrass (Pascopyrun smithii) and slender wheat grass (Elymus trachycaulus) (Ainslie, 1920). As settlers began moving west and small grain cereal crop production increased on the landscape, the native grasses that WSS foraged upon became quite rare (Ainslie, 1929). WSS was found damaging spring wheat for the first time in 1895 in Manitoba and Saskatchewan (Wallace and McNeal, 1966). With the diminishment of its natural hosts, WSS has adapted to infest the exotic non-cultivated and cultivated grasses now present (Criddle, 1923). WSS infestation has been shown in many species native to Montana including western wheatgrass (Pascopyrun smithii), slender wheat grass (Elymus trachycaulus) and bluebunch wheatgrass, Pseudoroegneria spicata (Cockrell et al., 2021).

Smooth brome (Bromus inermis L.) is a Eurasian cool-season rhizomatous grass introduced to the United States in 1884 from Hungary and Russia for soil conservation and use as livestock feed (Salesman and Jessica, 2011). Throughout the early 19th and 20th century, it was used throughout much of the upper half of North America. It is a perennial grass, and more drought resistant than other exotic grasses (Mapfumo et al., 2002). Due to its sod forming nature and large root system, it is extensively used to promote soil retention along highways (USDA-NRCS 2010).

Smooth brome was originally thought of as potentially useful as a trap crop in order to combat the growing WSS problem with cultural control practices (Farstad and Jacobson, 1945). However, due to its propensity to form monocultures and outcompete many native grasses, smooth brome was labeled by many as invasive (Carlson and Newell, 1985; Willson and Stubbendieck, 2000; Dillemuth et al., 2008). Smooth brome populations play a considerable role in altering native herbivore population dynamics and movement on the prairies of North America (Dillemuth et al., 2008).

## Biology of Wheat Stem Sawfly

### Adult

Adults are relatively small (8-13 mm) with a narrow elongated and slightly compressed black body, yellow legs, and three characteristic black bands across the abdomen. Females are larger than males and a visible long, dark, ovipositor. Both male and female adults are weak fliers, rarely flying long distances at one time (Criddle, 1923). They can be found on stems with their heads downward and legs aligned with their body, encircling the stem (Ainslie, 1920; Criddle, 1923). WSS are univoltine, having only one generation per year. Males are haploid (9 chromosomes), while females are diploid – females who do not find a mate will produce male offspring, while those who do produce female progeny (Holmes, 1978). Fully mature adults hatch in the spring once it has sufficiently warmed and the moisture levels in the soil reach threshold levels (Holmes, 1978; Perez-Mendoza and Weaver, 2006). In Montana, this period begins in mid- to late-May and ends early in July (Morrill and Kushnak, 1996; Weaver et al., 2005). Their lifespan usually lasts around five to eight days, emerging from May until June. Males emerge before the females, ensuring that many of the early females to hatch will find a mate and deposit eggs that will be female (Mackay, 2011). Copulation lasts less than one minute with the male holding the back of the female with their abdomen curved down under the female ovipositor. Fertilized females lay eggs several hours after copulation (Holmes, 1978).

### Oviposition

After locating a suitable host stem, female WSS oviposit an egg inside the host plant lumen. WSS larvae hatch inside the stem and feed on the stem’s parenchyma tissue (Beres et al., 2011). As the stem begins to senesce in the fall, the larvae travel to the base of the stem and makes a V-shaped groove (Criddle, 1923) above a constructed protective hibernaculum where they will overwinter (Holmes and Peterson, 1960). As a result of this deep groove, the stem weakens and topples easily. The resulting lodged stems are not only challenging for growers to harvest but also exhibit decreased kernel yield (Beres et al., 2011).

### Egg

Newly laid eggs are crescent in shape, milky-white in color, and range between 1 mm to 1.25 mm in length and 0.33 to 0.42 mm wide (Ainslie, 1920). After oviposition, the egg lies freely within the lumen of the host stem or in a hollow created by the female WSS ovipositor (Fulbright et al., 2017). Laval development occurs rapidly with the recognizable shape becoming apparent at day three. By day five, the jaws turn brown as the eye spots begin to appear and darken. By day six or seven, the larva begins to hatch using a series of convulsive movements and emerges into the stem cavity, with full functionality achieved by the seventh day (Ainslie, 1920).

### Larvae

After hatching and prior to feeding, the larvae are transparent and colorless (Ainslie, 1920; Criddle, 1923). WSS larvae are distinguishable from other larvae feeding in wheat and grasses by their highly sclerotized head capsule and large body size (Nelson and Farstad, 1953). As the larvae chew their way through the parenchymous and vascular tissues of the host stem, the larvae begin to develop a yellow-green color. As the larvae progress through the stem, they excrete frass; the digested plant tissue as well as metabolic breakdown products from the WSS (Beres et al., 2011). If multiple eggs are laid within the same stem, the largest (usually the first egg laid and hatched) will typically remain within a few weeks due to the larvae cannibalizing one another (Criddle, 1923; Seamans et al., 1944; Buteler et al., 2015). It remains unknown whether the consumption of other eggs and larvae is the result of intentional cannibalism or the indirect result of indiscriminate feeding (Buteler et al., 2015). As the host plant physiologically matures, and the larvae reaches its fifth instar, the insect is cued to travel to the base of stem and prepare for obligatory diapause (Beres et al., 2011). The descending movement of larvae and development of the hibernaculum are triggered by the host plant senescence. As the stem walls becoming increasingly transparent, they allow increased amounts of visible and infrared light into the stem (Holmes, 1977; Beres et al., 2011). Once the larvae reach the base of the stem, they construct an interior V-shaped groove, making the stem weak enough to lodge due to gravity or wind (Criddle, 1923). The larvae then plug the remaining stub with frass to insulate the hibernaculum during diapause (Holmes and Peterson, 1960). The larval stage of development can be as short as 30 days before entering diapause. Obligatory diapause lasts a minimum of 90 days (Salt and Hollick, 1947). After the larvae are exposed to temperatures less than 10C for 90 days, diapause is complete, and pupation can begin. Larvae can reenter diapause if temperatures approach 35C (Salt and Hollick, 1947) or if conditions are abnormally dry (Holmes, 1978) shortly after termination of diapause.

### Pupae

Once diapause is complete, pupal development begins. Pupation lasts for 21 days, beginning in early- to mid-May (Criddle, 1923). Initially, the pupa is milky-white in color (Ainslie, 1920), gradually changing to bright white as the wings develop (Fulbright et al., 2017). Additionally, as development continues, characteristic brown and black eye spots appear on the surface of the pupae. Once pupation is complete, the adult sawfly will chew through either the frass plug or the side of the stub, and emerge ready for flight (Ainslie, 1920; Holmes and Peterson, 1960).

## Damage Caused by Wheat Stem Sawfly

Wheat stem sawfly larvae travel throughout the stem of the host plant, feeding on the parenchymal tissue and chewing through the nodes. As a result, vascular translocation of water and nutrients within the plant is impaired (Macedo et al., 2007) and causes a notable reduction yield and quality of the grain produced (Seamans et al., 1944; Holmes, 1977; Morrill et al., 1992). In addition, the weakening of the stem due to WSS causes lodging prior to harvest, exacerbated by larvae stem girdling. Stem lodging causes a reduction in stem harvest efficiently (Ainslie, 1920), with yield lost due to unrecoverable lodged stem (Beres et al., 2007). Additionally, the reduction in stubble residues decreases snow retention which increases soil erosion and reduces soil moisture content (Keren et al., 2015).

## Management of Wheat Stem Sawfly

### Chemical Control

Because WSS are stem mining pests and spend most of their lives within the host plant stem, the use of traditional pest control methods such as insecticides have proven to be ineffective. The only life stage of WSS that takes place outside of the stem, the adult, is not targetable via chemical treatment due to adult emergence and oviposition taking place over many weeks (Knodel et al., 2010; Beres et al., 2011). WSS infestation chemical control methods were first tested as early as 1949 (Wallace, 1962). After using six different organochlorine and organophosphate insecticidal dust applied via a powder duster (Munro et al., 1949) the authors found “unsatisfactory results that did not control” WSS populations or limit infestations. In 1963, it was reported that a 73% control of WSS infestation could be achieved via granular heptachlor applied in the furrow with the wheat seed (Wallace, 1962). However, the findings of these tests were not able to be replicable in all scenarios of WSS infestation (Holmes et al., 1963). Heptachlor required light sawfly infestation – restricted to the lower internodes – to be effective. When infestation rates were high, and oviposition occurred in the upper internodes, the researchers saw little to no control. Similar results were found when testing with foliar insecticides, findings showing little differences in their effect when compared to controls (Blodgett et al., 1996). While the Montana Department of Agriculture has authorized the use of systemic organophosphate insecticide phorate (Thimet®) for use controlling WSS infestation in winter and spring wheat cultivars, it’s use is not considered environmentally friendly due to high toxicity to mammals, birds, and fish. Additionally, a preharvest interval of 85 days complicates harvest scheduling (Ginsberg, 2003; Mahajan et al., 2006), while the high toxicity may have potential adverse effects on beneficial parasitoid populations (Varella, 2016).

### Cultural Control

#### Host Plant Resistance

Mechanisms like antixenosis, antibiosis, and tolerance give a plant the ability to resist insect pests (Horber, 1980; Wilson et al., 1987). Antixenosis allows the host plant to trigger resistance by inducing non-preference in herbivores when they attempt to feed or oviposit. Recent work done by Achhami et al. (2020) found that Barely cultivars that closely resemble wheat show the greatest frequency of WSS oviposition. These findings suggest that semiochemicals produced by these barley cultivars, such as (Z)-3-hexenyl acetate (Piesik et al., 2008; Buteler et al., 2009) and (Z)-β-ocimene (Buteler and Weaver, 2012), are likely similar to those produced by wheat (Achhami et al., 2020). Antibiosis causes the improper development of herbivores, impacting survivability and reproduction. Tolerance allows the host plant to withstand infestation and overcome injuries (Van Emden, 1991; Van Zoeren and Guedot, 2017). Much of the research done on WSS resistant wheat cultivars focuses on solid stem trait and pith expression (Platt and Farstad, 1949; Varella et al., 2016; Varella et al., 2017). Solid stem varieties are more resistant to infestation, reducing WSS oviposition and increasing egg mortality (Holmes and Peterson, 1960; Varella et al., 2016). The increased pith expression also disrupts larvae movement within the stem, leading to higher larval mortality due to reduced feeding as well as smaller and less reproductively successful adults (Wallace and McNeal, 1966). In addition, greater pith expression increases stem strength, reducing losses due to lodged stems (Delaney et al., 2010). Additional factors including late planting and shorter crop duration, have been suggested as potential factors complicating antixenosis, leading to increased WSS oviposition and infestation (Achhami et al., 2020). The solid-stemmed trait has been developed in numerous spring and some winter wheat cultivars (Beres et al., 2011). While the use of solid-stemmed cultivars is most often recommended for WSS management (Beres et al., 2011), hollow-stemmed cultivars were found to exhibit greater parasitism when compared to solid-stemmed varieties (Holmes et al., 1963). It was previously suggested by Rand et al. (2012) that the thicker pith found in solid-stemmed cultivars makes sensing WSS larvae vibrations (Mankin et al., 2004) more difficult for female parasitoids. The inability to locate WSS hosts would then cause ineffective parasitism.

#### Tillage

Tillage is a potential control method for the WSS. Larvae are protected against both water loss and freezing temperatures by the soil surrounding the wheat stubble where the WSS overwinters. Tilling the soil exposes the wheat stubble to the soil surface, conditions which kill the hibernating larvae (Callenback and Hansmeier, 1944). Tillage of the WSS has been shown to be effective in decreasing the pest’s populations in both spring and winter wheat cultivars. Upwards of 90% mortality has been shown in North Dakota after fall tillage (Weiss et al., 1987). Many factors influence the effectiveness of tilling, including adequate exposure of wheat stubs on the soil surface and appropriate timing (Holmes and Farstad, 1956; Morrill et al., 1992; Weiss and Morrill, 1992). While the variability in effectiveness exists, some growers do consider it a crucial management tool (Blodgett et al., 1996; Lesieur et al., 2016). While effective in killing WSS larvae, there are negative impacts caused by this practice. Water loss, soil compaction, and erosion have all been shown to be dramatically increased by tilling activities (Phillips et al., 1980; Lal, 1991). In addition, tilling practices negatively impact beneficial populations of parasitoid wasps that target the WSS. WSS parasitoids overwinter higher in the stem compared to at the base like WSS (Morrill et al., 1998; Runyon et al., 2002a). By displacing soil when tilling, the upper portion of the plant is buried under the soil, preventing the parasitoid wasps from emerging in the spring (Runyon et al., 2002a; Weaver et al., 2004).

#### Plant Density and Spacing

Historically, it has been observed that wheat fields with wider row spacing (12 inches) have greater WSS cutting when compared to fields with reduced spacing (3 to 6 inches). Additionally, fields planted at greater seed density with solid stem cultivars exhibit lower WSS cutting compared to reduced seed density (Luginbill and McNeal, 1958). The reason for this relationship between decreased density and increased sawfly infestation is due to the moisture availability and height of the wheat stems. At higher densities, each stem has access to less water, in turn leading to the stem to develop a thicker pith – more solid stemmed. As discussed above, there is a negative linear relationship between an increase in the solid-stem characteristic and infestation (Kemp, 1934).

#### Crop Rotation

Continually planting wheat year after year creates ideal conditions for the WSS (Callenbach and Hansmeier, 1945). Rotating wheat with more resistant wheat varieties as well as with oats, or soybeans (crops that are less or not susceptible to WSS infestation) is recommended to decrease the year after year WSS infestation rates (Lin and Chen, 2014). The notable downside to utilizing crop rotation in a matter that is effective is the economic downside. Alternate-year, summer fallow production systems are typical for wheat growing practices across much of the northern great plains of the United States (Weiss and Morrill, 1992; Troeh et al., 1999).

#### Trap Crops

Trap cropping utilized non-economically important plantings to help reduce populations or block movement of pests into valuable crop areas (Hokkanen and Jokioinen, 1991). Effective trap crops of the WSS should have appealing characteristics to the WSS to attract the pest to the trap planting, and away from the wheat (Farstad, 1940). Historically, planted trap strips were used by attracting sawflies and destroying the strip at the end of the season, eliminating the larvae (Morrill et al., 1992). However, utilizing trap plantings of highly attractive wheat varieties has been shown to hold limited viability, as growers are not fond of destroying planted grain at the end of the growing season (Goosey, 1999). Alternative utilization of WSS traps entail planting and/or maintaining strips of smooth brome grass (Bromus inermis Leyss.) or other WSS host grasses (Farstad, 1940). Smooth brome in particular holds potential as it is a poor host to WSS larvae (Bhandari, 2020), yet WSS adults continue to oviposit the stems. This relationship of greater oviposition with increased mortality may lead to decreases in overall WSS populations.

#### Delayed Planting & Swathing

Wheat planted later in the growing season avoids much of the damage caused by the WSS as the wheat reaches stem elongation after many of the adult sawflies have already laid their eggs (Jacobson and Farstad, 1952). The problem with delayed planting is that the practice is known to make less efficient use the earl season moisture in the soil (Morrill and Kushnak, 1996). Growers may need to weigh the tradeoffs in delayed planting to reduce losses in areas with a history of severe infestation. Swathing, also known as windrowing, is the practice of cutting the wheat to accelerate the drying process and decrease the losses caused by WSS cutting (Holmes and Peterson, 1965; Carlson, 2019; McCullough et al., 2020). By cutting the wheat and letting it dry prior to sawfly cutting, swathing works by reducing losses due to lodging (Ainslie, 1920).

#### Biological Control Methods

The use of biocontrol in limiting WSS populations has been well studied with variable results. There are currently three types of organisms that have been reported as biocontrol agents of the WSS in wheat fields: Soil fungal pathogens, the predatory beetle Phyllobaenus dubius, and two parasitic Hymenoptera.

#### Soil Fungal Pathogens

Fungal pathogens are found throughout both the plant and animal kingdoms. Several fungi have been shown to negatively impact WSS larvae infestation within wheat stems. While highly lethal to sawfly larvae, F. graminearum, F. acuminatum, F. equiseti, and F. avenaceum also cause crown rot in the host plant. The trade-off between WSS control and crown rot may limit Fusarium spp. usefulness to WSS management. (Wenda-Piesik et al., 2009). Findings by Tangtrakulwanich et al. (2014) found that the fungi B. bassiana and M. brunneum effectively reduced damage caused by WSS. Both fungi kill insects by infecting via spore contact or by insect consumption of treated plant tissue (Portman et al., 2018).

#### Predatory Beetle

Phyllobaenus dubius (Wolcott) has been observed emerging from wheat stubble and within dissected wheat stems in Montana (Morrill et al., 2001). Further research is required on this coleopteran to determine its prospects going forward as an effective WSS management tool.

#### Parasitoids of the Wheat Stem Sawfly

Two parasitoid wasps are known target the wheat stem sawfly in wheat fields. The congeneric parasitoids, Bracon cephi (Gahan) and B. lissogaster Muesebeck, are the most effective agents of WSS biological control (Runyon et al., 2001). B. cephi and B. lissogaster, as distinguished by Runyon et al. (2001), were initially limited in their ability to parasitize WSS larvae within wheat stems. The wheat stem sawfly, originally known as the western grass-stem sawfly, was originally only found within native grasses throughout North America, existing in alongside B. cephi and B. lissogaster (Ainslie, 1920; Cárcamo et al., 2012). However, as agriculture increased and native grasses were replaced by large monoculture landscapes, the WSS moved into wheat (Farstad, 1940; Davis et al., 1955; Holmes, 1978) while the parasitoids were slower to adapt (Morrill et al., 1998).

## Biology of Bracon cephi and Bracon lissogaster

### Adult

Both B. cephi and B. lissogaster are protelean host-specific parasitoids with two generations per year (bivoltine) (Nelson and Farstad, 1953; Somsen and Luginbill, 1956; Holmes et al., 1963). They are ectoparasitoids and idiobionts, living on the integument of their hosts and immediately paralyzing their hosts in location and stage it was when it was attacked (Nelson and Farstad, 1953; Runyon et al., 2002b; Weaver et al., 2004). Male parasitoids are approximately 3.5 mm in length, while females are 4.1 mm. They are dark red, with black antennae, eyes, ovipositor and ocelli (Gahan, 1918). B. lissogaster and B. cephi are distinguishable by their head color and unique metasoma surface texture (Runyon et al., 2001). Adults emerge after completing a pupal stage within the host stem by chewing a hole through the stem wall. In Montana, the first generations are present from late June to late July, while the second generations are active in August and September (Somsen and Luginbill, 1956). Adult female B. cephi and B. lissogaster will locate a host stem using a combination of olfactory and volatile cues. The wasps will then locate a host sawfly larva using acoustic cues (Mankin et al., 2004) and use their ovipositor first paralyze, then deposit 1-4 eggs next to the paralyzed larva.

### Egg

B. cephi and B. lissogaster eggs are on average 0.86 mm in length, exhibiting an elongated shape and pale-yellow color. Females lay one to three eggs on the WSS larval integument or adjacent to the larva. The eggs develop for one to three days before hatching (Nelson and Farstad, 1953; Somsen and Luginbill, 1956).

### Larva

Parasitoid larvae are 1mm in length, with a translucent hue that turns increasingly brown as the larva progresses through five instar stages (Nelson and Farstad, 1953). The newly hatched larvae have no appendages, save for antennae, and they exhibit highly sclerotized mouthparts and well-developed mandibles. After hatching, the B. lissogaster larva attach themselves to the integument of the host larva and immediately begin feeding (Somsen and Luginbill, 1956). When feeding is complete, little remains of the host but the integument. Following feeding, the larva will spin a lightly brown colored cocoon over the course of several days and attach to the inside wall of the host plant stem. The larval stage takes an average of ten days between initial hatch and cocoon completion. Because there are two generations per year, the early summer offspring will hatch and emerge from the host stem. Offspring laid as a part of the second generation will overwinter within the cocoons and emerge in the spring. (Nelson and Farstad, 1953)

### WSS and Parasitoid Interactions with Smooth Brome

Bhandari (2020) has shown that oviposition occurs with similar frequency in wheat as it does in smooth brome, however, we see much higher rates of larval mortality within smooth brome stems. This has led researchers to hypothesize about the feasibility of using smooth brome as a trap cop (Perez-Mendoza et al., 2006; Lesieur et al., 2016). While feasible as a feed crop, as it holds considerably less economic value compared to wheat or other high value crops (Criddle, 1922). Smooth brome may be usable in trap strips along the edge of wheat fields (Hokkanen and Jokioinen, 1991). While a poor host for the wheat stem sawfly, surviving immature larvae found within brome stems can be parasitized by B. cephi and B. lissogaster. This combination of factors may indicate that smooth brome can function as a WSS sink and a parasitoid source, with very few WSS emerging from smooth brome patches, while also supporting populations of parasitoids that can then parasitize WSS in adjacent wheat fields.

## Plant Semiochemicals

Plants interact with other organisms through a complex system of chemical signals known as Semiochemicals. Semiochemicals are compounds or mixtures of compounds that are released from one organism that evoke a physiological or behavior response in another organism (Agelopoulos et al., 1999). Semiochemicals play a large role in both insect-insect and plant-insect interactions and are essential in insects locating feedings sites, mating sites, egg-laying sites, and/or refugia (Prokopy et al., 1984). While it is known that these chemicals play an important role in communication between plants, insects, and other organisms, there is still debate about the function of many of them (Reddy and Guerrero, 2004; Voelckel and Jander, 2018).

### Semiochemicals in Plant-Insect Systems

Within plant-insect systems, plants respond to insect herbivory by synthesizing and releasing volatile compounds and providing important signals to biocontrol agents to localize potential host insects (Sharma et al., 2019). In addition, semiochemicals provide localized signatures that allow insects to detect food sources, find mates, and locate preferential oviposition sites (Norin, 2007). Plants typically produce semiochemicals when defending themselves from herbivorous arthropods (Guerrieri et al., 1999) or pathogen attacks (Smart et al., 2014 Jan 1). These responses can be grouped into two categories, direct and indirect responses. Direct responses are compounds that the plant produces that have a direct effect on the herbivore or pathogen (Metcalf, 1998). Indirect responses are the compounds produced that have no impact directly on the pest, but instead may attract parasitoids, predators, or other biological control agents (Mayland et al., 2000; Colazza et al., 2004).

# Limitations And Key Assumptions

## Field Study

1. Time limits the number field sites that we can collect samples from throughout the summer.
2. Study area limited to western Montana – three regions – Big Sandy, Moccasin, and Amsterdam.
3. Our study will only take place over the course of 2-3 summers, thus, we are unable to make broader claims surrounding long term patterns of WSS and parasitoid interactions.  
   Green House Samples and Volatile Collection
4. We will assume that plants grown in the green house will be accurate and reasonable biological model representation of plants present in the field.
5. Volatiles will be collected from a maximum of 12 plants a day due to constraints with the collection system.

# Contributions to Knowledge of Potential Outcomes

## Potential outcomes

### Volatiles

We hope to observe a discernable difference in the volatile quantities produced by both infested smooth brome and winter wheat.

# CHAPTERS

## METHODS

### Central and Northern Montana Field Sites

#### Field Selection & Data Collection

To assess C cinctus infestation, larval mortality, and parasitoid prevalence within B. inermis and adjacent wheat fields, sites were chosen that had the best chance of accurately representing conditions throughout the Golden Triangle, a region of north-central Montana known for its dryland grain production. Samples were collected in early July and late August in 2021, 2022, and 2023 from sites in Big Sandy, Moccasin, and Amsterdam, MT, USA. B. inermis sites were set up as polygons along the edge of adjoining wheat fields measuring approximately 100 meters in area. Four collection squares of 1ft x 1ft were randomly partitioned within each polygon during both collection events each year. All stems within each 1 x 1 ft square were collected. The stems were then processed and split in the laboratory to assess infestation, live eggs, dead eggs, dead larvae, live larvae, parasitism, and cutting.

#### Statistical Analysis

Statistical analyses were conducted in R (R Core Team 2022) using RStudio (version 4.2.1) using linear modeling after log transformation to improve the normality and constant variance assumptions. ‘Site’ and ‘polygon’ were used as fixed effects (treatments) while the mean number of infested stems, parasitized stems, dead larvae, alive larvae, dead eggs, alive eggs awere used as the response variable to assess the differences in parameters among the sampling sites. ANOVA was used to caculate P-values using F-distribution to understand overall significant differences in mean C. cinctus infestation and parasitism among polygons. To assess the profile of C. cintus infestation, paraisitms, and larval presence by node, a linear model was fit for each sampling site. Further assessment was done on the resonpses between sampling sites using a Tukey test using the ‘TukeyHSD’ function.

## Controlled C. cinctus infestation of B. inermis

### Plant Selection and Field Cages

Assessment of C. cinctus infestation and mortality within B. inermis were assessed using a 34 x 60 ft plot at the Arthur H. Post Agronomy Farm (43°38’19.39”N, 116°14’28.86”W), an extension research station of Montana State University in Bozeman, MT. The cage structure was built using 1-inch PVC piping with the netting made using 530 Amber Lumite Screen (BioQuip Products, LLC). Twelve cages were built to dimensions of 6ft x 3ft x 3ft (L x W x H) with cage locations selected randomly based on the space available within the plot and arranged in sets of three. The cages were removed from the plot after 3 weeks of sawfly release.

### Insects

Wheat stem stubble was collected in Three Forks, MT, USA (43°38’19.39”N, 116°14’28.86”W) from fields that experienced high levels of C. cinctus infestation and cutting the year prior. Cut stubble, which contained C. cinctus larvae in diapause, were kept refrigerated between -2°C and 3°C for >100 days as required to complete obligatory larval diapause. As needed, stubs were removed from refrigeration and kept at 22-27°C for 4-5 weeks inside of 100 oz GladWare® storage containers (Glad®, Oakland, California USA). Stubs were added to cages once B. inermis stems reached six inches tall, with sawfly quantity treatments as follows: high (600 stubs), low (200 stubs), and control (0 stubs).

### Data Collection

Stems were collected from each cage site in late August. All stems from each 6 x 3 ft cage section were collected. Each stem was sliced open using X-Acto® knives to collect data on infestation, dead larvae, live larvae, and parasitism at each internode.

### Statistical Analysis

Statistical analyses were conducted in R (R Core Team 2022) using RStudio (version 4.2.1) using linear modeling after log transformation to improve the normality and constant variance assumptions. ‘Site’ and ‘polygon’ were used as fixed effects (treatments) while the mean number of infested stems, parasitized stems, dead larvae, alive larvae, dead eggs, alive eggs were used as the response variable to assess the differences in parameters among the sampling sites. ANOVA was used to calculate P-values using F-distribution to understand overall significant differences in mean C. cinctus infestation and parasitism among polygons. To assess the profile of C. cinctus infestation, parasitism, and larval presence by node, a linear model was fit for each sampling site. Further assessment was done on the responses between sampling sites using a Tukey test using the ‘TukeyHSD’ function.

## Organic Volatile Collection from C. cinctus Infested B. inermis and Winter Wheat

### Insects

Wheat stubble collected the year prior to each experiment were used to rear adult WSS. Wheat stubble was collected from Big Sandy (48.17754 N, -110.11406 E) and Three Forks (46.020216 N, -111.586582 E), MT, USA. Stubble was collected from fields that experienced high levels of WSS infestation and cutting the year prior. Cut stubble, which contained WSS larvae in diapause, were kept in a refrigerator between 0-4 C for >100 days as required to complete obligatory larval diapause. After completing diapause, immature WSS were kept at room temperature between 22-27 C for 4-5 weeks in sealed 70 cm x 35 cm x 20 cm plastic Tupperware bins to allow for pupation and emergence. Once emergence began, adults were collected daily and sorted in 2-liter glass mason jars sealed with filter paper to allow for airflow. Jars containing adults were refrigerated at 6-10C until they were needed for experiments. Unused adults were discarded after 48 hours of refrigeration.

### Plants

Smooth brome was grown from transplanted plants harvested from the Arthur H. Post Agronomy Farm (43°38’19.39”N, 116°14’28.86”W). Immediately following collection, plants were transplanted into 2.7 x 14 inch lightweight deepot cells (Stuewe & Sons Inc, Tangent, OR) and moved into the greenhouse. Wheat was grown using ‘Yellowstone’ winter wheat cultivar seed stock, started in 1 x 8 inch conical pots. The soil used consisted of MSU Plant Growth Center soil mix (equal parts sterilized Bozeman silt loam soil and washed concrete sand with Canadian sphagnum peat moss incorporated) and Sunshine Mix 1 (Canadian sphagnum peat moss, vermiculite, perlite, and Dolomite lime) in a 1:1 ratio. Fertilization was undergone once weekly using Jack’s Professional® Water-Soluble Fertilizer (20-20-20) (J.R. Peters Inc., Allentown, PA, USA). The plants were kept in a greenhouse in the Montana State University Plant Growth Center using supplemental light from model MVR1000/C/U GE Multi-Vapor Lamps (GE Lighting, General Electric Company, Cleveland, OH) under photoperiod of 16:8 (L:D) h at 22  2C and 20 – 40 % RH. After the plants reached Zadoks 12, they were moved into vernalization storage for 6 weeks to experience the length of dormancy needed for successful flower development. After the six-week period, wheat stems were transplanted into the larger, 2.7 x 14-inch Stuewe & Sons, Inc conical pots.

### Volatile Collection

Volatile compounds were collected from intact and healthy smooth brome and Yellowstone winter wheat. Collection was undergone on plants at Zadoks 49, for a period of six hours from 09:00 to 15:00. Collections were undergone three times a week, with twelve plants collected from each time. Each trial consisted of six smooth brome plants and six winter wheat, with four of each being infested, while the other two were controls.

Volatiles were collected from twelve plants per day using glass VCC’s that were 80 cm long and 4 cm in diameter. The top end of each VCC funneled into a threaded port that was 10 cm in diameter. Threaded onto the top of each VCC was an eight-port manifold adapter. Two glass filters (traps) (6.35 mm diameter x 76 mm length; Analytical Research Systems, Gainesville, FL) with 30 mg of Super-Q absorbent (Alltech Associates, Deerfield, IL) were inserted into two of the adapter ports. The remaining six collection ports were sealed to prevent ambient air exchange and pressure loss. One of the traps was used to collected extraneous volatiles from the initial experiment set up for the first ten minutes, whereas the other trap was used to collect volatile compounds emanating from plants beginning ten minutes after the system was turned on. An air source was connected to the base of each collection chamber with supply from charcoal air filters. A vacuum pump was used to maintain air pressure and flow rate (humidified air at 1.0 liter/min) within the collection system. A small piece of cotton encompassed by a Teflon® guillotine was used at the base of each plant to tightly seal the base of the system and prevent external air from entering. Laboratory tape was used to seal the edges of the guillotine and prevent contamination by ambient and soil derived volatiles.

Volatile collection and successive GC-MS analysis were conducted following methods described in Weaver et al. (2009). Glass volatile collection traps were eluted using 200 µL of dichloromethane into 2 mL screw top vials (Agilent Technologies, Inc., Santa Clara, CA), followed by a slow release of nitrogen to further clear the volatile trap. 10 µL of nonyl acetate solution was added to the eluted solution as internal standard to quantify compounds. Samples were then subject to gas chromatography (GC) on a J&W HP – 5ms; 30m x 0.25 µm film thickness column (Agilent Technologies, Inc., Santa Clara, CA). The GC unit (Agilent 6890; Agilent Technologies, Santa Clara, CA) was paired with a mass spectrometer (MS, Agilent 5973 instrument). Samples were injected into the column in pulse splitless mode, with the initial pressure of 82.7 kPa/m.

## PARASITOIDS

Parasitoids B. cephi and B. lissogaster were collected from wheat fields in Three Forks, MT (46.020216 N, -111.586582 E). Wheat stem residue from fields that experienced high levels of C. cinctus infestation were collected and stored inside 170-liter plastic barrel liners. Filled liners were stored in a cold storage room at 4C until mid or late spring of the following year. After the storage time exceeded the mandatory duration needed for obligatory diapause, the plastic barrel liner was removed from cold storage and the residue was placed inside 170 L trash barrels at room temperature and watered to encourage emergence. Collected parasitoids were kept at 6-10C until used.

### Plants

Smooth brome was grown from transplanted stems harvested from the Arthur H. Post Agronomy Farm (43°38’19.39”N, 116°14’28.86”W), an extension research station of Montana State University in Bozeman, MT. Immediately following collection, stems were transplanted into 2.7 x 14 inch lightweight deepot cells (Stuewe & Sons Inc, Tangent, OR). Winter wheat plants were grown using Yellowstone Winter Wheat cultivar seed stock, started in 1 x 8 inch conical pots. The soil used consisted of MSU Plant Growth Center soil mix (equal parts sterilized Bozeman silt loam soil and washed concrete sand with Canadian sphagnum peat moss incorporated) and Sunshine Mix 1 (Canadian sphagnum peat moss, vermiculite, perlite, and Dolomite lime) in a 1:1 ratio. Fertilization was undergone once weekly using Jack’s Professional® Water-Soluble Fertilizer (20-20-20) (J.R. Peters Inc., Allentown, PA, USA). The plants were kept in a greenhouse in the Montana State University Plant Growth Center using supplemental light from model MVR1000/C/U GE Multi-Vapor Lamps (GE Lighting, General Electric Company, Cleveland, OH) under photoperiod of 16:8 (L:D) h at 22  2C and 20 – 40 % RH. After the plants reached Zadoks 12, they were moved into vernalization storage for 6 weeks to experience the length of dormancy needed for successful flower development. After the six-week period, wheat stems were transplanted into the larger, 2.7 x 14-inch Stuewe & Sons, Inc conical pots.

## Behavioral Assessment of Parasitoids in Response to B. inermis Organic Volatile Compounds

### Insects

### Electroantennography

### Y- Tube Olfactometer Bioassay

Y-tube bioassays were conducted within the chemical ecology and insect behavior laboratory at the Plant Growth Center at Montana State University. Bioassays were undergone to determine behavioral choice preference response of individual female WSS to an airstream containing volatiles originating from smooth brome and a blank control. Bioassays were conducted using plants at growth stage Zadoks 49. The Y-tube system (Analytical Rsearch Systems, Micanopy, FL) used was similar as described in a previous study (Daisy et al., 2002) (Figure 1). A single plant was enclosed in a volatile collection chamber (VCC) (100 mm diameter, 254 mm length). Input air was charcoal-filtered and humidified, regulated by a diaphragm air pump that delivered air at a rate of 0.2 L min-1. Air was moved into the VCC through Teflon tubing connected with a 24/410 threaded glass joint. The bottom end of the VCC enclosed each plant and was tightly sealed by a Teflon guillotine (diameter 955 mm, 15 mm center opening), encircling the stem to prevent air leakage. The olfactory system used a Y-shaped glass tube that had two arms connected to the lower port of each VCC by Teflon tubing (diameter 0.64 cm), providing the airstream source from the plant. The VCC contained plant was illuminated by an enhanced spectrum LED grow light (sunshine Systems Grow UFO Light SS-Gu90w, Led Lighting4 Less, Paradise, CA) for one hour before the experiment to make the plant photosynthetically active.

Figure 1: Y-tube olfactory bioassay as constructed by Daisy et al. (2002). Used to measure insect behavior in the presence of volatile stimulus.

The Y-tube was made of bifurcated Corning® glass, measuring in at 28-mm diameter x 300 m length. The Y shape branched at an angle of 120, with each branch serving as an arm of the olfactometer. The stem of each Y-tube was 20 cm long. At the bifurcation, each branch extended 4 cm from the junction before ending in 10 cm parallel arms. The Y-tube was positioned horizontally inside a black box made of poster board (46 x 32 x 101.5 cm), designed to prevent the entry of unwanted ambient light. The black box had two holes on the upstream sie of the olfactometer, allowing a fiber optic illuminator (T-Q/FOI-1, Techni Quip Corp, El Segundo, CA) and Teflon tubing to pass through. The fiber optic illuminator was used to deliver light to both arms equally from the center of the bifurcation, while the Teflon tubing connected the VCC to the arms of the Y-tube. One arm of the Y-tube delivered volatiles from the enclosed plant while the other delivered filtered, pure air as a control. The movement of adult WSS is positively phototactic and the horizontally placed Y-tube was positioned such that the light distribution towards both arms was balanced to avoid bias. The arms of the Y-tube were oriented to minimize directional bias and the position of the plants was alternated between runs. For each assay, a single naïve female B. cephi or B. lissogaster parasitoid was introduced 1 cm into the unbranched end of the Y-tube. A 28-cm wire was used to facilitate parasitoid movement from the unbranched stem of the glass Y-tube to the test junction between the two upper arms. The order of individual test insects from a pool of naïve females was randomly assigned for each bioassay, and test plants were chosen randomly from the greenhouse culture before each experiment. All glassware was cleaned using dish soap and warm water, followed by solvent rinses with acetone and hexane. Cleaned and rinsed glassware was baked at 110C in a glassware oven for 24 hours before use. New plants were used for each experiment. Each individual parasitoid was allowed three minutes to respond to either airstream source. Once a positive response was determined to either source, the trail was ended. If no choice was made after five minutes, the trail was ended and the result was recorded as no response.

## Volatile Collection

### Sawfly Infestation

Winter wheat plants were deemed ready for infestation once they reached Zadoks 49. Both smooth brome and winter wheat plants were infested by placing a transparent plastic cylinder (60 x 3.8 cm) over the main stem of the plant. A 55-60 cm bamboo stick was added within each tube to help prevent toppling of the cylinder. Each cage had three holes, as well as an open top, covered with fine mesh cloth to allow for air circulation. Four females and two male WSS, newly emerged, were introduced into each tube via a small 1 cm opening. After which, the hole was plugged using a cotton ball covered in mesh cloth. Damp soil was mounded around the base of each tube to prevent insect escapees. Adult sawflies were left within infestation tubes for three days, after which the tubes were removed, and any surviving insects, as well as cadavers, were removed. After infestation, the plants were returned to normal water and fertilization schedules.

Infestation was undertaken three days a week, for five weeks. Each replicate included 6 ‘Yellowstone’ winter wheat plants at Zadoks 49 and 6 smooth brome plants exhibiting three nodes. For each replicate, three wheat and three smooth brome plants were infested, with three of each left uninfested. Infestation tubes were placed around all twelve plants to maintain control.

### Volatile Compound Collection

Volatile compounds were collected from intact and healthy smooth brome and ‘Yellowstone’ winter wheat. Collection was undergone on plants at Zadoks 49, for a period of six hours from 10:00 to 16:00. Collections were undergone three times a week, with twelve plants collected from each time. Each trail consisted of six smooth brome plants and six winter wheat, with three of each being infested, while the other three were a control.

Volatiles were collected from twelve plants per day using glass VCC’s that were 80 cm long and 4 cm in diameter. Volatile collection and successive GC-MS analysis were conducted following methods described in Weaver et al. (2009). The top end of each VCC funneled into a threaded port that was 10 cm in diameter. Threaded onto the top of each VCC was an eight-port manifold adapter. Two glass filters (traps) (6.35 mm diameter x 76 mm length; Analytical Research Systems, Gainesville, FL) with 30 mg of Super-Q absorbent (Alltech Associates, Deerfield, IL) were inserted into two of the adapter ports. The remaining six collection ports were sealed to prevent ambient air exchange and pressure loss. One of the traps was used to collected extraneous volatiles from the initial experiment set up for the first ten minutes, whereas the other trap was used to collect volatile compounds emanating from plants beginning ten minutes after the system was turned on. A vacuum pump was used to maintain air pressure and flow rate (humidified air at 1.0 liter/min) within the collection system. A Teflon guillotine was used at the base of each plant to tightly seal the base of the system and prevent external air from entering. Each plant stem was wrapped with a small piece of cotton prior to the application of the guillotine to further seal the base of the collection system and prevent contamination by ambient and soil derived volatiles.

### Volatile Elution

Volatile compounds were eluted from the glass traps using 200 L aliquot dichloromethane. Trapped volatiles were eluted slowly by adding dichloromethane and further clearing by using a slow release of nitrogen gas. The eluted samples were collected in a glass insert held within a 1.5-mL crimped top glass vial. 10 L of nonyl acetate were then added to the eluted samples as internal standard to quantify compound concentrations. The samples were processed using gas chromatography (GC) on a HP – 5MS; 30m x 0.25mm, 0.25 m film thickness column; (J and W Scientific, Folsom, CA). In order to analyze, the GC instrument (Agilent 6890; Agilent Technologies, Santa Clara, CA) was coupled to a mass spectrometer (MS, Agilent 5973 instrument). Each sample was injected using the automated system in pulse splitless mode, with the initial pressure set to 82.7 kPa per minute. The inlet temperature of the GC was set to 250C while the column temperature was 50C for 4 minutes. The temperature was set to increase at a rate of 5C per minute until it reached 160C, after which it increased to a rate of 25C per minute until it reached 280C. The temperature of the transfer line leading to the mass selective detector (MSD) was set at 300C. Helium was used as a carrier gas to maintain the flow rate of samples within the column at 1.2 mL/min. The MSD was set in ‘SCAN’ mode running from 50 – 300 m/z.

### Volatile Analysis

The compounds collected from both the smooth brome and wheat samples were identified by comparing mass spectra and retention times using the National Institute of Standard and Technology (NIST) library. Peaks and volatiles were analyzed using MSD ChemStation (Agilent Technologies, Inc., Santa Clara, CA). The data obtained from the mass-spectrometer was transferred to a Microsoft® Excel spreadsheet where the compounds were quantified by comparison with the internal standard peak area. The amount obtained for each compound was then transformed to the amount produced in six hours. The true quantity of compounds was calculated relative to the internal standard.

### Electroantennogram

Specimens were prepared by excising the head from the body using fine micro-dissection scissors. For each specimen, a micro-capillarity tube was pulled in half using a pipette puller (Model PP-830, Narishige Scientific Instrument Lab, Tokyo, Japan). Each half was cut to the appropriate length and angle, then filled with saline solution. The parasitoid head was mounted on the broken end of one of the capillary tubes, then the tube was place on one of the conducting electrode wires connected to a micromanipulator, type INR-5 (Narishige MN-151). The second half of the capillary tube was place on the other electrode wire, and one insect antennae was positioned to touch the empty capillary tube, thus connecting the circuit. The amplifier transferred the information to a computer interface with an EAG version 2.7 software (Syntech NL 2001, Hilversum, Netherlands). This apparatus and method has been consistently used previously in assessing insect volatile response (Agelopoulos et al., 1999).

A consistent stream of purified, humidified air (20 mL sec-1) was delivered onto the antennae. The test compound was delivered via airborne stimulus released from a glass pipette with filter paper treated with 10 L of the test compound. The pipette was connected to a Stimulus Controller CS-55 (Syntech NL 2010, Hilversum, The Netherlands). The equipment was programmed to deliver a 0.4 second puff of air through a Teflon tube and through the glass pipette. The electrical response of the antenna to the test compound was detected by the recording electrode and transferred to the amplifier, increasing the signal and delivering the information to the computer. Figure 2: Electroantennogram connected to GCMS. Useful for connecting insect electrochemical response to host plant produced volatile compounds. List of compounds used.

## Data Analysis

### Field Survey

### Greenhouse Experiments

#### Comparative Analysis of Collection of Volatiles

The concentration of volatile compounds gathered was determined as nanogram per gram per hour (ng-1g1hr1), accounting for plant biomass adjustments. Data were analyzed using R Studio version 2023.12.0+369 (R Core team 2023). Data were first transformed via centered-log ratio (CLR) transformation, a common technique for compositional data (Brückner and Heethoff, 2017). The data underwent both Permutational Analysis of Variance (PERMANOVA) and Permutational Analysis of Multivariate Dispersions (PERMDISP). PERMANOVA was used to determine differences among identified plant volatile compounds between the two plant types (Yellowstone winter wheat, B. inermis) at two infestation levels (Infested, control). PERMDISP was used to assess the variance of volatile quantities within our different groups.

#### EAG-FID

#### Organic Volatile Collections

##### Centered Log-Ratio Transformation

The data was transformed using a centered log-ratio transformation (CLR). This method is commonly used for multivariate compositional data (Brückner and Heethoff, 2017). CLR works by taking the natural logarithm of the ratios between the values of individual components and their geometric mean across samples. This helps to mitigate spurious correlations and allows the application of standard statistical techniques to our compositional data.

##### PERMANOVA

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