POPULATION DYNAMICS OF WHEAT STEM SAWFLY, *CEPHUS CINCTUS* NORTON, IN BARLEY IN MONTANA

By

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DEDICATION

I dedicate this dissertation to my family members. Without their love and support, my dissertation would have never been completed. My parents, late Chakra Bahadur Achhami and Suk Maya Achhami, and my sister, Sitali Achhami and brother will always hold a special place in my heart, and I owe for their encouragement and love over the years. I owe special thanks to our kids, Ishab and Ishan, for being calm and adaptive at home in absence of me. Finally, my wife, Yisha Hitanga who has provided tireless patience and encouragement throughout my journey of graduate school, for that I will always be grateful.

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ABSTRACT

Wheat stem sawfly (WSS) is an economically devastating pest of cereals grown in North America. The larva is the only feeding stage and remains confined within a host stem until it emerges as an adult the following year. This limited mobility increases larval vulnerability to mortality factors when host plant traits are hostile to survival. For instance, larval mortality is greater in barley than in solid stemmed wheat cultivars known to be resistant. Both solid stemmed wheat and barley kill neonates via host plant resistance traits. Traditionally, barley was recommended as an alternative rotational crop to prevent WSS outbreaks in wheat crops. There is limited data available regarding barley host plant resistance and questions persist. Has barley resistance changed over time? What is the impact of larval feeding injury on grain yield of barley? Do females display similar oviposition behaviors across barley cultivars that vary in susceptibility? To answer these questions. I conducted field experiments to assess resistance and possible tolerance to WSS in eight barley cultivars. Based on the number of eggs, ‘Hockett’ was the most attractive cultivar to WSS female (less antixenosis), while ‘Craft’ and ‘Celebration’ killed the greatest number of neonates due to antibiosis. Multiple decrement life table studies revealed that plant defense and cannibalism were two major causes of larval mortality. We measured greater yield in infested stems with dead larvae (potential tolerance) than for cut stems and both were greater than uninfested stems in all cultivars except 'Celebration’. A greenhouse study revealed that females preferred ‘Hockett’ over ‘Craft’ in frequencies of oviposition behaviors and numbers of eggs deposited. Additionally, a greater amount of the WSS attractant (*Z*)-3-hexenyl acetate was found in aerations from ‘Hockett’ plants than from ‘Craft.’ The amount of defensive compound linalool was greater in aerations from ‘Craft’ than from ‘Hockett.’ These results suggest that barley cultivars are equipped with varying levels of antibiosis, antixenosis, and tolerance traits against WSS. Thus, we can exploit these traits in the development of cultivars which can reduce WSS populations and decrease economic loss caused by this species.

CHAPTER ONE

INTRODUCTION

Wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), is successfully adapting to both cultivated and native grasses (Cockrell et al. 2017) over an increasing geographic area. It is an economically devastating pest of cereals grown in the Northern Great Plains (NGP) of the United States and southern parts of the Canadian Prairie Provinces (Beres et al. 2011a, 2011b). *Cephus cinctus* (hereafter WSS) is a native species in the NGP (Lesieur et al. 2016).

WSS larvae that hatch approximately a week after oviposition begin to devour the interior culm of host plants. The feeding injuries in the culm reduce translocation of water and minerals to the photosynthetic regions of wheat plants. The feeding injuries in stems reduce photosynthetic assimilation (Macedo et al. 2005, 2006, 2007), and subsequently reduce grain yield (Delaney et al. 2010) in wheat. Additionally, this reduced photosynthetic assimilation may alter protein: carbohydrate ratios in grain. The protein: carbohydrate ratio is a key decisive parameter for determining the market value of grains, especially in malt barley (Briggs 1998).

Mature larvae make stems susceptible to lodging. Once the host plant reaches maturity, the larva moves to the base of the stem to prepare a structure for hibernation. To make this hibernaculum, it creates a v-shaped groove in the interior of the stem. An interior circumference of the stem is girdled by chewing a uniform gouge that encircles the entire stem. This gouge leads to the stem breaking and when severed, this is called a ‘cut’ stem. These cut stems increase stem lodging on the ground; making it difficult to recover grains using combines at harvest. The basal part of the cut stem that contains an overwintering larva within its hibernaculum is known as a ‘stub’. On average, yield loss due to WSS in wheat is estimated to be approximately $80 million (USD) per year in the state of Montana (Bekkerman 2014, Fulbright et al. 2017, Bekkerman and Weaver 2018) and overall costs are estimated at $350 million per year across the NGP (Beres et al. 2011a, 2011b).

The grains that are not recovered at harvest, can germinate in the fall and in the following spring. These volunteer seedlings become a green bridge for the wheat curl mites, *Aceria tosichella* Keifer (Prostigmata: Eriophyidae), a vector of wheat streak mosaic virus. Potential economic losses caused by this and similar viruses in barley has not been explored. However, in addition to direct monetary loss due to larval feeding injuries and stem lodging, WSS can cause other socioeconomic problems by decreasing the potential market value of grain (Bekkerman and Weaver 2018).

Several techniques—spanning chemical, mechanical, cultural, and biological controls—are available to reduce the economic losses caused by WSS. But grower adoption of these techniques is limited, which could be due to variation in efficacy and risks posed to non-target organisms.

For example, foliar-application of insecticides (e.g. lambda-cyhalothrin and thiamethoxam) is not only ineffective in reducing WSS populations (Knodel et al. 2009), but is also hazardous to beneficial and other non-target organisms.

Cultural control methods, such as adjusting seeding dates to escape WSS oviposition period (Criddle 1922, Farstad et al. 1945) lead to reduced grain yield in spring wheat (Morrill and Kushnak 1999). Furthermore, deep plowing at the end of the cropping cycle to bury stubble and overwintered larvae; as well as shallow plowing the following spring to expose overwintered larvae to cold temperatures are practiced in some parts of the NGP. Repeated plowing in a field leads to soil erosion (Morrill et al. 1993) and reduces the survival of WSS natural enemies (Runyon et al. 2002).

The conservation of natural enemies is another technique used to manage WSS. Two congeneric natural enemies, the braconid parasitoids—*Bracon cephi* (Gahan) and *Bracon lissogaster* Muesebeck (Hymenoptera: Braconidae)—parasitize WSS larvae. But the parasitism rates of these is highly varied across locations and years (Runyon et al. 2002, Morrill et al. 1998, Weaver et al. 2004, 2005). Other groups of natural enemies—entomopathogenic nematodes and fungi—are found effective to kill small cohorts of overwintering larvae; but development of these entomopathogens at a scale suitable for broad application across the NGP has not been achieved (Portman et al. 2016, Portman et al. 2018).

In addition to variations in the effectiveness of applied techniques, weather parameters also both negatively and positively affect WSS survival. Weather parameters, such as precipitation, ambient temperature, and humidity regulate WSS populations directly and indirectly. For instance, host stem dryness at crop maturity triggers the larval diapause (Church 1955). Similarly, ideal weather conditions like a good balance of sunny and rainy days during the growth period accelerates crop growth that is favorable for larval growth and development (Seamans 1945). However, the impact of ambient temperature and precipitation on larval development and survival has not been considered in barley as it has in wheat crops. For instance, in solid-stem wheat, heavy precipitation in June reduces stem pith development that increases the larval survival compared to survival in fully developed pith (Beres et al. 2017). Thus, a study on the impact of precipitation and temperature for WSS larval growth and development in barley will provide insights to formulate WSS management program.

So far, in wheat, current WSS control measures rely on the deployment of host plant resistance traits, for example, solid stem cultivars. Solid stem cultivars not only reduce larval survival, but can actually reduce the percentage of cut stems when compared to hollow stem cultivars (Beres et al. 2009, 2013, Talbert et al. 2014, Varella et al. 2016). But, the amount of pith in the stem is highly weather dependent

(Beres et al. 2017) and the numbers of high yielding solid stem wheat cultivars are limited compared with hollow stem cultivars (Beres et al. 2011). As a result, management of WSS by only cultivating solid stem wheat cultivars cannot be achieved immediately (Bekkerman and Weaver 2018). Thus, for WSS management, developing alternative tools other than those currently available is imperative.

Alternatively, WSS oviposition rates are lower and larval mortality is greater in barley than in any solid-stem wheat cultivar (Beres et al. 2013, Varella et al. 2018). Studies have reported that WSS infestation rate—the presence of egg or larva or frass in stem—in barley ranged 0–53% (Farstad and Platt 1946), 0–2 % (Callenbach et al. 1945), and 1–49% (Varella et al. 2018). All these infestation ranges are lower than those that can occur in solid stem wheat cultivars, but all currently cultivated barley has hollow stems. This means that the lower oviposition rate in barley is associated with traits other than the stem solidness.

Overall, WSS is a problematic pest across the NGP that is now widening its geographical adaptation to eastern Colorado and northwestern Kansas (Lesieur et al. 2016 and currently applied management practices are ineffective (Knodel et al. 2009; Morrill and Kushnak 1999, Morrill et al. 1993). In addition, solid stemmed wheat cultivars are relatively resistant to WSS, but the number and yield of cultivars is limited relative to hollow stem cultivars; thus, the economic management of WSS by planting solid stemmed wheat cultivars is not easily achieved (Bekkerman and Weaver 2018). On the other hand, barley was not only recommended as a rotational crop to prevent the outbreak of WSS in wheat cropping (Farstad and Platt 1946), but is also superior to solid stemmed wheat in killing neonates (Varella et al. 2018). All cultivated barley has hollow stems that cause greater neonate mortality than is caused by resistant stem architecture in wheat. Therefore, we executed several field and greenhouse studies using eight cultivars of barley to assess responses to WSS. The overall goal of this study was to assess barley traits as viable options for the sustainable management of WSS across the NGP. To achieve the goal, the formulated objectives are as follows:

i) to assess infestation by counting eggs, larvae and cut stems in cultivars from different classes of barley;

ii) to compare summer and postdiapause larval mortality in these cultivars;

iii) to observe the impact of precipitation, plus barley growing degree days on larval survival;

iv) to explore age- and cause-specific mortality of WSS larvae in barley cultivars;

v) to compare barley grain yields across different levels of larval injury in stems;

vi) to observe WSS host choice and oviposition behaviors in two barley cultivars (one resistant and one susceptible to WSS); and

vii) to compare the plant volatile profiles from these same two cultivars.

To meet these objectives, we selected cultivars from three different classes of barley. These classes were i) Malt barley: Hockett, AC Metcalfe, Conrad, Craft, Moravian 115, Celebration, Tradition, and Innovation; ii) Feed barley: Haxby and

Champion; and iii) Forage barley: Haybet and Lavina. We sowed these cultivars in three locations in Montana: near Amsterdam (45°45'27.3"N, 111°24'00.9"W), near Conrad (48°14'34.4"N 111°22'33.4"W), and near Gildford (48°23'31.3"N 110°20'55.0"W) in 2016. But we sowed only eight cultivars near Amsterdam (45°45’27.3” N, 111°24’00.9” W) and near Big Sandy (48°15’42.1” N, 110°22’19.1” W) in 2017 because of a similar pattern of WSS infestation and cut stem proportions across the remaining cultivars. The excluded cultivars for 2017 planting were ‘AC Metcalfe,’ ‘Conrad,’ ‘Moravian 115,’ and ‘Innovation.’

CHAPTER TWO

LITERATURE REVIEW

Biology of Wheat Stem Sawfly

Wheat stem sawfly (WSS) is a univoltine hymenopteran insect. WSS females lay eggs in the lumen of the host stem. On average, an adult female can lay as many as 50 eggs in her life (Ainslie 1920, Criddle 1922, Carcamo et al. 2016). Typically, eggs are crescent shaped, glossy on the outer surface, with a milky white interior (Ainslie 1920). Approximately seven days after oviposition, a neonate emerges from the egg. The newly emerged neonate starts to devour parenchymous tissue—the main source of food for its growth and development—in the stem (Ainslie 1920). A fully developed larva varies in size from 8–14-mm long and 1–2-mm in diameter (Ainslie 1920). The larva keeps devouring tissues in the stem lumen until the host plant reaches its maturity, ripens and desiccates. Host plant maturity and the depletion of moisture in the stem triggers the larva to initiate larval overwintering processes (Holmes 1979). In this process, the larva makes a “v” shape cut around the stem internally; while at the cut, it plugs both the upper and lower parts of the stem with frass and plant material to create an overwintering structure—a hibernaculum (Ainslie 1920, Donald 1926, Farstad et al. 1949). Inside the hibernaculum, the larva ceases to be a pre-pupa at the end of winter and becomes a pupa when warming the following spring. The pupa is milky white, slender, and is approximately 12-mm long and 1.5-mm wide (Ainslie 1920). After 10–21 days, depending on temperature, an adult emerges from the pupa (Seamans 1945). Adult eclosion usually occurs in late May or early June (Munro 1947) and adults only survive up to 8 days (Wallace and McNeal 1966). The adults are active on warm and sunny days (Ainslie 1920) at temperatures from 17–32^{o}C, but remain inactive on windy days (Morrill et al. 1992).

Damage due to Wheat Stem Sawfly

Adult WSS are not known to actively feed on host plants (Sing 2002), but larval feeding injuries are economically devastating in three ways. First, the larvae devour parenchymous tissues and bore through vascular bundles in the host stem; the broken tissues reduce water and nutrient transport in the stems and also reduce photosynthetic assimilation and other metabolic processes (Macedo et al. 2005, 2006, 2007). As a result of reduced photosynthetic rates, the estimated grain weight loss is 10–30% (Holmes 1977, Morrill et al. 1992, Delaney et al. 2010). Furthermore, grain yield reductions accelerate if the injured stem experiences stresses, such as the depletion of water or phosphorus availability to the plants (Delaney et al. 2010).

Second, as the host plant reaches its maturity, the larva makes a “v” shape cut internally encircling the stem. This cut at the base of the stem causes stem lodging by gravity or when the conditions vary from gentle breezes to high winds.

The lodged stems are difficult to recover using a combine harvester. Overall grain yield loss due to WSS is estimated to approach $80 million (USD) per year in Montana alone (Bekkerman 2014, Fulbright et al. 2017).

Third, as an indirect loss, the lodged stems with ears remain on the ground and the remaining grains germinate the following spring as volunteer plants. The volunteer plants and other wild grasses become a host for the survival of wheat curl mites (*Aceria tosichella* Keifer) which is a vector of wheat streak mosaic viruses in wheat (McMechan et al. 2014). In addition, the subsequent volunteer crop uses valuable soil moisture (Bekkerman and Weaver 2018).

However, all current data on losses due to WSS are based on wheat crops; but the extent of local losses due to WSS in barley is comparable with wheat and could be greater due to strict grain quality requirements for maltsters. Additionally, the mechanism of transmission and magnitude of economic loss due to the wheat streak mosaic virus and other related viruses in barley has yet to be assessed.

Management of Wheat Stem Sawfly

Multiple management techniques: spanning cultural, chemical, biological, and host plant resistance can be practiced by growers. Cultural practices from early research in Canada include plowing a field to a depth of 15-cm in August and in June of the following year to bury stubble, mowing the edge of the field (Criddle 1922), early swathing of the crop (Holmes and Peterson 1965), and narrow row spacing as well as high seed rate sowing (Luginbill and McNeal 1958, Beres et al. 2011b). But repeated plowing of fields aggravates problems such as soil erosion and equipment wear (Bekkerman and Weaver 2018). Thus, the adoption rate for cultural control practices is limited among growers across the NGP.

Insecticides for seed treatments (Skoog and Wallace 1963, Knodel et al. 2009), for foliar and furrow applications (Wallace 1962), and for foliar applications (Goosey 1999, Knodel et al. 2009) are not widely applicable practices used to suppress WSS in the NGP. Repeated application of these synthetic insecticides creates problems on the farm and in the environment. For instance, although soil application of phorate (Thimet®20-G) reduces cut stems 64–100% in Montana (Wanner and Tharp 2015, Montana Department of Agriculture 2015), it poses grave risks to other non-target organisms (fish, birds, and mammals). Thus, phorate was only recommended to apply by incorporation into the soil 85 days before crop harvest, but limited use of the procedure resulted in no renewal after expiration at the end of December 2019.

Besides the insecticides being potentially toxic to non-target organisms, possible impacts of these insecticides on larvae are not well known because the larvae are protected inside the host stem. It may be possible that applied insecticides may not reach larvae in the quantity required to be lethal; or larvae have mechanisms to counter the lethal effect of these insecticides. The foliar application of insecticides also poses a greater threat to established natural enemies of WSS (Beres et al. 2011a). Thus, insecticides are ineffective for WSS as well as posing a greater risk to the environment and non-target organisms, including effective natural enemies of WSS.

Nine species of hymenopteran parasitoids are known to parasitize WSS (Morrill et al. 1998). Among them, *Bracon cephi* and *Bracon lissogaster* are two important native parasitoids of WSS larvae (Morrill 1997). Although parasitism ranged up to 90% due to these two parasitoids (Varella et al. 2018, Peterson et al. 2011, Rand et al. 2017), the rates are largely spatially limited (Morrill et al. 1998, Weaver et al. 2004, Peterson et al. 2011, Buteler et al. 2015). Besides the variation in parasitism by native parasitoids, another study showed that the emergence of the imported larval parasitoid, *Collyria catoptron* (Hymenoptera: Ichneumonidae), did not synchronize with WSS larval phenology (Rand et al. 2016a); thus, it would be unable to establish in the NGP and the project was abandoned (Rand et al. 2016b).

Entomopathogenic nematodes (*Heterohabditis bacteriophora, Steinernema feltiae* and *S. riobrave*) (Portman et al. 2016, Tangtrakulwanich et al. 2014) and isolates of entomopathogenic fungi (*Beauveria* and *Metarhizium*) (Portman et al. 2018) both can kill overwintered WSS larvae, but these results are from laboratory studies. Thus, achieving the goal of killing WSS by applying these entomopathogenic organisms over a broad area remains a challenge.

It seems natural enemies are an integral part of WSS management. Some groups of natural enemies need further study to validate on a wider scale, while other groups of natural enemies display erratic performance over the locations and year. The inconsistent patterns of parasitism could be due to several reasons. First, farming operations, such as crop harvesting near the soil surface and compressing or chopping the harvested straw may be detrimental. These operations reduce the chances of parasitoid survival through the following spring because parasitoid cocoons can be located anywhere from the first to fifth internodes in infested stems (Meers 2005). Second, the braconid parasitoids are bivoltine while their host—WSS—is univoltine (Criddle 1923). In this instance, the newly emerged second-generation parasitoid adults are unable to find a suitable host because the WSS larvae are either already parasitized or already overwintered and inaccessible (Holmes et al. 1963). Third, higher seeding rates reduces the number of tillers and ultimately reduces the number of suitable stems available for oviposition. Fewer infested stems imply fewer larval hosts available for parasitoids. Fourth, extreme weather like hailstorms also impacts parasitoid populations and causes inconsistent rates of parasitism (Holmes et al. 1963). Besides these inconsistencies in effectiveness, parasitoids are indeed an important component for the management of WSS (Rand et al. 2017).

Plant Defenses

Plant defense mechanisms could be a tool to reliably suppress WSS populations below the economic injury level. Every plant has a wide variety of traits that confer resistance to herbivore injuries (Karban and Baldwin 1997, Schoonhoven et al. 2005). Based on the mechanism of resistance, the traits are categorized into three groups: antixenosis (non-preference), antibiosis, and tolerance (Painter 1951). In many instances, these mechanisms are indistinguishable (Stout 2013).

In WSS, the females select an appropriate host that is not only suitable to her for oviposition, but also supportive of larval growth and development (Varella et al. 2016). To select a host, WSS females exploit both chemical and morphological cues of a host plant. As chemical cues, WSS females are attracted to plants that emit volatile compounds including (*E*)- and (*Z*)-β-ocimene (Buteler and Weaver 2012), (*Z*)-3-hexenyl acetate, (*Z*)-3 hexenol, and 6-methyl-5-hepten-2-one as (Piesik et al. 2008, Weaver et al. 2009). As morphological cues, WSS females are attracted to taller stems with greater stem diameter. Additionally, the host plant should be at an appropriate growth stage, a stem must have at least two nodes for oviposition (Buteler et al. 2010). Comparatively, the quantity of (*Z*)-3-hexenyl acetate released by a host plant combined with any of the above-mentioned morphological traits will attract gravid WSS females and result in oviposition (Weaver et al. 2009, Buteler and Weaver 2012). Although host plant traits have a substantial influence on WSS female oviposition behavior (Varella et al. 2017), the role of these traits is yet to be fully understood in wheat (Weaver et al. 2009). And host selection behaviors of WSS in barley are virtually unknown.

Plant factors that reduce the growth, survival, and fecundity of herbivores are called antibiosis traits (Painter 1951). Host plants, such as downy bromegrass (*Bromus tectorum* L., Cyperales: Poaceae), solid-stem wheat, and barley cause a substantial reduction of larval growth and development and no larvae survived until maturity in oat species (Farstad 1944, Weaver et al. 2004). The reduced rate of growth and development due to host plant defenses can be assessed by measuring the width of the head capsule of larvae (Weitzner and Whalon 1987). The larvae that were developed in flax, *Linum usitatissimum* L. (Malpighiales: Linaceae) were smaller than larvae that were developed in wheat (Farstad 1944). Similarly, larval body weights from developing in spring wheat were four times greater than the body weight of larvae that developed in downy brome (Perez-Mendoza et al. 2006). Similarly, female-biased WSS populations were observed in wheat cultivars with larger size stems, while male-biased populations were observed in wheat cultivars with smaller size stems (Morrill et al. 2000a, Morrill and Weaver 2000). In addition to growth and development, the size and quality of stems are equally influential factors to WSS fitness: body weight, body length, and fecundity. A reduction in fitness was observed in the females that were developed from solid-stem wheat cultivars compared with the fitness of females that were developed from hollow-stem wheat cultivars (Cárcamo et al. 2005). A positive correlation between WSS adult body weight and fecundity was observed, while a negative correlation between the WSS adult body weight and the intensity of stem solidness was also observed (Cárcamo et al. 2008). The nutritional composition of host stems and dietary requirement of WSS larvae have yet to be fully studied, but it is clear that host traits negatively impact the life history of WSS.

Besides selecting an appropriate host for oviposition, WSS females deposit multiple eggs in a stem because they fail to discriminate stems that have already been oviposited in by a conspecific female (Buteler et al. 2009). It is not clear yet whether failure to discriminate is due to female behavior or the lack of an infested host antixenosis trait against WSS or both. However, at the end of larval development towards maturity only one larva will survive in a stem due to obligate cannibalism (Wallace and McNeal 1966, Buteler et al. 2015).

In addition to obligate cannibalism, host plant mediated parasitism and diseases are additional causes of WSS mortality (Buteler et al. 2015). For instance, female parasitoids attracted toward a host due to herbivore-induced plant volatiles (HIPV) that are released by plants in response to larval feeding injuries (Perez 2006). Calling larval parasitoids is an indirect defense against larvae by the host plants. Furthermore, some plants harbor entomopathogenic fungi without developing symptoms, but the fungi in the plant stem can kill the developing larvae (Wenda-Piesik et al. 2009).

As a direct effect, a large proportion of neonates die in plant stems. Yet, WSS females oviposit eggs in barley stems at rates greater than 50%, while the rate of stem cutting is less than < 15 % (Callenbach and Hansmeier 1945, Farstad and Platt

1945, Varella et al. 2018). This indicates that a large proportion of larvae die before becoming fully mature and cutting the stems.

Tolerance traits are present in host plants that can produce a similar quantity and quality of agronomic yield irrespective of herbivore injuries (Painter 1951). Tolerance traits are less studied than antibiosis and antixenosis, but it is the most sustainable approach of pest management in comparison to antixenosis and antibiosis (Peterson et al. 2017). The tolerance traits of wheat and barley to larval feeding injuries in stems have yet to be fully studied. Larval feeding injuries in stems that do not influence the quality and quantity of grain are a function of tolerance traits. For instance, fewer seeds and fewer fertile spikelets per head were recovered from stems injured by larval feeding compared with the number of seeds and fertile spikelets from uninjured wheat stems (Buteler et al. 2008). In other studies, wheat grain weight was reduced by 30% in WSS infested stems compared to grain weight in uninfested stems (Delaney et al. 2010). However, it is not mentioned if reduced grain weighs were truly due to reduced photosynthetic rates or because the injured plant used additional resources to deploy more metabolites to repair the injured stems. Thus, there is a need to understand tolerance traits in wheat and barley that are induced in response to larval feeding injury in stems.

Barley and Wheat Stem Sawfly

Barley, *Hordeum vulgare* L. (Poaceae) is the fourth most important cereal crop in the world (FAOSTAT 2018). In the USA growers harvested 883,024 ha (2.18 million acres) of barley in 2019 (US Department of Agriculture 2020). The state of Montana, where growers harvested 229,467 ha of barley in 2019, is the second-highest barley producing state in the US (US Department of Agriculture 2020). Additionally, in Montana, more than 53 licensed breweries used over seven million pounds of malted grain, which is half of the barley grain production in the state (Lavey 2018). Barley is a well-adapted crop for both dryland and irrigated conditions; thus, it is known as one of the most suitable crops in Montana's cropping system.

Barley is used to make malt for beer, feed for animals, and food for humans (McVay et al. 2009). These uses are categorized based on the starch and protein composition in barley grains. For instance, a typical malt barley grain contains 60–65% starch and 10.5–13.5% protein (Schwarz and Li 2011). The ratio of protein and starch compositions in grains is critical for breweries. Conversely, protein content in feed barley can range from 10–15% (Robertson and Wesenberg 2003). Besides malt and feed, barley grains are also used as food and have medicinal uses. For example, barley grain is rich in tocochromanols which enhances reproductive health in mammals (Graebner et al. 2015). Thus, barley can be considered as a source for food and medicine, a source for feed, a source for the production of beer and has considerable economic value.

A single seed of barley produces several tillers. The number of tillers per seed varies by field condition, plant density, time and quantity of irrigation, sowing date, and cultivar types (Simmons et al.1982). Each tiller consists of several cylindrical hollow internodes and solid nodes. A leaf is derived from each node (Robertson and Wesenberg 2003, Briggs 1978). In the beginning of growth, stem length and stem dry weight increase rapidly by reserving extra photosynthetic metabolites in the stems. Thus, stem reserves reach a maximum level when the head or spike is completely emerged or at the flowering stage. Thereafter, redistribution of the stem reserves to spikes begins at the grain fill. A positive relationship occurs between large stem size and higher grain yield. Although the mechanisms behind it are yet to be deciphered, a larger stem reserves a larger quantity of photosynthetic metabolites and redistributes the reserved metabolites proportionally in a larger quantity to grain, when compared to a smaller stem (Daniels and Alcock 1982). Additionally, all the tillers are not capable of bearing heads and all the heads do not produce fertile spikelets (Briggs 1978). As a result, relatively few tillers contribute to grain yield in barley even if the barley has a prolific tillering trait. The number of grains per ear is an equally important factor affecting higher grain yield.

The number of grains per ear varies based on the type of barley cultivar: six-row or two-row barley. The spike of barley bears a series of spikelets in a set of three. Based on the fertile rows in spikelets, barley is categorized into two groups: two-row and six-row barley. In two-row barley, only the central spikelets at each rachis node are fertile and develop into two rows of kernels. In six-row barley, all three spikelets are fertile and develop into six rows of kernels (von Bothmer and Komatsuda 2011). Under favorable growing conditions, grains per spike in six-row barley are three times greater than grains per spike in two-row barley; however, the number of tillers per hill and the average grain weight per head is typically greater in two-row rather than six-row cultivars (von Bothmer and Komatsuda 2011). Therefore, in theory, grain yield per unit area is relatively similar in both two-row and six-row barley cultivars, but other biotic factors, such as insect injuries can influence total grain yield across these cultivar types.

The incidence of insect pests in barley varies by type of cultivar and geographical location. More than 100 insect-pests are reported that infest barley, but Russian wheat aphid (*Diuraphis noxia* Kurdjumov), bird cherry-oat aphid (*Rhopalosiphum padi* L.), and greenbug (*Schizaphis graminum* Rondani) are the most common insect pests in barley (Mornhinweg 2011). In Montana, WSS is becoming a serious pest in barley because a decade long (2005–2017) study showed an increase to a rate of 5–25% cut stems (Varella et al. 2018). Historically, barley was recommended as an alternative crop with wheat crops to suppress WSS in the Northern Great Plains (Criddle 1922).

Previous studies recorded WSS infestation rate (presence of WSS egg, larva or larval feeding injuries in stem) 54–97% in barley cultivars ‘Trebi’ and ‘Hannchen’ in Canada, while six other cultivars had an infestation rate intermediate between these two cultivars (Farstad and Platt 1946). Although the infestation rate could be as high as 97%, the cut stems rate was only 2% in ‘Trebi’ and 22% in ‘Hannchen.’ Similarly, the cut stem rates in the other remaining six cultivars were intermediate between these two cultivars (Farstad and Platt 1946). A similar pattern of a higher percentage of infestation but a lower percentage of cut stems in barley was recorded in Montana: the highest infestation rate was 49% in ‘Hockett’ and the lowest infestation rate was 27% in ‘Craft’; cut stems rate was 14% and 1% in these cultivars, respectively. Both infestation and cut stems percentage in the cultivars ‘Conrad’ and ‘Harrington’ had an intermediate infestation rate between 1–13% (Varella et al. 2018). In Canada, ‘Harrington,’ a malt barley cultivar, had 11% cut stems (Beres et al. 2013). These results indicate that larval mortality rates were greater in barley before the larvae were fully mature and prepared for their hibernation. In contrast, a decade long (2005 to 2017) field survey in barley has shown that larval mortality rates were decreasing relative to those recorded previously in Montana (Varella et al. 2018). As a result, the historical classification of barley crops as a resistant to WSS is no longer valid. Although the larval mortality rate is decreasing in barley, mortality rates do vary by cultivar. Thus, we can infer that some barley cultivars possess traits that impede the WSS larval establishment and survival rate (Varella et al. 2018).

Host Plant Defense Mechanisms in Barley

Plants contain a plethora of metabolites and some metabolites could play a major role to deter insect herbivores away from the plants (Mithofer and Boland 2012). By the nature of biosynthesis triggers within the plant, the metabolites are divided into two groups: induced and constitutive defense metabolites.

The constitutive defense metabolites are synthesized and distributed within the plant system irrespective of herbivore incidence. For instance, the Poaceae plant family synthesizes silica compounds as a regular process of the plant's growth and development; but the silica acts as a defense compound against number of biting and chewing herbivores (Cooke and Leishman 2011).

Plants can also synthesize metabolites in response to herbivore injury and these are called induced defenses (Walling 2000). For instance, barley plants synthesize chitinase and β-1,3-glucanase proteins after infestation by bird cherry-oat aphid or infection by a fungal pathogen and these act as defense proteins against bird cherry-oat aphid (Forslund et al. 2000). The impact of potential constitutive and induced defense mechanisms against WSS larval feeding injuries in barley has yet to be explored.

Several studies have shown that the majority of WSS larvae die before they become fully mature in barley (Criddle 1922, Farstad and Platt 1946, Varella et al. 2018). For instance, barley stems are bounded with silicified epidermis (Arber 1937) that may hinder oviposition by WSS females and possibly impair larvae. Similarly, the barley plant accumulates a higher quantity of silica in its stems (Barber and Shone 1966). Excessive amounts of silica in stems reduces palatability and digestibility of plant tissues to herbivores by wearing down the mandibles (Kvedaras et al. 2009). The WSS larvae chew on parenchyma tissue while feeding as well as chewing through nodes to search for more parenchyma or rival larvae and also do so to move down to the base of the stem to make a hibernaculum.

Similarly, two alkaloids: gramine and hordenine, are commonly found in stems and leaves of barley (Smith 1977, Lovett and Hoult 1994). Hordenine acts as a feeding deterrent for chewing herbivores (Balmer et al. 2013), while the role of gramine in herbivores has yet to be explored. Varying levels of these and other alkaloids in barley cultivars could possibly be factors that cause the death of many neonates in barley stems (Farstad and Platt 1946, Varella et al. 2018).

Host plant defense mechanisms play a major role in causing a higher proportion of WSS larval mortality compared with other causes of mortality in solid stem wheat (Buteler et al. 2015); however, the larval mortality rate in barley due to host plant resistance is greater than in wheat (Varella et al. 2018). In both cases, the host plant resistance could be both the physical and chemical traits of the host plants. For instance, the defense-related compounds: DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one), tricetin, and lignin are down-regulated in WSS infested wheat plants compared to plants without infestation (Biyiklioglu et al. 2018). Additionally, benzoxazinoids have diverse roles in insect communities, such as toxicity to herbivores, repelling herbivores, attraction of natural enemies, in addition to serving as growth-promoting factors and signaling molecules within the plant (Niculaes et al. 2018). Our understanding of the association between these alkaloids and transcriptome factors with variations in WSS larval growth and development in barley is limited. Our knowledge of the quantity of these alkaloids in stems of commercially cultivated barley is also limited.

The above mentioned physical and chemical compounds play a direct role to deter or to suppress herbivores. But these compounds, in some instances, play an indirect role in suppressing these herbivores. Chewing herbivores cause extensive tissue damage by their mandibles while feeding and also release a myriad of cues that alter the host plant's defense response. The cues can be derived from oral secretions, salivary glands, frass, and herbivore-associated endosymbionts and can both be favorable or unfavorable for their growth and development (Basu et al. 2018). For instance, oral secretions of *Spodoptera littoralis* Boisduval and *Pieris brassicae* L. contain an effector that suppresses proteinase inhibitors in *Arabidopsis* (Consales et al. 2012). The proteinase inhibitor exists in barley as trypsin inhibitor (Odani et al. 1983) that impairs protein digestion in herbivores (Hartl et al. 2011).

Similarly, the saliva of *Helicoverpa zea* L. contains glucose oxidase that suppresses the induced defense and nicotine production in *Nicotiana tabacum* (Musser et al. 2002). The frass of *Spodoptera frugiperda* J. E. Smith suppressed herbivore-induced plant defenses in maize (Ray et al. 2015, 2016a). Although WSS larvae suppress the biosynthetic pathway of defense metabolites, such as DIMBOA, tricetin, and lignin in wheat (Biyiklioglu et al. 2018), it is still necessary to elucidate which forms of larval elicitors: oral secretion, saliva, or frass might play a role in downregulating these defense metabolites in wheat. Regarding these mechanisms, the knowledge for barley is more limited than for wheat.

Multiple Decrement Life Table of Wheat Stem Sawfly in Barley

The classical single-decrement insect life table only provides information about age-specific mortality and survival (Carey 2001) but does not explicitly indicate the causes of mortality at each stage of an insect's life cycle. In comparison, multiple-decrement life tables (MDLT) allow for estimating the probability of death in the presence or absence of one factor, or for a combination of mortality factors (Carey 1993, Peterson et al. 2009, Davis et al. 2011). Contribution to the death of an insect due to one mortality factor and the contribution of that mortality factor to cause the death of the insect cannot be replaced by available other mortality factors; this is called irreplaceable mortality of that factor. Additionally, life table study provides insights to measure the effectiveness of pest control and to measure pest fitness—fecundity, adult life span, and immature development time of the pest depending upon the host plant (Naranjo 2018, Ning et al. 2017). By knowing the mortality rate of insect herbivores by each mortality factor, we can adjust pest management programs to the targeted pest based on the contributions of each mortality factor to the mortality of that pest. To estimate the proportion of mortality by each mortality factor for the insects that are protected inside the host plant and have a univoltine life cycle, there is an advantage in assessing causation because evidence persists.

WSS is a univoltine insect (Holmes 1975) and the larva is the longest and the most active stage in the WSS life cycle. At crop maturity, the larva turns into a pre-pupa that overwinters within a hibernaculum. Recording larval mortality factors from pre-diapause through the emergence of the adult post-diapause, we can exploit these mortality factors to determine if we achieve a desired level of WSS suppression. For instance, larval parasitism rates up to 90% were recorded (Varella et al. 2018, Rand et al. 2017). However, the mortality due to parasitism was calculated in the presence of other mortality factors and calculated without using multiple-decrement life tables. Thus, the actual contribution of parasitism to larval mortality was not represented which creates a false assumption for use in implementing a biological control strategy to control WSS.

These MLDT allow the calculation of irreplaceable mortality—the fraction of mortality caused by one cause of mortality that cannot be replaced by other causes of mortality— for a given mortality factor. For example, irreplaceable mortality due to cannibalism caused 7–15%, plant response caused 5–31%, predation caused 0.1–0.5%, parasitism caused 2.3–11%, and unknown factors caused 1.7–8.2% in wheat (Buteler et al. 2015). Using MDLT to explore cause- and age-specific mortality rates of WSS is an important factor to decipher the gradient of mortality due to each mortality factor and at each growth stage. So far, only two studies have been conducted to construct MDLT of WSS in wheat (Peterson et al. 2011, Buteler et al. 2015), and these have not been prepared using barley as a host. The results from wheat may not be directly applicable in the case of barley because barley is quite different from wheat, even though both crops are grown in a similar agroclimatic region. First, barley has historically been categorized as a resistant crop to WSS and at present the WSS infestation rate is increasing, while the larval mortality rate is decreasing. Second, barley has hollow stems, but the larval mortality rate is higher than for any solid-stem wheat cultivars (Varella et al. 2018). Thus, the causes of larval mortality in wheat are completely different from the causes of larval mortality in barley. Furthermore, nothing has been reported on potential differences in causes of mortality across multiple cultivars in barley. Thus, if we can enhance the age- and cause-specific mortality of WSS, we could develop effective management techniques for WSS.

Host Selection and Oviposition Choice

Host selection processes in insects are complex and follow programmed behaviors in a repeated but fixed order (Atkins 1980). Also, host plant volatile concentrations (Cha et al. 2011) and plant architecture (Rudgers and Whitney 2006) are key factors in host selection. In addition, host plant defenses are influenced by environmental factors such as drought stress, adult female life span and flight capacity (Larsson and Ekbom 1995) and offspring survival rates (Gripenberg et al. 2010).

After initially selecting a host, finding a specific oviposition site and judging for oviposition suitability all precede oviposition. Buteler et al. (2009) recorded step by step behaviors after host selection that led to oviposition by WSS in wheat. These behaviors are quiescence on leaves or stems, walking up and down on leaves or stems, abdominal tapping on the stem, and finally ovipositor insertion and egg deposition. However, inserting an ovipositor initiates the possibility of oviposition, it is not necessarily followed by placing an egg, sometimes it occurs only to then judge the site or even the host inappropriate (Varella et al. 2016). Additionally, WSS females prefer not to oviposit in stems from near-isogenic lines that are derived from a parental line of resistant solid stem wheat cultivar when compared to those from a susceptible parental line. Overall, the number of eggs in solid stem wheat is fewer than in hollow stem wheat, but there are even fewer in barley.

Even though the female selects an appropriate host, larval survival is conditional for two reasons. First, the larva is obligated to spend its entire larval and pupal period in the stem that it emerges from as an adult. Thus, both induced and constitutive defense traits could potentially be deleterious to larval survival. Additionally, larval cannibalism is obligate if multiple eggs are oviposited within a stem (Wallace and McNeal 1996). Such host selection and survival rate discrepancies have been observed in several host plants. For instance, the larval survival rate is comparatively higher in hollow stem wheat than in solid stem wheat (Varella et al. 2015, Talbert et al. 2014), than in downy brome (Perez-Mendoza et al. 2006), and in barley (Varella et al. 2018). Female WSS also occasionally oviposit in oat stems in which all larvae die (Criddle 1922).

Additionally, females fail to discriminate a host that has already been oviposited by conspecific females; such behaviors lead to multiple eggs within a stem and subsequently reduces larval survival by obligate cannibalism. But the survivors from the cannibalism receive additional nutritional resources from the victims. Thus, such indiscriminate oviposition in already infested stems is possibly a deliberate strategy that is yet to be deciphered or perhaps it is just random behavior.

It seems that WSS females can distinguish unsuitable and supportive hosts for larvae while ovipositing. However, the certainty of judging host plants based on the larval survival rate is not explicitly explainable. Thus, deciphering host choice and oviposition behaviors could provide some valuable insights that could be used to develop alternative management tools for WSS.

CHAPTER THREE

ANTIXENOSIS, ANTIBIOSIS, AND POTENTIAL YIELD COMPENSATORY RESPONSES IN BARLEY CULTIVARS EXPOSED TO WHEAT STEM SAWFLY UNDER FIELD CONDITIONS

Contribution of Authors and Co-Authors

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Manuscript Information Page

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Abstract

Wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera), females require favorable host cues to locate and oviposit in host plants. Larvae of *C. cinctus* injure the host plant by feeding inside the stem and by girdling the base of ripened stems before obligate diapause. Barley, *Hordeum vulgare* L. (Gramineae), cultivars were previously planted as resistant crops in rotations to manage *C. cinctus*, but due to increasing levels of injury in crop fields this is no longer a valid management tactic in Montana. Therefore, we aimed to understand antixenosis (behavioral preference), antibiosis (mortality), and potential yield compensation (increased productivity in response to stem injuries) in barley exposed to *C. cinctus*. We examined these resistance traits in eight barley cultivars spanning malt, forage, and feed classes. Antixenosis was assessed by counting the number of eggs per stem and antibiosis was assessed by counting infested stems, dead larvae, and stems cut by mature larvae. Potential yield compensation was evaluated by comparing grain yield from three categories of stem infestation: 1) uninfested, 2) infested but the larva died, and 3) infested with a surviving larva that cut the ripened stem at crop maturity. We found the greatest number of eggs per infested stem (1.80 ± 0.04), the highest proportion of infested stems (0.63 ± 0.01), the lowest proportion of dead larvae (0.31 ± 0.01), and the highest proportion of cut stems (0.33 ± 0.01) in the malt cultivar 'Hockett'. This popular cultivar, by production acreage, was highly attractive to *C. cinctus* females, and subsequent larval mortality in 'Hockett' stems was lower than for other cultivars planted. Seven out of eight cultivars had greater grain weight for infested stems than for uninfested stems. These cultivars may have compensatory responses to larval feeding injury, but it is difficult to separate this from preference for larger stems by ovipositing females. Overall, these barley cultivars contain varying levels of antixenosis to adult females, varying antibiosis to feeding larvae, and different yield compensation patterns in response to larval feeding injuries. Our results provide a foundation of knowledge to explore resistance cues in barley and subsequently provide a framework to further develop *C. cinctus* resistant or tolerant barley cultivars.

Key words: egg, larva, stem injuries, cut stem, grain weight

Introduction

Complex and intertwined relationships exist between phytophagous insects and their host plants. Direct and indirect defenses of host plants are often induced in response to insect injury (Dicke 2009, Turlings and Erb 2018, Piesik et al. 2011, 2013). Defense responses such as the synthesis of toxic secondary metabolites can negatively impact insect growth and development (Kos et al. 2012) but these processes can also have negative, constant, or positive effects on the host plant itself (Agrawal 1998). There is a well-known tradeoff between growth and plant defenses that typically reduce yield (Ballhorn et al. 2014). Feeding insects remove and ingest plant tissues or mobilize tissue contents which usually creates plant stress (Peterson and Higley 2000); however, sometimes stress outcomes are of little consequence to yield in the presence of insect injury. This type of resistance is called tolerance (Pedigo and Higley 1992, Pedigo 1995, Peterson et al. 2017). If host plant yield can be positively correlated with plant injury, this phenomenon is called overcompensation (Pedigo et al. 1986). Understanding the relationships between host plant responses and feeding injury is necessary to develop sustainable management strategies for most phytophagous insects. This study focuses on interactions between barley (*Hordeum vulgare* L.) and the wheat stem sawfly and reports on the relative roles of antixenosis, antibiosis, and possible yield compensation in innate plant defenses to this insect.

Cephus cinctus Norton, the wheat stem sawfly, is an economically devastating pest of wheat grown on the northern Great Plains of North America (Beres et al. 2011a). This native species (Lesieur et al. 2016) has a host range that includes both cultivated small grain cereals and native grasses over a large area of US and Canadian wheat production (Cockrell et al. 2017, Criddle 1923). Female *C. cinctus* begins to deposit eggs in host stems early in the stem elongation process. At hatch, the larva starts to feed on the stem lining near the point of emergence from the egg. The resulting feeding injuries to this parenchymous tissue, and subsequently to vascular structures at the stem nodes may reduce the translocation of water and minerals from roots to shoots (Morrill et al. 1994, Delaney et al. 2010). As a result, the photosynthetic rate can be reduced in wheat (Macedo et al. 2005, 2006, 2007, Delaney et al. 2010).

As the host plant ripens, the mature larva moves to the base of the stem and makes a v-shaped groove around the interior of the stem. It does this by chewing a uniform gouge that encircles the stem interior and leads to lodging of the upper stems (Ainslie 1920, Weiss and Morrill 1992). In response to wind and gravity these break off and are commonly called “cut stems.” Lodged, cut stems lower grain recovery at harvest (Holmes and Peterson 1965, Beres et al. 2007). As a result of physiological and physical impacts of herbivory, estimated losses can range from approximately 50–80 million (USD) per year in Montana alone (Bekkerman 2014, Fulbright et al. 2017) and overall, can cost $350 million per year (Beres et al. 2011a, 2011b).

Grain yield in barley is a collective form of the number of seed heads per unit area, the number of seeds per head, and weight per seed. Additionally, grain weight is a function of the amount of photosynthetic assimilates translocated to the seeds at grain fill (Gallagher et al. 1976). The amount of translocated assimilate allocated to grain fill is based on a priority system for tillers where tillers that are further away from the main stem are lower priority and can suffer from carbohydrate starvation at grain fill (Gonzalez et al. 2011). As a result, many spikelets on more distal tillers are aborted and unable to bear grain (Simmons et al. 1982). This can happen in wheat injured by *C. cinctus* if feeding by larger larvae is not curtailed (Buteler et al. 2008, Bekkerman and Weaver 2018).

In barley, photosynthetic rates are greater in plants with partially removed spikelets (physical injury) than in plants with intact spikelets (Serrago et al. 2013). Thus, minor plant injury may not be directly relevant to the reduction in grain yield in barley. Several studies have shown that *C. cinctus* injury reduces grain yield from 2 to 30% in wheat heads (Holmes 1977, Morrill et al. 1992, Delaney et al. 2010, Beres et al. 2007). Currently, there are no data reported for potential yield losses due to larval stem-mining injury in barley stems, nor is there any information on potential tolerance or induced compensatory responses due to this type of injury.

Several management strategies can be implemented to limit losses due to *C. cinctus* injury but there has been limited success. For instance, insecticides application (Knodel et al. 2009, Beres et al. 2011a); adjustment of planting dates to escape the peak of the *C. cinctus* oviposition period (Morrill and Kushnak 1999); deep plowing to bury stubble and shallow plowing to expose overwintered larvae to low temperatures and desiccation (Morrill et al. 1993); and temporal and spatial variation in parasitism rates (Morrill et al. 1998, Weaver et al. 2004, 2005). Nonetheless, host plant resistance that is due to the presence of solid pith in the stem can be relatively successful for reducing stem cutting in wheat (Beres et al. 2013, Talbert et al. 2014, Varella et al. 2016, Adhikari et al. 2018), but this may be compromised by environmental effects on pith expression (Beres et al. 2009, 2017) and relative expression of the trait in different backgrounds (Cook et al. 2019, Varella et al. 2019, Bainsla et al. 2020).

In addition to stem solidness, other defense related metabolites, such as DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one), tricetin, and lignin are downregulated in *C. cinctus* infested wheat stems when compared to uninfested stems (Biyiklioglu et al. 2018). Among these identified compounds, DIMBOA is well-investigated as a toxin from cereals for different classes of herbivores, notably aphids and caterpillars (Martos et al. 1992, Niemeyer 2009, Erb et al. 2009). Tricetin activates lignin biosynthesis in secondary cell wall production (Moheb et al. 2013). However, *C. cinctus* feeding downregulated the synthesis of these metabolites, perhaps supporting the idea that these larvae challenge components in the specific biosynthetic pathways, disrupting signals and limiting subsequent synthesis of these metabolites (Biyiklioglu et al. 2018). Cultivated barley has lost the biosynthetic capability to produce DIMBOA (Nomura et al. 2002, Grun et al. 2005), but there are other groups of compounds, such as benzoxazinoids, flavonoids, and alkaloids that may be synthesized to ward off insect pests (Balmer et al. 2013). The role of specific secondary metabolites leading to greater larval mortality for *C. cinctus* larvae in barley than in resistant wheat cultivars has yet to be explored (Varella et al. 2018).

Cultivated barley has hollow stems, yet the proportion of stems cut by *C. cinctus* larvae is lower in barley than in solid-stem wheat cultivars (Beres et al. 2013, Varella et al. 2018), primarily because in barley a greater proportion of larvae die before becoming mature enough to cut stems. Thus, the larval mortality in barley is based on factors other than the stem architecture. It is important to note that syntenic comparisons indicated that considerable similarity existed between certain genetic sequences in barley and the solid stem loci known from bread and durum wheat (Biyiklioglu et al. 2018), but there is no evidence of this trait in domestic barley. Historically, larval mortality rates up to 100% has been recorded in barley (Farstad and Platt 1946). A decade-long comparison has shown that infestation rates have been increasing and larval mortality rates have been decreasing trend in *C. cinctus* infested barley in Montana (Varella et al. 2018). The conflict between recent findings and early records could be due to diverse, overlapping *C. cinctus* populations (Lesieur et al. 2016) that facilitate better adaptation, first from grass to wheat (Ainslie 1920, Morrill and Kushnak 1996) and now to barley in Montana. Potentially this could be due to loss of significant sources of antixenosis and antibiosis in modern barley; like the biosynthesis of benzoxazinoids (Grun et al. 2005). These two factors could also interact and cause the current trends. Increasing *C. cinctus* damage poses a serious threat to Montana barley production, which is third highest in the US (US Department of Agriculture 2020).

We conducted a field study in Gallatin and Choteau counties of Montana to explore antibiosis, antixenosis, and yield compensation traits in barley cultivars in 2016 and 2017. The specific objectives of the study were: i) to determine the relative attractiveness of barley cultivars to *C. cinctus* females for oviposition (antixenosis); ii) to assess larval death rate in stems (antibiosis); and iii) to compare grain yield among uninfested and different categories of infestation (potential yield compensation).

This study will provide an understanding of these key types of host plant resistance in different barley classes, which should be the foundation of an integrated pest management (IPM) strategy (Pedigo 1995, Peterson et al. 2018) for *C. cinctus* (Beres et al. 2011b). Our findings will bolster efforts to incorporate these traits when developing barley cultivars, which will ultimately lead to a significant reduction in future economic losses incurred due to *C. cinctus*.

Materials and Methods

Research Sites and Experimental Design

We assessed *C. cinctus* infested stems by counting the number of eggs and the proportion of dead larvae in the cultivars that were seeded in the spring season at three sites in the state of Montana: near Amsterdam in 2016 (45°45’27.3” N, 111°24’00.9” W) and 2017 (45°45’33.2” N, 111°23’50.0” W), and also near Big Sandy in 2017 (48°15’42.1” N, 110°22’19.1” W). Each planting of the experiment was conducted using a randomized complete block design. At a site, each block was equally divided into three blocks based on field variability due to slope. Each block was divided into eight plots with a size of 1.8 m × 3.6 m with 0.3 m spacing between each plot and block. The barley cultivars grown at all sites were: Celebration (PI6B01-2218), a six-row malt barley; Champion (YU501-385), a two-row feed barley; Craft (PI646158), a two-row malt barley; Haxby (MT950186), a two-row feed barley; Haybet (CI16569), a two-row forage barley; Hockett (PI657121), a two-row malt barley; Lavina (MT981397), a two-row forage barley; and Tradition (B2482), another six-row malt barley. We seeded each cultivar at a rate of 0.9 g per square meter per plot. A self-propelled, seven-row seeder (Fabro Enterprises Limited, Swift Current, SK, Canada) was used for seeding near Amsterdam in 2016 and 2017, while a custom built, six-row self-propelled cone seeder with an Atom Jet paired row opener (BNM Customs, Havre, MT) was used near Big Sandy in 2017. Seeding was completed near Amsterdam in 2016 on April 4, near Amsterdam in 2017 on May 4, and near Big Sandy in 2017 on April 13. Local best management practices followed by participating wheat and barley growers were used to prepare and fertilize the soil before planting, but several manual weeding operations were subsequently performed to maintain better crop stands at all sites.

Sample Collections and Dissection

We began sample collections when plants were Zadoks stage 32–33 (Zadoks et al. 1974), which was from 43–59 days after seeding (DAS) (at Big Sandy - 43 DAS; at Amsterdam 2016 - 59 DAS; and 2017 - 51 DAS) because this was when *C. cinctus* infestation occurred. All plants within a 0.3 m row length in each plot were uprooted and wrapped in labelled paper bags. This sampling procedure was repeated 3 times in each plot for each week of sampling. Samples were collected in 9 consecutive weeks but were categorized into 2 groups from each site: green samples (stems from week 1 to week 8 samples) and harvest samples (samples collected at crop maturity in week 9). From the green samples, we selected 35 primary stems from each sample bag (105 stems per plot) and dissected all stems lengthwise from base to head. Each dissected stem was visually examined to count the number of eggs and larvae and numbers of live and dead for each life stage were recorded. It was not possible to dissect all the collected samples on the date of collection, so samples were stored at 4^{o}C for dissection the following day. To prevent inflated counts of dead larvae due to prolonged storage, we selected only 35 primary stems from each sample bag (105 stems per plot). By doing this, we were able to dissect all the stems while they were in good condition (not withered or deteriorated) to get a precise determination of mortality for the *C. cinctus* eggs and larvae at the time of sample collection.

From every stem in each harvest sample, we removed the seed head and saved it in a uniquely labelled envelope. Stem diameter was measured by using a micrometer (Mitutoyo Outside Micrometer, Billings, MT) as per Buteler and Weaver (2012). Individual grain numbers from each seed heed were recorded for each head using a grain counter (Masch. Nr. 77314, Pfeuffer GmbH, Kitzingen, Germany) with the following settings: container 2, speed-80, sizes-24, and present-3000. Total grain weight for each head was recorded using a digital balance (model SC 2020, Merck KGaA, Damstadt, Germany). For the variables grain weight, grain number, and stem diameter, the stems were separated into one of three categories based on their infestation status: i) infested with a cut stem (stem girdled at the base that resulted in a mature larva in a hibernaculum); ii) infested with a dead larva (the stem contained larvae that died before maturity); and iii) uninfested stems (stem with no evidence of a larva or larval feeding). To compare grain weight and grain number per head based on infestation status, we removed occasional outliers with the smallest and the largest stem diameters. Additionally, we excluded stems with less than seven grains per head which indicated a rare loss of grains during handling.

Next, we compared grain weight and grain number based on stem infestation status within a cultivar. We did not compare grain yield among the cultivars, irrespective of *C. cinctus* stem injuries because we included different classes of barley cultivars, such as two-row and six-row malt barley, as well as cultivars from the feed and forage classes. With different end uses intended, these barley classes have different grain yield potential, irrespective of *C. cinctus* stem injury.

We calculated the proportion of infested stems by recording the number of infested and uninfested stems. A stem was categorized as infested stem if it contained a live or dead egg, larval frass, or either live or dead larvae. Otherwise, the stem was categorized as uninfested. Similarly, the proportion of dead larvae was calculated by first assessing all larvae in each cultivar. Thereafter, we categorized the dead larvae into three categories: i) dead due to plant factors if the larva died within one internode early in development; ii) dead due to parasitism, if a stem contained parasitoid larvae or cocoons or a parasitoid emergence hole; and iii) dead due to other factors, a later instar larva died from other than the above mentioned reasons.

Statistical Analyses

We used a generalized linear model (GLM) (Marschner 2011) with a quasi-Poisson distribution for number of eggs per stem, number of eggs per infested stem (after correcting for stems without eggs), and GLM with a binomial distribution for proportion of infested stems, proportion of dead larvae, and proportion of cut stems. Additionally, a linear regression model was used to compare grain weight within a cultivar among the stem infestation status. Regression coefficients of grain weight and stem diameter with different infestation status were compared using the function lstrends in lsmeans package (Lenth 2016). Post-hoc tests were conducted by using Tukey's HSD test in multcomp (Hothorn et al. 2008). Tukey HSD was used to reduce error due to unequal sample sizes across cultivars. All the statistical analysis and data visualizations were performed using ggpubr (Kassambara 2018) and ggplot2 (Wickham 2016) in R version 3.6.2 (2019).

Results

Mean Number of Eggs

A total of 26,632 stems were split to assess the number of eggs per stem among eight cultivars over 3 sites × years. Most of the cultivars had eggs during the first four weeks of sampling (from 1st to 4th week), with the exception being at Amsterdam 2017 (Fig. 1). The mean number of eggs per sampled stem differed by cultivars and by sites (GLM, $F_{14, 164} = 3.52$, $P < 0.001$). A similar pattern was found for the mean number of eggs per infested stems, where there was an interaction between cultivars and sites (GLM, $F_{14, 164} = 3.65$, $P < 0.001$). Overall, 'Hockett' had the greatest number of eggs per stem and per infested stem, relative to the other cultivars (Tables 1and 2).

Proportion of Dead Larva per Stem

We observed the first dead larvae in all cultivars and sites during the second sampling week, except for in ‘Celebration’ and ‘Champion’ in Amsterdam 2016 (data not shown). We found an interaction between cultivar and site for proportion of dead larvae (GLM, $F_{14,164} = 3.02$, $P < 0.001$). The proportion of dead larvae was greatest in ‘Craft’ and least in ‘Hockett’ at all sites; while the rest of the cultivars were intermediate (Fig. 2).

Categories of Larval Mortality

There was no interaction between cultivar and site for percentage of larvae dying within a single internode (GLM, $F_{14,102} = 1.67$, $P = 0.07$); similarly, we did not found an interaction between cultivar and site for percentage mortality due to parasitism (GLM, $F_{14,102} = 0.74$, $P = 0.73$) or percentage mortality due to other factors (GLM, $F_{14,102} = 0.83$, $P = 0.64$). However, the greatest percentage of larvae dying within a single internode occurred in ‘Craft’ stems (79.4 ± 2.4) and the lowest percentage in ‘Hockett’ stems (37.2 ± 4.4) (Table 3). There was no difference among cultivars for percentage mortality due to parasitism. However, ‘Hockett’ had the highest percentage of larval mortality due to other factors (58.1 ± 3.8), while the lowest was in ‘Craft’ (18.8 ± 3.1) (Table 3).

Proportion of Infested Stems

A total of 25,108 and 15,843 stems were dissected for Amsterdam 2016 and 2017 respectively, while 23,125 stems were dissected from Big Sandy in 2017 to assess the proportion of infested stems. The proportion of infested stems differed by cultivars and by sites (GLM, $F_{14, 164} = 6.17$, $P < 0.001$ (Fig. 3). Overall, a lower proportion of infested stems (< 50% except for Haxby and Hockett) was found near Big Sandy in 2017.

Proportion of Cut Stems

A total of 3,960 and 2,514 stems were collected at harvest for subsequent dissection from near Amsterdam in 2016 and 2017 respectively, while 4,255 stems from near Big Sandy were similarly collected and dissected in 2017 to assess the proportion of stems cut by mature larvae (Table 4). There was an interaction between cultivar and site for the proportion of cut stems (GLM, $F_{14,164} = 2.96$, $P < 0.001$). The proportion of cut stems was less in all cultivars near Big Sandy in 2017 than it was near Amsterdam in 2016 and 2017. Survival to harvest was greatest in ‘Hockett’, which had the greatest proportion of cut stems compared to other cultivars at all site × years (Fig. 4).

Grain Yield per Stem

Across cultivars, there was a mixed trend for grain weight per head in stems based on infestation status. However, whenever there was a difference in grain yield per stem by stem infestation status, a greater grain weight per head occurred in stems containing dead larvae and in stems cut by mature larvae than for uninfested stems across site × years (Fig. 5). The six-row malt barley 'Celebration' had a similar grain weight per head across infestation status and site × year (lm, $F_{4,793} = 1.31$, $P = 0.33$). The remaining cultivars displayed interactions between infestation status and site (Champion: lm, $F_{4,1239} = 15.42$, $P < 0.001$; Craft: lm, $F_{4,1292} = 3.12$, $P = 0.01$; Haxby: lm, $F_{4,1456} = 4.76$, $P < 0.001$; Haybet: lm, $F_{4,1119} = 4.61$, $P < 0.001$; Hockett: lm, $F_{4,1247} = 3.72$, $P = 0.005$; Lavina: lm, $F_{4,1582} = 19.35$, $P < 0.001$; Tradition: lm, $F_{4,826} = 4.99$, $P < 0.001$).

Correlation between Grain Weight and Stem Diameter

All cultivars displayed a strong positive correlation (correlation coefficient from 0.59 to 0.80) between per stem diameter and per stem grain weight that was independent of stem infestation status and cultivar (Fig. 6). Similarly, a strong correlation (correlation coefficient from 0.73 to 0.97) (Supp. Figure S1) between grain weight and grain number was also found irrespective of stem infestation status. The pairwise comparisons of regression coefficients for stem diameter that were made for: uninfested stems vs infested stems with dead larvae; uninfested stems vs infested stems cut by mature larvae; and infested stems with dead larvae vs infested stems cut by mature larvae, were similar in all cultivars except in 'Celebration' (near Amsterdam 2016 and 2017), 'Hockett' and 'Tradition' (near

Amsterdam 2017), and ‘Champion,’ ‘Haxby,’ and ‘Hockett’ (near Big Sandy 2017) (Supp. Table S2).

Discussion

Barley Antixenosis

We aimed to explore antixenosis that determines *C. cinctus* host selection behavior and oviposition and antibiosis that reflects larval mortality and the number of nodes injured by feeding. We also measured potential yield compensation that could indicate a variation in responses to larval feeding injuries. The results provide a foundational characterization of traits to target for development of resistant barley cultivars to reduce economic losses incurred by *C. cinctus* in barley.

The experimental design we used allows us to determine that a greater number of eggs in a stem indicates an oviposition preference for stems of that cultivar. We found that ‘Hockett’ had the greatest number of eggs per stem and the greatest number of eggs per infested stem relative to all other cultivars across site × years (Table 2). This indicates that ‘Hockett’ barley has traits, similar to those reported for wheat, that are more attractive to foraging *C. cinctus* females (Weaver et al. 2009, Buteler et al. 2010, Buteler and Weaver 2012) and lead to oviposition in the stem lumen after exploration (Buteler et al. 2009, Varella et al. 2017) . Previous studies have shown that gravid females select stems using specific cues. For instance, females are attracted to wheat plants that release large amounts of several compounds, including (*Z*)-3-hexenyl acetate (Piesik et al. 2008, Weaver et al. 2009, Buteler et al. 2010) and (*E*)- and (*Z*)-β-ocimene (Buteler and Weaver 2012), and also prefer stems that are taller (Buteler et al. 2009) as well as those with less stem solidness (Varella et al. 2017, 2018).

Overall, we found a higher proportion of infested stems and more eggs per stem near Amsterdam in 2017 compared to near Amsterdam in 2016 and near Big Sandy in 2017 (Table 1, 2 and Fig. 3). This could be due to a shorter crop duration (110 days vs 140 days), because re-seeding was necessary near Amsterdam in 2017. A potential reason could be that the late planting provided an ample number of succulents, newly elongating stems that are preferred for oviposition (Seamans 1928, Holmes and Peterson 1960, Weaver et al. 2009, Buteler et al. 2010). This is because plants experienced higher temperatures and required amounts of rainfall which accelerated growth and development in the later 2017 planting but resulted in oviposition occurring in only 2 sampled weeks rather than in all 4 (Fig. 2). In contrast, the 2016 plantings, yielded earlier plants that were exposed to less than optimal temperatures from evening to early morning in the early stages of development (this is normal for spring planted barley). As a result, those plants produced an adequate, but lower number of tillers that were suitable for oviposition. Further study is needed to assess the production of volatile attractants in field grown barley, which will complement results from laboratory efforts (Piesik et al. 2011, 2013). Similarly, detailed characterizations of stem exploration leading to oviposition in barley by gravid *C. cinctus* is warranted.

Barley Antibiosis

The primary source of nutrition for developing larvae includes parenchymous tissue and vascular bundles at the stem node (Macedo et al. 2005, 2007). We found the greatest proportion of larval mortality occurs within a single internode near where the neonate emerges from the egg, and relatively soon after larval feeding begins (Table 4). If the larva survives initial feeding, it must eventually proceed into obligate diapause after a mature larva makes a girdling cut around the interior base of the stem. The lowest percentage of cut stems, 6.5 ± 3.0 (mean ± SE), occurred in 'Craft' near Big Sandy in 2017, and the highest percentage of cut stems, 50.0 ± 4.1 occurred in 'Hockett' near Amsterdam 2017 (Fig. 4). Additionally, the proportion of infested stems was 45.6 ± 1.4 in 'Craft' near Big Sandy in 2017 and was 77.5 ± 3.3 in 'Hockett' near Amsterdam in 2017 (Fig. 3). We can see a clear difference between the patterns for the proportion of infested stems and the proportion of cut stems in these two cultivars at corresponding sites, indicating that a greater number of larvae died before they were fully matured in 'Craft' compared to 'Hockett.'

Further, greater proportions of dead larvae within one internode could be due to traits that limit further development of neonates via toxins or a critical limit of essential nutrients (Perez-Mendoza et al. 2006, Holmes 1982). Similar results were recorded in a field survey of barley in Montana, with larval mortality ranging from 51 to 100% (Varella et al. 2018). One major factor causing larval mortality is host plant antibiosis in barley and certain wheat cultivars (Wallace and McNeal 1966, Buteler et al. 2015, Varella et al. 2018). Some defense related secondary metabolites, such as DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one), tricetin, and lignin are downregulated in *C. cinctus* infested wheat stems (Biyiklioglu et al. 2018). This indicates that these plants are likely to synthesize secondary metabolites in response to *C. cinctus* injury. Although cultivated barley has lost the ability to biosynthesize DIMBOA (Nomura et al. 2002, Grun et al. 2005), other groups of defensive compounds, such as benzoxazinoids, flavonoids, alkaloids can be synthesized to ward off insect pests (Balmer et al. 2013). For instance, two alkaloids: hordenine (N, N-dimethyltriamine) and gramine (N, N-dimethylindolemethyl-amine) are feeding deterrents to grasshoppers (Bernays and Chapman 1977, Westcott et al. 1992), *Heliothis* (Bernays et al. 2000) and aphids (Zuniga et al. 1988). We did not analyze the amounts of these secondary metabolite(s) that were potentially associated with larval mortality; however, our results showed compelling evidence that the antibiotic trait plays a key role in overall larval mortality in barley.

Yield Compensation

The trends in grain weight per stem were dissimilar across cultivars when considering the stem infestation status (Figs. 5 and 6). When there was a difference due to stem infestation status within a cultivar, we found a greater grain weight per stem for both infested stems containing dead larvae and infested stems cut by surviving larvae, relative to uninfested stems (Fig. 5). Despite larval feeding injuries in the stem interior, infested stems attained a greater grain weight than uninfested stems. This could be caused in two ways. First, *C. cinctus* females are biased to select the best and most productive main stems for oviposition (Buteler et al. 2009, 2010) and second, larval feeding injuries in vascular tissues might stimulate compensatory growth via the infested stems. This type of compensatory growth after vascular tissue injury occurs in rice, *Oryza sativa* L., plants infested by yellow stem borer, *Scirpophaga incertulas* Walker (Rubia et al. 1996). The stem borer injured plants produce a greater number of tillers than uninfested plants (Rubia et al. 1996).

First, our results show a strong positive correlation between grain weight and stem diameter in all the cultivars, as expected (Fig. 6, Supp. Figure S1). In barley, the primary spike is the main factor in increasing grain yield (Alqudah and Schnurbusch 2014), while the other tillers are only supportive components in gaining greater overall yield (Sakamoto and Matsuoka 2004, Sreenivasulu and Schnurbusch 2012). Further, not all tillers are able to produce grains because the more distal florets of the tillers are not directly connected to the primary phloem (Hanif and Langer 1972). As a result, the tertiary tillers or distal florets are aborted due to an insufficient amount of carbohydrates at anthesis (Gonzalez et al. 2011, Sakamoto and Matsuoka

2004, Mäkelä and Muurinen 2011), which leads to reduced grain yield in tillers, but could contribute to increased weight in the primary stem.

Second, overcompensating and relocating resources in response to *C. cinctus* larval injuries could play a role in an increased grain yield in the infested stems compared with the uninfested stems. Despite larval injury in the vascular tissues, the infested plants might accumulate excess amount of photosynthetic assimilates from the vegetative stage to recover the loss due to injured areas within the stem. Later, the stem remobilizes assimilates, a common trait in cereals (Rae et al. 2005, Saint Pierre et al. 2010, Bainsla et al. 2020). The stored assimilates contribute heavily to grain fill which improves yield stability in these crops (Slewinski 2012). A supporting study showed that the barley plants had higher photosynthetic assimilation in plants with partial spikelet removal than in plants with intact spikelets (Serrago et al. 2013). Thus, in our results, some of the cultivars may have the capacity to deliver larger amounts of photosynthetic assimilates in response to larval stem injuries; which could lead to greater grain yield in these cultivars.

Both factors, the female bias to select robust stems and yield compensation, are likely and could act together in response to *C. cinctus* stem injury and grain weight in barley. We found a stronger correlation between stem diameter and grain yield in the stems with dead larvae compared with the other two categories, either uninfested stems or stems with the greatest injury due to cutting by mature larvae.

A weaker correlation between stem diameter and grain weight in uninfested stems indicates that there was a narrow range of stem diameter or grain weight (Fig. 6). This was because most of the robust primary stems were oviposited in by females, with only a few primary stems and many tertiary stems remaining uninfested, causing a weaker correlation.

In the infested stems cut by mature larvae, weaker correlations between stem diameter and grain weight could be due to a compensatory mechanism insufficient to recover the physiological loss due to stem mining (Fig. 6). We found that in stems cut by mature larvae, the stem mining injured 3.3 ± 0.04 (mean ± SE) nodes per stem; while the number of injured nodes in infested stems with dead larvae was 2.5 ± 0.03 (mean ± SE) (Supp. Table S1). Continuous stem mining by feeding larvae in stems impeded potential compensation in stems that were cut by mature larvae. As a result, the growth of the stems as well as the increase in grain weight was not less than for the stems with dead larvae even though the females had selected the primary and most productive stems for oviposition for both outcomes.

We found some discrepancies in cultivar responses to *C. cinctus* in fields near Amsterdam compared to the field near Big Sandy. It could be because the *C. cinctus* population in the Amsterdam area has been exposed continuously to barley for more than two decades (personal communication - M. Flikkema), while *C. cinctus* population exposure to barely crop near the Big Sandy area is relatively naïve, with no barley grown in nearly four decades (personal communication - L. Edwards). Overall, the levels of *C. cinctus* injury were lower across cultivars near Big Sandy. Yet, even for pest populations that had never encountered this host, 'Hockett' experienced the greatest injury. This pattern remains true at sites near Amsterdam with continuous exposure of the pest to barley, but the magnitude of injury is greater. Since the increase in damaging populations is relatively recent (Varella et al. 2018), the greater vulnerability of 'Hockett' is worrisome given the popularity of this cultivar with barley growers. By area of production, 'Hockett' was the third most grown malt barley cultivar in Montana in 2019 (AMBA 2020).

Although there were some discrepancies within cultivars against *C. cinctus*, we can conclude that 'Hockett' had the most eggs per infested stem compared to all other cultivars across sites (Table 2). Further study may provide insights into the cultivar's traits—visual, chemical, and tactile cues or weaker antixenosis traits relative to other cultivars—that attract a higher number of gravid females compare with other cultivars. Additionally, the highest proportion of dead larvae (Fig. 2) and highest percentage of dead larvae occurring within a single internode was in 'Craft' relative to all other cultivars across site × years (Table 3). This could be a result of more antibiotic compounds or a greater deficiency of essential nutrients, which are explicit mechanisms of antibiosis. Heritability of antibiosis and antixenosis in the biotic interaction between barley and *C. cinctus* is a promising area for future study. In terms of grain yield, understanding underlying compensatory mechanisms in response to larval feeding injury could provide opportunities to develop *C. cinctus* tolerant barley cultivars in the future. Further studies exploring remobilization of stored photosynthetic assimilates to grain fill in response to stem injury in barley cultivars would also be an asset towards developing more stable barley production.

Finally, these findings in barley cultivars show varying levels of antixenosis, antibiosis, and potential yield compensation traits that prepare a foundation to understand the specificity of host plant interactions. Knowledge of these interactions can be advanced by conducting transcriptomic, proteomic, and metabolomic studies that can help to decipher key resistance pathways in these cultivars. After doing these, we can better incorporate the selected traits for developing novel barley cultivars, and possibly other cereal grain cultivars, with greater resistance to *C. cinctus*.

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Table 1. 1 Mean (± standard error, SE) number of eggs per sampled stem

Cultivar	Amsterdam 2016	Amsterdam 2017	Big Sandy 2017	Mean
Celebration	0.01±0.012^{a}	0.30±0.025^{a}	0.25±0.022^{a}	0.2±0.011
Champion	0.21±0.015^{bc}	0.78±0.042^{ac}	0.27±0.022^{ab}	0.38±0.015
Craft	0.09±0.008^{a}	0.46±0.032^{ab}	0.38±0.026^{ac}	0.28±0.013
Haxby	0.17±0.012^{ac}	0.76±0.043^{bc}	0.47±0.029^{bc}	0.42±0.016
Haybet	0.17±0.012^{ac}	0.90±0.052^{c}	0.24±0.017^{a}	0.36±0.015
Hockett	0.30±0.017^{c}	0.82±0.048^{c}	0.50±0.031^{c}	0.49±0.018
Lavina	0.13±0.010^{ab}	0.45±0.031^{ab}	0.25±0.016^{a}	0.25±0.01
Tradition	0.08±0.008^{a}	0.58±0.040^{bc}	0.28±0.024^{ac}	0.27±0.014
F-value	F$_{7,60}$ = 6.76	F$_{7,37}$ = 8.66	F$_{7,60}$ = 5.35	
P-value	<0.001	<0.001	<0.001	

Mean values with different letters are different (P<0.05) within a site according to Tukey HSD.

Table 1. 2 Mean (± SE) number of eggs per infested stem

Cultivar	Amsterdam 2016	Amsterdam 2017	Big Sandy 2017	Mean
Celebration	1.202±0.085^{ab}	1.523±0.064^{a}	1.469±0.075^{ab}	1.429±0.043
Champion	1.274±0.038^{ab}	2.038±0.064^{bc}	1.520±0.070^{ac}	1.669±0.037
Craft	1.026±0.015^{a}	1.728±0.065^{ab}	1.672±0.068^{bc}	1.564±0.039
Haxby	1.150±0.028^{ab}	2.089±0.070^{bc}	1.636±0.064^{bc}	1.681±0.038
Haybet	1.201±0.035^{ab}	2.349±0.088^{c}	1.335±0.048^{ab}	1.721±0.045
Hockett	1.345±0.038^{b}	2.261±0.081^{bc}	1.810±0.074^{c}	1.798±0.040
Lavina	1.157±0.031^{ab}	1.712±0.066^{ab}	1.230±0.037^{a}	1.376±0.029
Tradition	1.010±0.010^{a}	2.048±0.084^{ac}	1.487±0.083^{ab}	1.647±0.051
F-value	F$_{7,60}$ = 2.94	F$_{7,37}$ = 4.69	F$_{7,60}$ = 5.63	
P-value	0.01	<0.001	<0.001	

Mean values with different letters are different (P<0.05) within a site according to Tukey HSD.

Table 1. 3 Mean (± standard error of SE) percentage larval mortality by types of mortality

Cultivar	Type of mortality Dead in one internode	Parasitism	Other factors
Celebration	68.3 ±3.6^{b}	3.3±1.0^{a}	28.4±3.1^{b}
Champion	69.3±3.8^{a}	3.0±1.4^{a}	27.8±3.4^{c}
Craft	79.4±2.4^{d}	1.9±1.3^{a}	18.8±3.1^{a}
Haxby	60.4±4.2^{a}	3.0±1.1^{a}	36.5±4.2^{c}
Haybet	46.3±5.5^{b}	4.7±2.3^{a}	49±4.8^{b}
Hockett	37.2±4.4^{c}	4.7±2.3^{a}	58.1±3.8^{d}
Lavina	54.9±4.7^{b}	5.6±2.6^{a}	39.5±4.3^{b}
Tradition	56.3±4.8^{ab}	3.7±1.5^{a}	40±4.5^{bc}
F-value	$F_{7,102}$ = 20.06	$F_{7,102}$ = 2.0	$F_{7,102}$ = 16.87
p-value	<0.001	0.06	<0.001

Column with different letters are different (P<0.05) within type of mortality, using a Tukey HSD.

Table 1. 4 Number of stems cut by mature larvae and the number of stems collected at harvest by cultivar and site across 3 site*years.

Cultivar	Amsterdam 2016	Big Sandy 2017	Amsterdam 2017	Total sampled stems
Celebration	83 (297)[†]	15 (271)	19 (169)	854
Champion	96 (394)	69 (514)	51 (213)	1337
Craft	40 (493)	32 (447)	52 (304)	1368
Haxby	142 (474)	72 (559)	71 (283)	1601
Haybet	194 (206)	72 (571)	98 (244)	1385
Hockett	272 (270)	98 (463)	137 (286)	1526
Lavina	144 (481)	82 (659)	70 (319)	1755
Tradition	26 (348)	39 (292)	65 (133)	903
Total stems	3960	4255	2514	

[†] Numbers in parentheses is the sum of uninfested stems and infested stems with dead larvae.

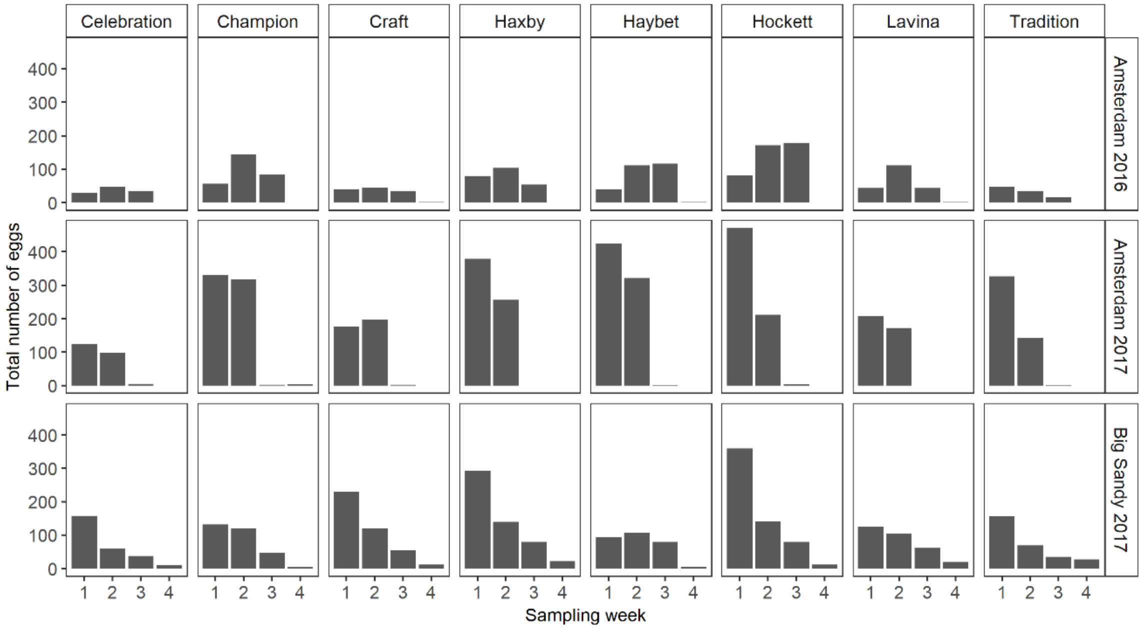

Figure 1. 1: Number of eggs by cultivar and site across sampling week over 3 site*years. Sampling week equivalents for **Amsterdam 2016** [1 = 59 days after seeding (DAS), 2 = 67 DAS, 3 = 73 DAS, 4 = 81DAS], **Amsterdam 2017** [1 = 51 DAS, 2 = 58 DAS, 3 = 65 DAS, and 4 = 72 DAS], and **Big Sandy 2017** [1 = 43 DAS, 2 = 50 DAS, 3 = 57 DAS, and 4 = 64 DAS].

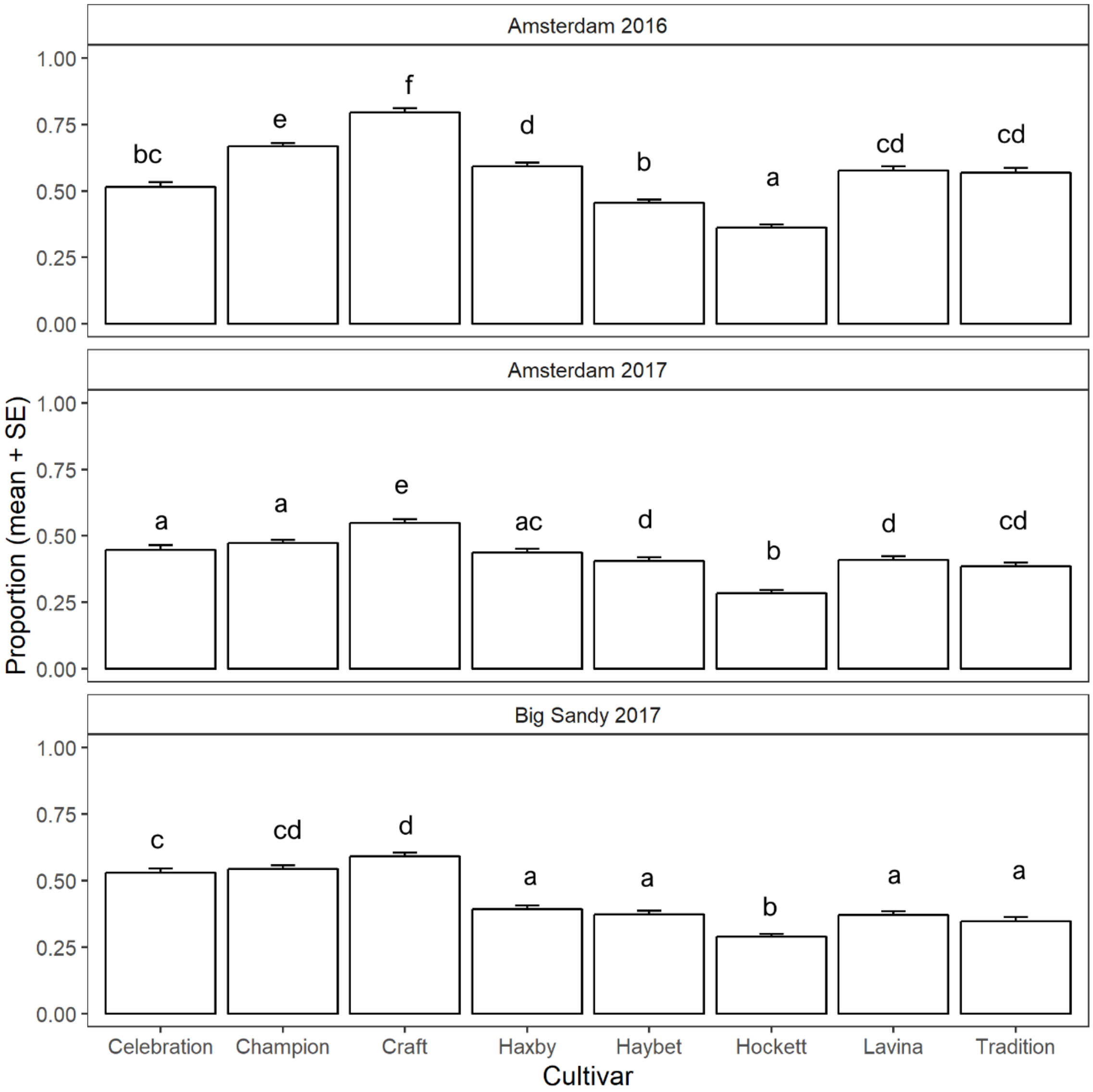

Figure 1. 2: Proportion (Mean + SE) of dead larva by cultivar and site for 3 site*years. **Amsterdam 2016** ($F_{7,60} = 19.44$, $P<0.001$), **Amsterdam 2017** ($F_{7,37} = 16.35$, $P<0.001$), and **Big Sandy 2017** ($F_{7,60} = 20.94$, $P<0.001$). Bars with different letters are different ($P < 0.05$) within a site*year using a Tukey HSD.

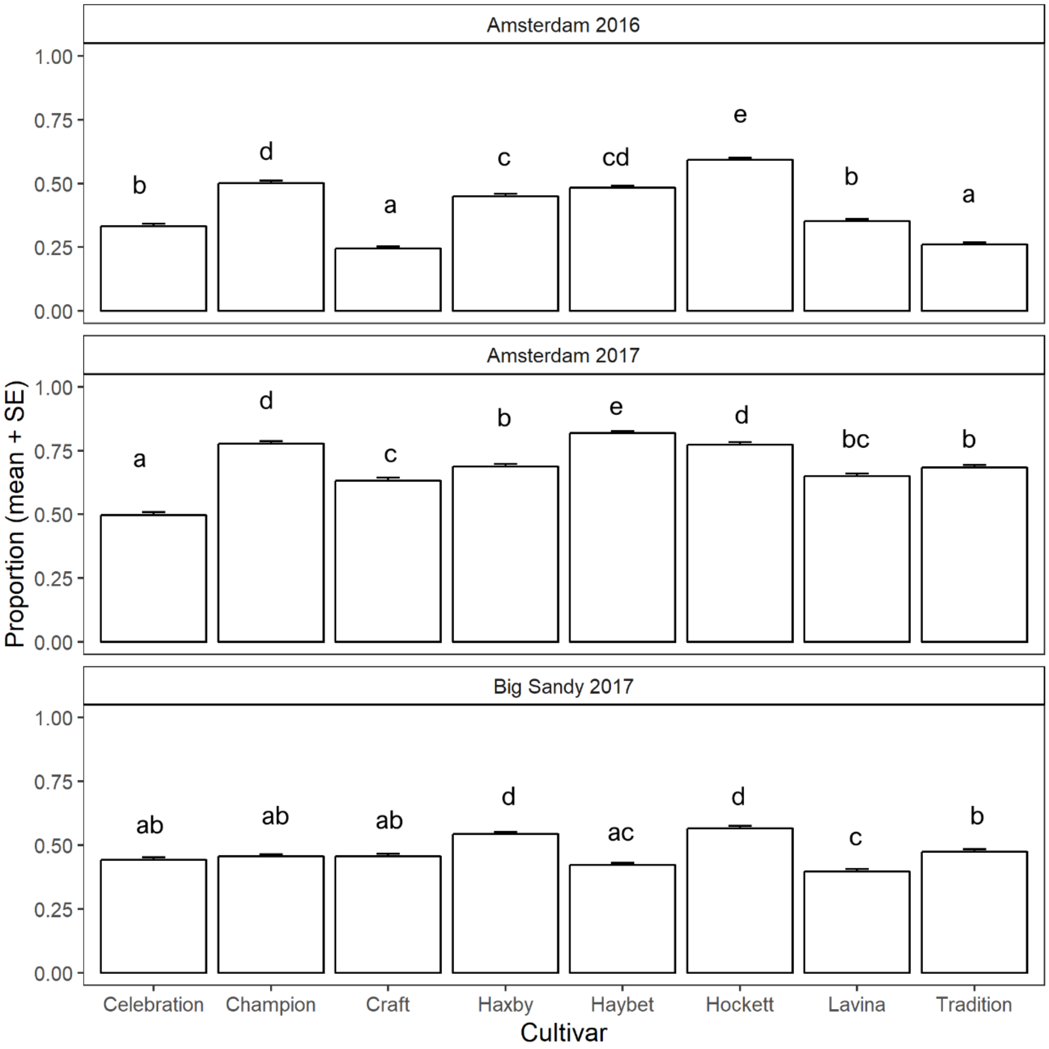

Figure 1. 3: Proportion of infested stems (mean +SE) by cultivar and site across 3 site*years in Montana. **Amsterdam 2016** ($F_{7,60}$ = 16.40, P<0.001), **Amsterdam 2017** ($F_{7,37}$ = 8.24, P<0.001), and **Big Sandy 2017** ($F_{7,60}$ = 8.81, P<0.001). Bars with different letters are different (P< 0.05) within a site, according to Tukey HSD.

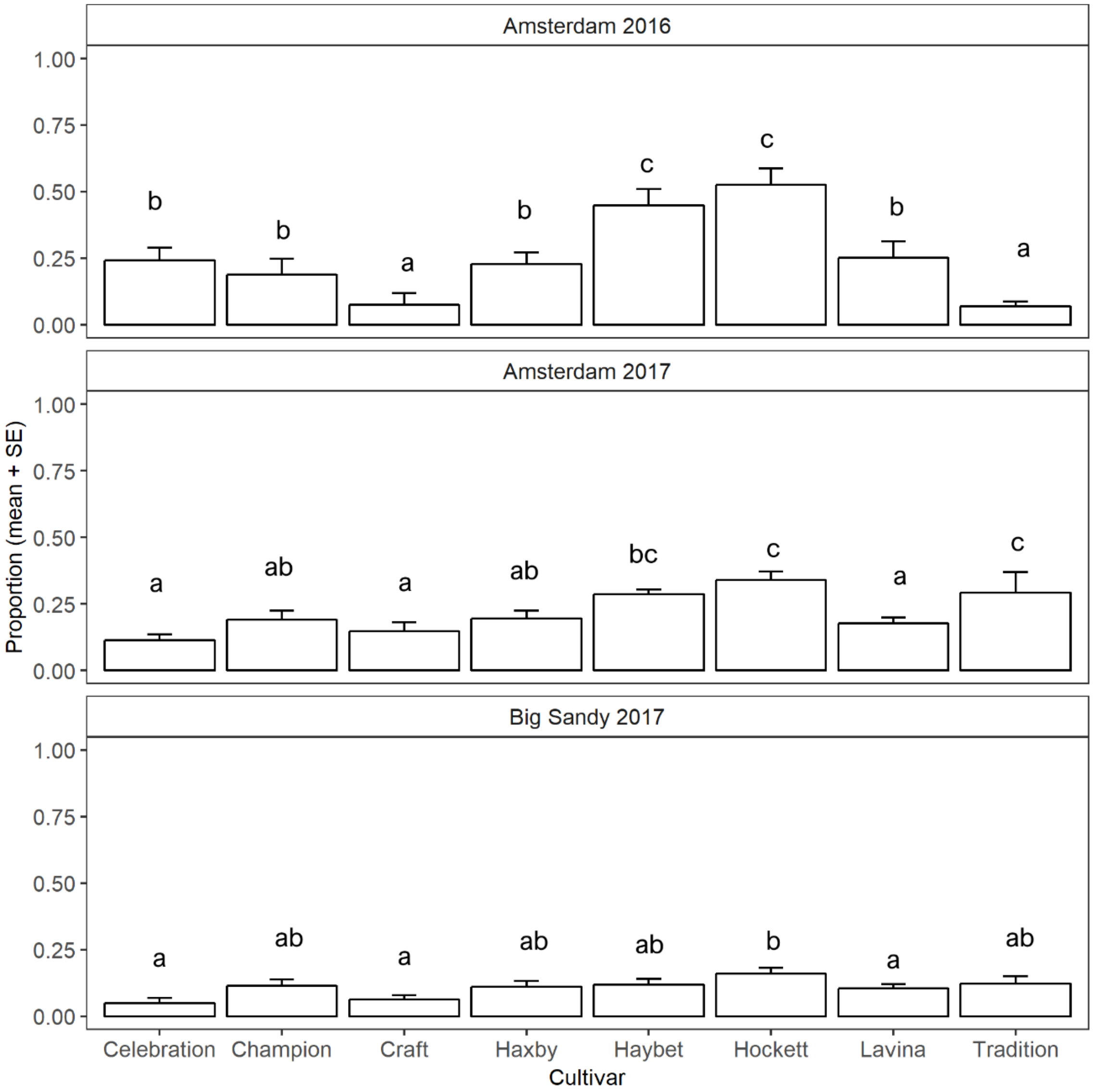

Figure 1. 4: Proportion of cut stems (mean +SE) by cultivar and site across 3 site*years in Montana. Amsterdam 2016 (F7,60 = 8.88, P<0.001), Amsterdam 2017 (F7,37 = 4.61, P<0.001), and Big Sandy 2017 (F7,60 = 2.69, P = 0.01). Bars with different letters are different (P < 0.05) within a site, according to Tukey HSD.

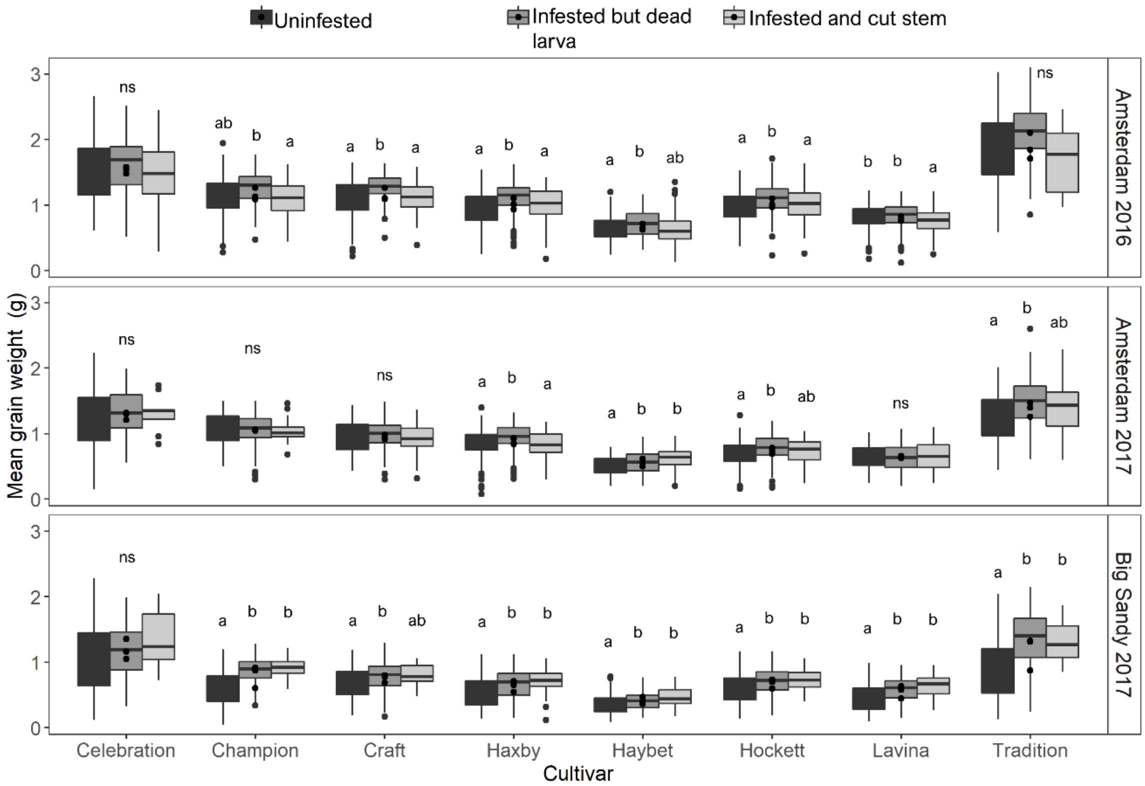

Figure 1. 5: Distribution of grain weight per head in gram by stem infestation status (uninfested, infested but dead larva, and infested and cut stems) by cultivar and site across 3 site*years in Montana. Middle line each box plot represents the mean within each infestation status. **Amsterdam 2016** (Celebration: lm, $F_{2,348}$ = 0.83, P = 0.44; Champion: lm, $F_{2,452}$ = 15.07, P <0.001; Craft: lm, $F_{2,510}$ = 14.03, P <0.001; Haxby: lm, $F_{2,570}$ = 32.60, P <0.001; Haybet: lm, $F_{2,282}$ = 5.21, P = 0.006 ; Hockett: lm, $F_{2,403}$ = 11.59, P<0.001; Lavina: lm, $F_{2,575}$ = 4.48, P = 0.01; Tradition: lm, $F_{2,357}$ = 9.57, P<0.001), **Amsterdam 2017** (Celebration: lm, $F_{2,174}$ = 1.05, P = 0.35; Champion: lm, $F_{2,237}$ = 0.08, P = 0.91; Craft: lm, $F_{2,334}$ = 1.95, P = 0.14; Haxby: lm, $F_{2,331}$ = 4.76, P = 0.009; Haybet: lm, $F_{2,296}$ = 7.58, P<0.001; Hockett: lm, $F_{2,369}$ = 6.17, P =0.002; Lavina: lm, $F_{2,360}$ = 0.80, P = 0.45; Tradition: lm, $F_{2,173}$ = 5.02, P = 0.007), **Big Sandy 2017** (Celebration: lm, $F_{2,270}$ = 3.2, P = 0.04; Champion: lm, $F_{2,549}$ = 102.77, P <0.001; Craft: lm, $F_{2,447}$ = 8.45, P<0.001; Haxby: lm, $F_{2,554}$ = 20.71, P<0.001; Haybet: lm, $F_{2,540}$ = 13.64, P<0.001; Hockett: lm, $F_{2,474}$ = 17.60, P<0.001; Lavina: lm, $F_{2,646}$ = 45.87, P <0.001; Tradition: lm, $F_{2,295}$ = 31.55, P <0.001). Bars with different letters are different (P < 0.05) within cultivar and within site, according to Tukey HSD.

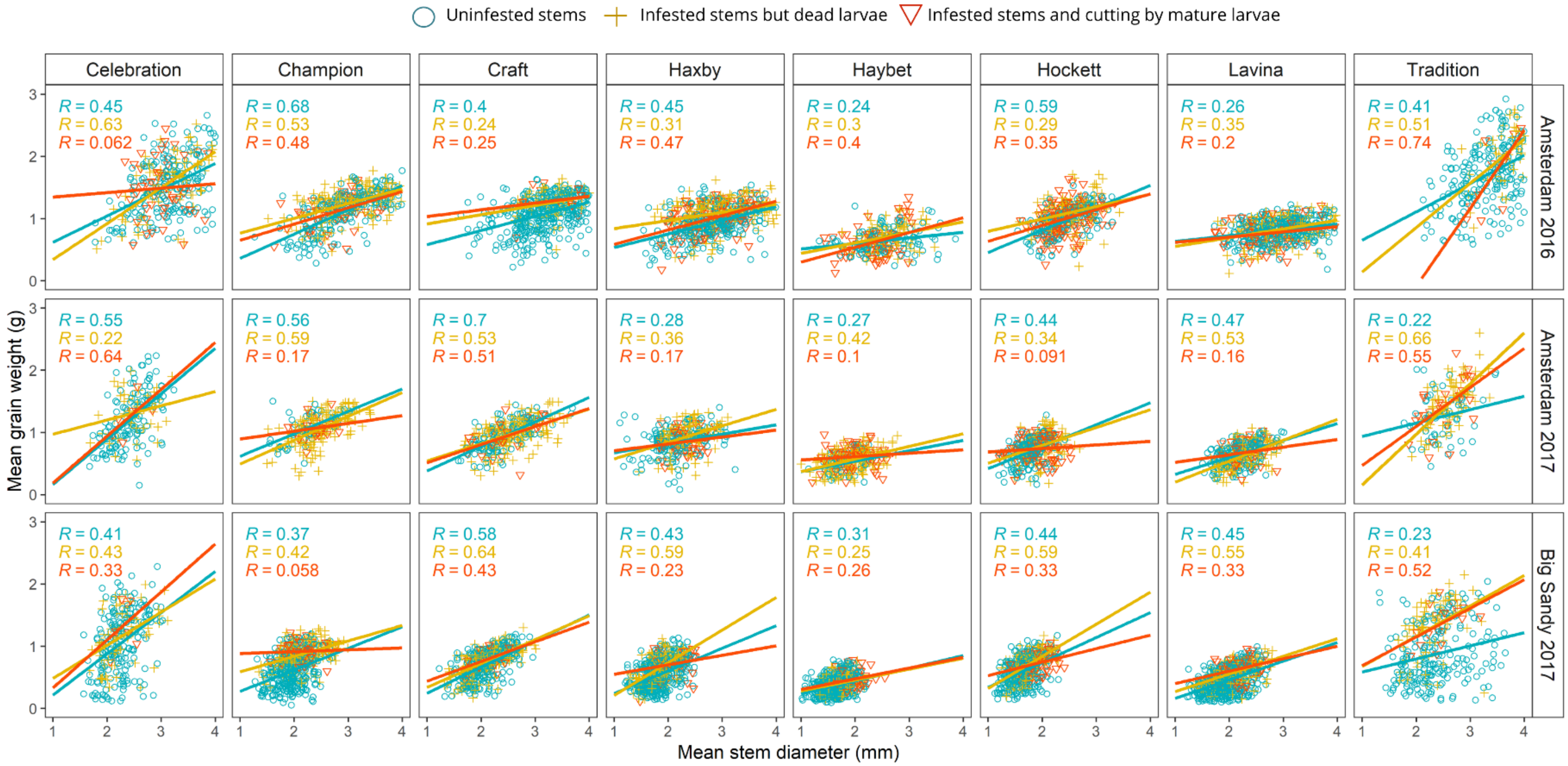

Figure 1. 6: Correlation of stem diameter and grain yield by stem infestation status (uninfested, infested but dead larva, and infested and cut stems) by cultivar and sites across 3 sites × years in Montana. R value indicate the correlation coefficient in respective cultivar under and are arrange by from top to bottom; first uninfested stems, second infested stems and dead larvae, and third infested and stem cutting by mature larvae.

CHAPTER FOUR

EFFECT OF PRECIPITATION AND TEMPERATURE ON LARVAL SURVIVAL OF *CEPHUS CINCTUS* (HYMENOPTERA: CEPHIDAE) IN BARLEY CULTIVARS

Contribution of Authors and Co-Authors

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Contributions: obtained funding for the project, supervised the work, assisted in preparation of the manuscript.

Manuscript Information Page

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Abstract

Host plant traits strongly affect survivorship of insect herbivores, and host suitability is especially important for the wheat stem sawfly (WSS), *Cephus cinctus* Norton (Hymenoptera: Cephidae), which spends its entire egg, larval, and pupal periods in a single stem. Measuring larval survival inside stems from egg hatch through diapause-mediated dormancy is a potential measure of population size for the next year but is also useful in assessing effects of growing season precipitation and temperature. Larval growth is synchronized with host plant growth, and the larva cannot switch hosts. Thus, incorporating plant physiological time, as growing degree days, may yield a better prediction of larval survival. Therefore, we assessed WSS survival from early larval growth to the beginning of autumnal diapause in barley cultivars selected from across feed, forage, and two- or six-row malt groups. Field experiments were conducted in Gallatin and Chouteau counties, Montana in 2016 and 2017. We used Kaplan-Meier estimation to assess larval survival among cultivars. We found that the survival of pre-diapause larvae was greatest in 'Hockett' (36.5%) and lowest in 'Celebration' (15.4%). Precipitation and temperature during the growing season affected temporal patterns for larval survival across study sites. Adjusting survivorship curves using site-specific growing degree day accumulation allowed cultivar-specific survivorship to be estimated more precisely for each site, despite differing environmental influences. Our findings suggest that measuring WSS survival across barley cultivars and standardizing by site-specific growing degree days may provide better recommendations on barley cultivars that impede WSS population growth and reduce economic losses.

Key words: mortality, survivorship curve, demography, *Hordeum vulgare*, Kaplan-Meier

Introduction

Wheat stem sawfly (*Cephus cinctus* Norton, hereafter 'WSS') is a major pest of wheat and barley in the Northern and Central Great Plains of the USA and the Canadian Prairie Provinces (Beres et al. 2011a, Cockrell et al. 2017). Losses have been estimated to be $44–80 million (USD) per year in Montana alone (Fulbright et al. 2017, Bekkerman and Weaver 2018). Localized host adaptation and movement of WSS from native grasses to spring wheat and subsequently to winter wheat (Morrill and Kushnak 1996, Lesieur et al. 2016) has occurred and is the main reason for spread of damaging populations of WSS (Lestina et al. 2016, Olfert et al. 2019). Similarly, greater WSS infestations in barley (*Hordeum vulgare* L.) have been recorded in Montana since 2005 (Varella et al. 2018). Since barley has historically been considered resistant to WSS (Criddle 1922, Callenbach and Hansmeier 1944), investigations that determine the reasons for an increase in WSS damage have not occurred, especially in relation to the duration of the larval stage.

Larvae of WSS negatively impact yields. They mine host stems, reducing plant vigor by reducing photosynthate assimilation, altering protein quality, and accelerating senescence (Macedo et al. 2005, Macedo et al. 2006, 2007). In addition, larvae cause stem lodging due to a v-shaped groove they make around the interior of the stem when undergoing obligate diapause. Lodged stems lying on the ground are more difficult, or even impossible, to recover at harvest.

For oviposition, females locate host plants that emit a higher concentration of attractants (Weaver et al. 2009), typically preferring varieties with taller (Buteler et al. 2009), and less solid (Varella et al. 2018), stems at an appropriate developmental stage (Holmes and Peterson 1960). However, oviposition in a suitable host does not guarantee successful larval establishment and survival. For example, female WSS cannot discriminate host plants that have already been infested by conspecifics (Buteler et al. 2009) and cannibalism results in the survival of a single larva (Buteler et al. 2015).

In addition to cannibalism, larval survival can also be reduced due to host plant traits (Buteler et al. 2015). Host plants upregulate the production of secondary metabolites in response to larval injury, which can reduce the larval survival. For instance, no WSS larval survival was reported in oat (Criddle 1923) and mortality approached 100% in barley (Farstad and Platt 1946). Reduced larval survival is likely to be due to constitutive defense compounds in host plants (Osbourn et al. 2003), but the specific compounds limiting WSS survival have not yet been identified.

In 2015 and 2016, cumulative mortality summed over larval instars varied among barley cultivars over locations in Montana, ranging from 88–95% in 'Craft' and 86–97% in 'Conrad,' to 51–74% in 'Hockett' (Varella et al. 2018). The causes for mortality observed include host plant resistance, cannibalism, and parasitism events that occur singly or in combination across a cohort. However, these levels of larval mortality were recorded only at the end of the growing season (Varella et al. 2018), which does not allow for temporal estimation of the pattern of larval survival in that cultivar. Temporal estimation allows better identification of the causes of mortality, such as plant resistance, cannibalism or parasitism. For example, larval mortality due to cannibalism cannot be accurately determined at the end of the growing season because cannibalism occurs at any time from first to final instar, but this can be reported when recording mortality factors in repeated sequential samplings.

In addition to time series mortality estimation, temperature and precipitation may influence the larval survival. For instance, extreme temperatures (> 38^oC) are lethal to WSS larvae and pupae in diapause (Seamans 1945, Robert 2006). Since larvae are inside the host stem, they might not experience similar temperatures even though the outside temperature is lethal. But high outside temperatures could have sublethal effects on larval survival across cultivars. In addition to temperature, precipitation might affect larval survival by influencing host plant traits. For instance, wheat stem pith development is negatively correlated with precipitation and the amount of pith is negatively correlated with larval survival (Beres et al. 2011b, Beres et al. 2017).

Temperature and precipitation are indirectly related to WSS survival but growing degree days (GDD) is a better predictor for WSS development (Beres et al. 2011b). Peak emergence of adult WSS varied by calendar week in field studies conducted from 2004–2006, but peak emergence was very similar in terms of GDD, ranging from 578–595 GDD over three years (Beres et al. 2011b). We do not know yet if there are interactions between growing season temperature or precipitation and larval survival across barley cultivars, let alone whether GDD calibrates larval survival across locations. In addition, no replicated experiments have been conducted to investigate temporal dynamics of WSS larval survival in association with rainfall patterns and temperatures in commonly grown barley cultivars. Therefore, in this study, we estimated larval survival rates in barley cultivars that are currently grown under rain-fed conditions in Montana. The objective was to estimate larval survival probabilities in barley while accounting for varying temperature and rainfall in the field. We assessed larval survival rates in barley cultivars across the entire growing season to determine 1) how WSS larval survival varied among cultivars and 2) if weather affected WSS larval survival within cultivars.

Materials and Methods

Field Location, Preparation, and Seeding

To estimate WSS larval survival rates in barley, observations were made in Montana over three site-years: Amsterdam in 2016 (45°45’27.3” N, 111°24’00.9” W) and 2017 (45°45’33.2” N, 111°23’50.0” W), and Big Sandy (48°15’42.1” N, 110°22’19.1” W). All sites were rainfed. For each site-year (Amsterdam #1 in 2016, Amsterdam #2 in 2017, and Big Sandy in 2017), we planted eight barley cultivars in the spring. The cultivars selected were currently grown for feed (Haxby and Champion), forage or hay (Haybet and Lavina), and malt (Tradition, Hockett, Craft, and Celebration). All the cultivars were two-rowed seeded heads except ‘Celebration’ and ‘Tradition’, which had six-rowed heads.

Each trial used a randomized complete block design. Every block was divided into eight plots and each plot was 1.8 × 3.6 m. The spacing between plots and between rows was 0.3-m. At the two Amsterdam sites, we randomly seeded each plot at nine g/m^2 using a self-propelled, seven-row seeder (Fabro Enterprises Limited, Swift Current, SK, Canada). At the Big Sandy site, seeding was conducted with a custom-built, six-row, self-propelled cone seeder with Atom Jet paired row openers. We finished seeding on April 11, 2016 at the Amsterdam #1 site and on April 20, 2017 at Big Sandy. However, seeding was not finished until May 3, 2017 at the Amsterdam #2 site because irregular germination of the initial planting in April required reseeding. Plots were manually weeded as needed to maintain a better crop stand.

Determination of Larval Density and Survival in Stems

Stem sampling began at stem elongation (Zadoks et al. 1974), which is the first stage when WSS infestation can occur. Stem elongation occurred 43–59 days after seeding (DAS) (Amsterdam #1, 2016: 59 DAS; Amsterdam #2, 2017: 51 DAS; and Big Sandy: 43 DAS). To select stems for sampling in each plot, we randomly selected a 0.3-m section of row by throwing a wooden measuring stick into the plot (30×2.5×2.5 cm) and uprooted all stems adjacent to the measured length. This procedure was repeated three times in each plot. All uprooted stems were placed in labeled paper bags with block number, plot number, cultivar name, and date of sampling. We collected 3 bags of samples per plot.

In the laboratory, from each sample bag, we randomly chose 35 primary stems (105 per plot) that were suitable for WSS infestation (with at least three nodes per primary stem). These were then dissected lengthwise along the entire length (Buteler et al. 2015). The dissected stems were examined visually to record the number of WSS immatures and the status of each WSS (either dead or alive). In total, we dissected 105 stems from each plot (35 per bag) each week from the first to eighth week of sampling. We selected only 35 primary stems from each bag to ensure that all dissected stems were not wilted or deteriorated, specifically because increased sample size prolonged the time required for dissection of all samples. If green stems were stored more than three days, the larvae might die in storage and incorrectly inflate the record of larval mortality. At crop maturity, we dissected all mature stems (no longer green) in harvest samples taken in the ninth week of sampling.

Collection of Rainfall and Temperature Data

We retrieved daily weather (rainfall and temperatures) data from the weather stations nearest to our field sites (Amsterdam #1 and #2: 7.88 km and Big Sandy: 1.77 km) (Supp. Figures S1–S3). Archived data were retrieved 10 March 2018 from an online database (https://www.wunderground.com). For each location, we calculated the average maximum temperature (Tmax), average minimum temperature (Tmin), and total precipitation for each week of sampling from the archived data.

Statistical Analysis

We compared Tmax, Tmin, and total precipitation by sampling weeks and experimental locations using analysis of multivariate data and repeated measures design (MANOVA). To compare the overall impact of Tmax, Tmin, and total precipitation on proportion survival, we used a regression model created using the package car (Fox and Weisberg, 2019) in R version 3.4.4 (2017).

To better account for the impact of Tmax and Tmin on larval survival proportion, we calculated growing degree-days (GDD) from seeding to harvesting date at each location. For this, we used 0^{o}C as a base temperature for barley (Miller et al. 2001) where:

$$GDD = \frac{Daily\ Maximum\ Temperature + Daily\ Minimum\ Temperature}{2} - Developmental\ Threshold$$

In addition, we visualized weekly total precipitation and survival proportion by sampling week using ggpubr (Kassambara 2018) and ggplot2 (Wickham 2016). To assess the impact of GDD and total precipitation on larval survival, we conducted principal component analysis (PCA) using FactoMineR (Le et al. 2008) and used Factoextra (Kassambara and Mundt 2017) to extract and visualize results.

We subsequently used Kaplan-Meier estimation (Kaplan and Meier 1958) to calculate larval survival probability by cultivar and experimental site. This estimator is a non-parametric approach to estimating survival rate. To estimate a cultivar specific survival rate, we determined the mortality event if dead and censored WSS larvae if alive at the time of stem dissection. We then determined median survival probability and final (pre-diapause) survival probability using GDD by locations and cultivars. In addition, pairwise comparisons were made between the cultivars using a Log-Rank test to compare pre-diapause survival probability between two cultivars. We used the packages dplyr (Wickham et al. 2019), prodlim (Gerds 2018), survival (Therneau 2015), and survminer (Kassambara and Kosinski 2018) for analysis and data presentation. All data analysis and graphing were done in R version 3.4.4 (2017).

Results

Weather Parameters and Larval Survival

Average maximum temperature (Tmax), average minimum temperature (Tmin), and total precipitation differed with sampling week (Wilks' Lambda = 0.27; df = 8, 205; $P < 0.001$) and location (Wilks' Lambda = 0.92; df = 2, 205; $P = 0.009$). Overall WSS larval survival was best explained by a regression model with Tmax, Tmin, and total precipitation as dependent variables (F = 17.36; df = 3, 212; $P < 0.001$) (Table 1). However, the individual weather parameters (either total precipitation, Tmax, or

Tmin), one by one, accounted for less than 5% each of the total variation. Increased weekly total precipitation increased subsequent larval survival relative to weeks without precipitation events (Fig. 1). However, the combination of Tmax and Tmin, expressed as degree days, had a stronger influence on the larval survival rate. There was a strong negative correlation between cumulative GDD and larval survival and a weaker, but positive correlation between total precipitation and survivorship proportion (Supp. Figure S4).

Pre-diapause Survival by Cultivar

The larval records at harvest were used to estimate survival at crop maturity, when larvae were preparing to overwinter. The number of larvae remaining in the cohort at the end of sampling varied by cultivar, from 2,873 (Celebration) to 5,348 (Hockett) (Table 2). Overall, pre-diapause survival varied by cultivar, with the highest survival rate of 36.2% (34.2–38.8) in Hockett and the lowest of 15.4% (13.3–17.6) in Celebration (Table 2). All the assessed cultivars were different in pairwise comparisons of larval survival except for the pairs of ‘Craft’ and ‘Celebration’, and of ‘Tradition’ and ‘Haxby’ (Table 3).

Survival by Cultivar and Location

Median larval survival (50%) occurred at different times in different cultivars. At Amsterdam #1, in 2016, WSS larvae in ‘Hockett’ reached median survival at crop maturity, at 1700 GDD (Fig. 2), whereas in ‘Celebration’, ‘Tradition’,’ Haxby’, ‘Haybet’, and ‘Lavina’ median survival occurred earlier, at 1550 GDD. The most rapid pattern of mortality occurred in ‘Champion’ and ‘Craft’, with median survival occurring at 1400 GDD (Fig. 2).

At Amsterdam #2, in 2017, median survival was recorded at two time points (Fig. 3) across cultivars. For ‘Haybet’ and ‘Hockett’ 50% larval survival occurred late, at crop maturity (1900 GDD), while larval survival in all other cultivars reached median mortality sooner, at 1600 GDD (Fig. 3).

For Big Sandy in 2017, there were three time points of median larval survival by cultivar (Fig. 4). For ‘Celebration’ and ‘Craft’ median survival occurred at 1350 GDD, whereas for ‘Champion’, ‘Haxby’, ‘Haybet’, and ‘Tradition’ it occurred at slightly more than 1500 GDD. Finally, in ‘Hockett’ and ‘Lavina’, median larval survival occurred at crop maturity at 1600 GDD (Fig. 4).

Discussion

The survival of WSS larvae varied by barley cultivar across experimental sites. Our assessment of varietal effects of WSS was based on only pre-diapause larvae at the end of the feeding period. These larvae are the source of adults of the WSS population the following year (Peterson et al. 2011, Rand et al. 2017). This variable is a useful estimator of intergenerational WSS population trends. It may not possible, using current technology, to determine temporal sublethal effects that might be carried over from the harvest to the following spring by sampling larvae during winter diapause. We found that individual weather parameters representing weather extremes (such as Tmax, Tmin, and total precipitation) had a smaller impact on temporal larval survival than accumulated degree-days (Table 2, Fig. 1, Supp. Figure S4). Although actual lethal temperatures for larvae feeding in growing stems are unknown, soil temperatures over 38°C can be lethal to WSS larvae in stubs, where diapausing larvae overwinter (Seamans 1945). In addition, air or soil temperature may not adequately represent temperatures that larvae experience while feeding in stems because of shading by adjacent tillers and buffering by the stem itself. During research at these sites, air temperature never exceeded these lethal levels (Supp. Figures S1–S3). Similarly, the differences between canopy temperature and air temperature recorded typically range from –4°C to +3.8°C in wheat (Neukam et al. 2016). For example, at 47°C canopy temperature, the temperatures inside and on the surface of the stem were measured at 39.9 ± 1.8°C and 41.5 ± 1.3°C, respectively, at a stem height of 42 cm; while these were 25.4 ± 0.6°C and 25.5 ± 1.7°C, respectively, at the soil surface in a greenhouse (Robert 2006). These records support that WSS larvae inside the stem experience lower temperatures than the surrounding air. Thus, we expect that canopy temperatures experienced by feeding larvae were below lethal levels for all site-years. In the absence of extreme temperatures, we found only a minimal influence of daily temperature on larval survival. However, when we calculated degree-days, we found a stronger relationship for larval survival across cultivars.

We found that the pre-diapause larval survival was lowest in ‘Celebration’ (15%) and highest in ‘Hockett’ (36%) (Table 2) and larval survival varies across cultivars. Varella et al. (2018) found survival ranged from 40–60% in the resistant wheat variety ‘Choteau’ and from 46–85% in the more susceptible wheat variety ‘McNeal’, while for observations in paired barley fields (cropped to either Craft, Conrad, Harrington, or Hockett), survival ranged from 0 to 49%.

Previous data emphasize a major role of host plant traits such as stem solidness for larval mortality in wheat (Buteler et al. 2015, Varella et al. 2018). However, all barley cultivars reported in the earlier field monitoring of Varella et al. (2018) or in our experimental plots have hollow stems, suggesting that larval mortality is due to factor(s) other than stem architecture. The barley cultivars we evaluated can be categorized into three groups based on end-use: feed, malt, and forage and can also differ based on fertile spikelet rows: either two-rowed or six-rowed. These differences in intended end use may give rise to differing composition of structural and secondary metabolite profiles in stems. Two-row barley varieties produce more tillers than six-row barley, but average stem diameter is greater in six-row than in two-row cultivars (von Bothmer and Komatsuda 2011). In wheat, WSS females prefer to oviposit in taller plants (Buteler et al. 2010) with wider stems (Buteler and Weaver 2012), so these same factors may be used in determining suitability of barley hosts.

Rainfall could also play a role in larval survival because the week after rainfall, the larval survival rate increased relative to weeks without rainfall events. For example, at Big Sandy and at Amsterdam #2 in 2017, it was relatively dry after the fourth sampling week, while at Amsterdam #1 in 2016 drought started after the seventh sampling week (Supp. Figure S1). Differences in precipitation at different crop stages could alter the stem composition and affect larval survival. Specific precipitation patterns during the wheat growing season can reduce pith development in solid stem wheat cultivars, increasing larval survival and subsequently increasing the proportion of cut stems (Beres et al. 2017). All barley cultivars have hollow stems, but we observed that precipitation had a variable effect on larval survival. Figure 1 indicates two patterns of precipitation and larval survival when precipitation was greatest in the sixth sampling week in 2016, while precipitation was greatest in the first and third sampling weeks (early growth stages) at Amsterdam and Big Sandy, respectively, in 2017. A change in the plotted survival rate occurred after the largest precipitation event at Amsterdam #1 in 2016. Further study is needed to determine the impact of precipitation on stem chemical composition, especially in relation to larval survival and diet. For instance, as suggested by the findings of Beres et al. (2017), later precipitation events may also play a role in increased survival in solid-stem wheat that is not due to decreased pith expression.

Even though the growing conditions for both fields in Amsterdam were similar, the cultivars did not show a similar pattern in terms of larval survival (Figure 2 and 3). In assessing for a year effect, the duration of crop growth may play a role in the survival rate. The duration of crop growth was 140 days in 2016 and only 110 days in 2017. The trade-off between growth and defense in plants (Stamp 2003, Barton 2007) suggests that shorter growth periods tend to result in greater photosynthetic assimilate allocation to growth and development rather than defense against herbivory, which our data support. Additionally, the growing season at Amsterdam was 30 days shorter in 2017 due to improper germination at initial planting requiring a later reseeding date. The shorter crop duration, coupled with greater average temperature, increased GDD at Amsterdam 2017 compared to 2016. The differing GDD patterns for Amsterdam in 2016 and 2017 support that larval survival changes in response to growing conditions because these two experimental sites are so much closer to each other than to Big Sandy. Thus, uneven growth patterns might change the overall response of each cultivar to herbivory, which then altered larval survival.

The differing patterns observed in larval survival could be associated with specific defenses in the barley cultivars or possibly represent a deficiency in nutrients essential for larval growth and development – or both. Considering these factors was beyond the scope of this study. All our trial areas were on rainfed farms, so our study may provide a foundation to explore nutrient ecology for WSS larval growth in barley under rainfed conditions and help to predict future WSS population dynamics.

For WSS larvae, final survival at the onset of diapause is the key factor affecting the rate of population growth for the next year because of the univoltine life cycle. Our results suggest that WSS larvae in ‘Hockett’ had almost twice the final overall survival compared to larvae in ‘Craft’ and ‘Celebration’ from the same cohort (Table 2). This is the first study rigorously assessing WSS larval survival in barley cultivars. Our work opens an area of study where predicting WSS populations in barley fields sown to particular cultivars is possible, with known weather patterns. Our findings suggest ‘Hockett’ is the most suitable host for WSS populations in barley, an important consideration for Montana barley producers, because this cultivar is the third leading malting barley (AMBA, 2020). Our recommendation is to plant a barley cultivar with lower larval survival in areas where highly damaging populations of WSS are evident.

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Table 2. 1: Regression statistics for WSS larval survival in relation to temperature (Tmax and Tmin) and total precipitation

Variables	Parameter	Estimate	Standard Error	Partial R^2
	Intercept	1.279	0.177	
Tmax	β_1	-0.015	0.008	0.014
Tmin	β_2	-0.023	0.013	0.013
Total precipitation	β_3	0.003	0.002	0.014

Table 2. 2: End of season survival of pre-diapause larvae in barley. The data in parenthesis represent the 95% confidence interval

SN	Cultivar	% Survival	*n*
1	Celebration	15.4 (13.3–17.6)	2873
2	Champion	16.6 (15.0–18.1)	4684
3	Craft	15.9 (14.2–17.5)	3364
4	Haxby	18.5 (16.8–20.3)	4711
5	Haybet	32.2 (30.1–34.3)	4574
6	Hockett	36.5 (34.2–38.8)	5348
7	Lavina	24.3 (22.1–26.4)	3741
8	Tradition	20.2 (17.7–22.7)	3151

Table 2. 3: Pairwise comparison of final survivorship rate of larvae

	Celebration	Champion	Craft	Haxby	Haybet	Hockett	Lavina	Tradition
Celebration	—	3.91	-2.42	0.81	4.71	15.70	12.90	-0.80
Champion	0.003	—	-6.33	-3.10	0.80	11.79	8.99	-4.71
Craft	0.60	<0.001	—	3.23	7.13	18.13	15.33	1.62
Haxby	<0.001	0.013	<0.001	—	3.90	14.90	12.09	-1.61
Haybet	<0.001	<0.001	<0.001	<0.001	—	10.99	8.19	-5.51
Hockett	<0.001	<0.001	<0.001	<0.001	<0.001	—	-2.80	-16.50
Lavina	<0.001	<0.001	<0.001	<0.001	0.012	<0.001	—	-13.70
Tradition	<0.001	<0.001	<0.001	0.048	<0.001	<0.001	<0.001	—

Estimates for the difference in final survivorship rates of larvae for pairs of cultivars are above the diagonal dash line and the corresponding *p*-value is below the diagonal line of dashed entries. Differences are calculated by subtracting final survivorship rates for cultivars listed vertically from those listed horizontally. A negative value means that final survivorship is greater for the upper listed cultivar.

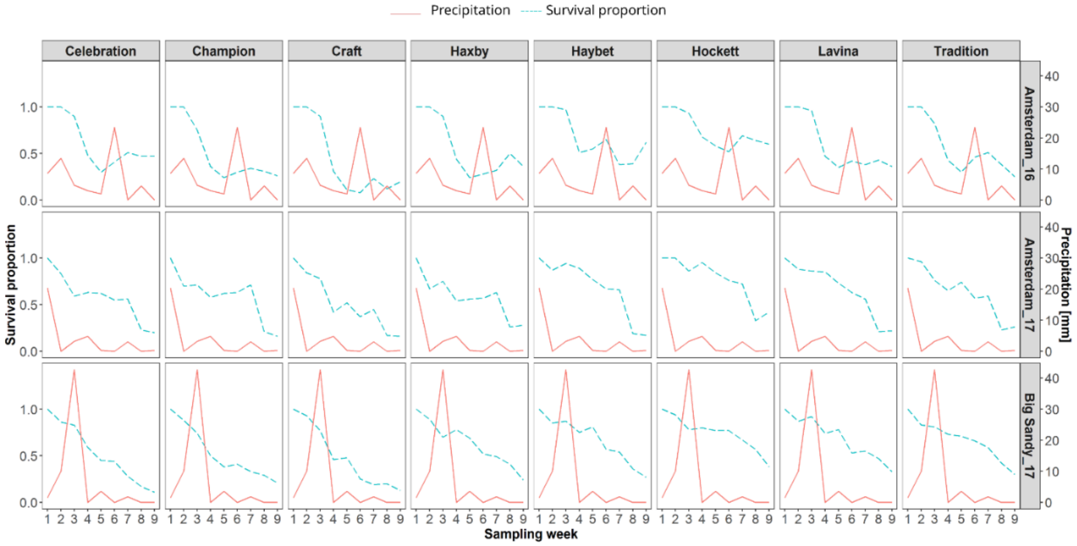

Figure 2. 1: Relation between larval survival and total precipitation, divided by sampling week, for each cultivar and location. Sampling week equivalents are as follows: Amsterdam #1, 2016 [1 = 59 days after seeding (DAS), 9 = 115 DAS; Amsterdam #2, 2017 [1 = 51 DAS, 9 = 114 DAS); and Big Sandy [1 = 43 DAS, 9 = 99 DAS).

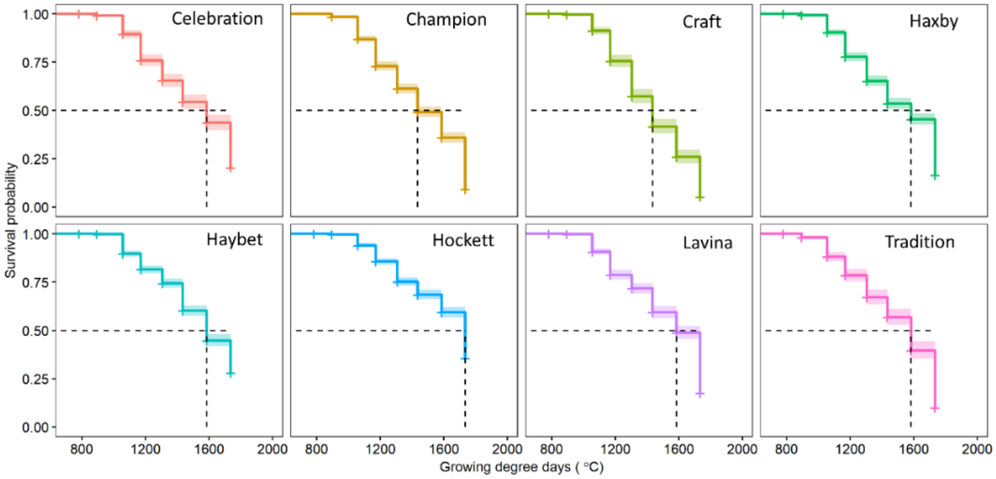

Figure 2. 2: Survival probability of wheat stem sawfly in different cultivars barley in Amsterdam #1, MT, 2016

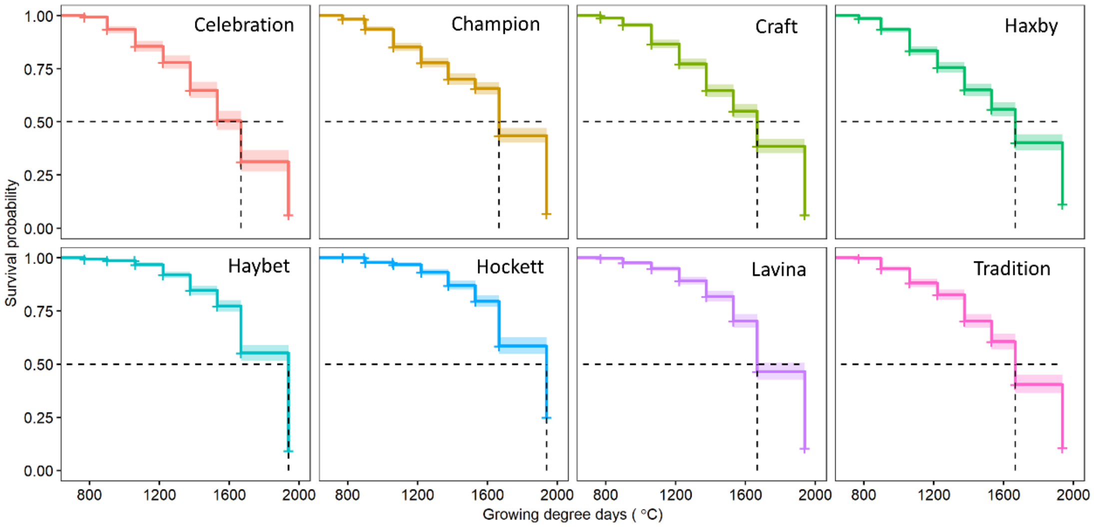

Figure 2. 3: Survival probability of wheat stem sawfly larvae in barley cultivars in Amsterdam, MT, 2017

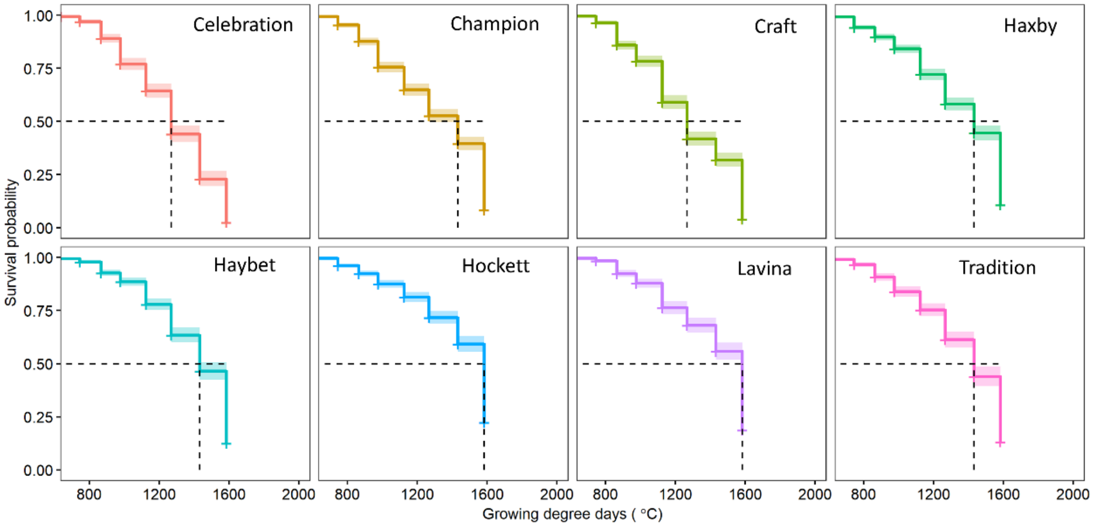

Figure 2. 4: Survival of wheat stem sawfly larvae in barley cultivars at Big Sandy, MT, 2017

CHAPTER FIVE

MULTIPLE DECREMENT LIFE TABLES OF *CEPHUS CINCTUS* NORTON (HYMENOPTERA: CEPHIDAE) ACROSS A SET OF BARLEY CULTIVARS: THE IMPORTANCE OF PLANT DEFENSE VERSUS CANNIBALISM

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Manuscript Information Page

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Abstract

Accurately estimating cause-specific mortality for immature insect herbivores is usually difficult because feeding stages are exposed to abiotic and biotic mortality factors, causing cadavers to simply disappear before cause of mortality can be recorded. Also, herbivores are often highly mobile on hosts, making it difficult to follow patterns for individuals through time. In contrast, the wheat stem sawfly, *Cephus cinctus* Norton, spends its entire egg, larval, and pupal period inside a host stem. Therefore, with periodic sampling stage-specific causes of mortality can be ascertained. Consequently, we examined *C. cinctus* mortality in eight barley, *Hordeum vulgare* L., cultivars in two locations in Montana from 2016 to 2018 by collecting stem samples from stem elongation to crop maturity at weekly intervals, and collecting overwintered barley stubs the following spring and summer from the same plots. If larvae were present, we examined larval status—dead or alive—and categorized dead individuals into one of 5 mortality categories: plant defense, cannibalism, parasitism, pathogens, and unknown factors. We used multiple decrement life tables to estimate cause-specific mortality and irreplaceable mortality (the proportion of mortality from a given cause that cannot be replaced by other causes of mortality). Plant defense (antibiosis) caused 85.7 ± 3.6%, cannibalism (governed by antixenosis) caused 70.1 ± 7.6%, parasitism caused 13.8 ± 5.9%, unknown factors caused 38.5 ± 7.6%, and pathogens caused 14.7 ± 8.5% mortality in the presence of all causes of mortality. Similarly, irreplaceable mortality due to plant defense was 22.3 ± 6.4%, cannibalism was 29.1± 4.2%, unknown factors was 6.2 ± 1.8%, pathogens was 0.9 ± 0.5%, and parasitism was 1. 5 ± 0. 6%. Antibiosis traits primarily killed newly emerged larvae, while other traits supported more favorable oviposition decisions by females, increasing mortality by obligate cannibalism. Our results suggest that breeding barley for resistance to *C. cinctus* is a highly valuable tactic for management of this important pest.

Key words: cannibalism, host plant resistance, irreplaceable mortality, replaceable mortality

Introduction

Wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), successfully colonizes ancestral wild grass hosts through domesticated spring and winter wheat cultivars (Morrill and Kushnak 1996). In addition, this species is not only widening its range southward to Colorado and Kansas (Lesieur et al. 2016), but it also is increasing infestation and larval survival in barley in Montana (Varella et al. 2018, Achhami et al. 2020 accepted: Journal of Economic Entomology). To mitigate economic loss, an integrated pest management (IPM) approach has been recommended for wheat across the northern Great Plains (Beres et al. 2011a). However, to further formulate and implement IPM tools against this pest, it is crucial to estimate the mortality of *C. cinctus* across life stages with other crop species in multiple environments.

Cephus cinctus is a univoltine insect. In spring, when plant hosts are at the stem-elongation stage, females lay eggs in suitable host stems. Approximately one week after oviposition, a larva emerges. The larva feeds primarily on the parenchymous tissues of the stem until the host plant reaches maturity. Once the host plant ripens and desiccates, the larva moves to the base of the stem, makes a v-shaped groove by chewing a uniform gouge around the stem interior, and plugs the stem below this groove with frass and plant materials to make a hibernaculum. The v-shaped groove contributes to stem lodging when gravity and wind impact the ripened stem. The remaining part of the lodged stem that is intact in the soil and contains an overwintering larva is called a “stub.” Within this stub, the larva moves close to the root crown where it hibernates and spends 8−9 months as a prepupa. The following spring, and after sufficient warming, the hibernated prepupa fully metamorphoses before it chews through the frass plug in the stub and emerges as an adult (Church 1955, Holmes 1975).

The probability of death of an individual at each developmental stage due to cause-specific mortality can be estimated using a multiple decrement life table (Carey 1989). The multiple decrement life table mathematically accounts for a number of mutually exclusive causes of mortality. In addition, the multiple decrement life table can be used to estimate the contribution of each cause of mortality in the presence and absence of other mortality causes.

Parasitism, host plant resistance, and cannibalism are three major causes of mortality of *C. cinctus* in wheat (Buteler et al. 2015). However, the mortality due to parasitism varies by locations and by host types (Runyon et al. 2002, Peterson et al. 2011, Buteler et al. 2015). These three major causes of mortality act on different stages of *C. cinctus* larvae. For instance, host plant defense kills mostly neonates, at an early stage of development, while cannibalism and parasitism occur in instars larger and later than neonates. The larvae that survive from the plant defense mechanisms are still vulnerable to parasitism, and more vulnerable to cannibalism if multiple live larvae inhabit a stem. Thus, cannibalism and plant defense are important factors influencing for *C. cinctus* fitness at both the individual and population levels. However, the role and importance of these three causes of mortality for *C. cinctus* developing in a barley host is yet to be determined.

Although barley was historically categorized as resistant to *C. cinctus*, a decade-long study revealed an increasing infestation rate and larval survival rate of *C. cinctus* in barley in Montana (Varella et al. 2018). Montana ranked second among barley producing states based on the amount of production in the US in 2019 (USDA 2020).

Growing solid-stem cultivars and swathing are two tools that can mitigate economic loss incurred by *C. cinctus* in Montana wheat crops (Bekkerman and Weaver, 2018). However, both management tactics are not implementable for barley because all currently cultivated barley cultivars have hollow stems and swathing can substantially reduce grain quality, especially in malt barley. In Montana, malt barley was 61.8% of total barley production by hectare in 2019 (AMBA 2020).

To reduce the increasing economic loss caused by *C. cinctus* in Montana, estimating age- and cause-specific mortality in populations is crucial. Cannibalism and host plant resistance are two major causes of mortality in wheat (Buteler et al. 2015). Both causes of mortality are mediated by host plant traits. For instance, cannibalism ultimately occurs because the host plants release an attractive compound like (*Z*)-3-hexenyl acetate (Piesik et al. 2008, Weaver et al. 2009) and (*E*)-and (*Z*)-β-ocimene (Buteler and Weaver 2012). These, along with other physical characters, stimulate the abundant gravid females to lay numerous eggs in a stem at high population densities (Buteler et al. 2009, Buteler et al. 2012). In wheat, stem solidness obstructs larval movement and most of the larvae eventually die (Holmes 1954, Talbert et al. 2014). The pith also plays a role in maternal oviposition decisions in stems with varying solidness (Varella et al. 2017). Therefore, estimating causes of mortality by barley cultivars will provide a foundation to estimate cultivar-specific mortality and may allow for the development of cultivars that cause consistently greater levels of larval mortality. Consequently, in this study, we estimated age- and cause-specific mortality of *C. cinctus* in eight barley cultivars (Celebration, Champion, Craft, Haxby, Haybet, Hockett, Lavina, and Tradition) at two locations in Montana.

Materials and Methods

Field Site, Field Preparation, and Seeding

To estimate *C. cinctus* mortality in barley, we conducted a field study in Montana near Amsterdam in 2016 (45°45’27.3” N, 111°24’00.9” W) and 2017 (45°45’33.2” N, 111°23’50.0” W), and near Big Sandy in 2017 (48°15’42.1” N, 110°22’19.1” W). In each site-year, we planted eight barley cultivars in the spring. The cultivars were ‘Haxby’ and ‘Champion’ (feed barley); ‘Haybet’ and ‘Lavina’ (forage or hay barley); and ‘Celebration,’ ‘Craft,’ ‘Hockett,’ and ‘Tradition’ (malt barley). All cultivars were two-rowed seeded heads except ‘Celebration and ‘Tradition’, which had six-rowed heads.

Each study used a randomized complete block design. The study field was divided into three blocks based on field variability due to slope. Each block was divided into eight plots and each plot was 1.8 m × 3.6 m with a 0.3-m spacing between each plot. The seeding rate was 9 g/m^2. We performed several manual weeding operations to maintain a better crop stand.

Summer Samples

Stem sampling was conducted as described previously (Achhami et al. accepted: Journal of Economic Entomology). Briefly, we began 59 days after seeding because more than 50% of plants were at stem elongation (Zadoks et al. 1974), the growth stage of the host plant when *C. cinctus* female begins to lay eggs. Sampling continued to crop maturity; in total, we collected for 9 consecutive weeks. For each sampling week, we randomly measured a 0.3-m row length in each plot, uprooted all the plants within the measured length, and wrapped the uprooted plants in a uniquely labelled paper bag. We repeated this uprooting procedure 3 times within a plot; so that each plot had 3 bags of samples per sampling week. Subsequently, we dissected the collected samples lengthwise and, if infested, recorded the status of *C. cinctus* by counting eggs and dead or live larvae. We selected only 35 primary stems from the samples (the samples that we collected until the 8th week) because all samples could not be dissected on the day they were collected. Unprocessed samples were stored in a 4^{o}C storage room and we dissected them as rapidly as possible on the following day to prevent wilting and to maintain larval status as when collected. However, we dissected all ripened stems from the harvest samples collected at crop maturity (9th week of sampling).

Spring and Summer Stub Samples

We collected stub samples from each plot where we collected the stem samples during the previous summer. We collected stubs twice, at both before and after the flight period of adults. Once again, these stubs are the remining lower part of a cut stem where the hibernating larva is located.

The first collection of stubs was conducted in the first week of the following April or May (Amsterdam 2016: 4 April 2017; Amsterdam 2017: 28 April 2018; Big Sandy 2017: 3 May 2018). In mid to late May through early July, female adults emerge, mate, and begin laying eggs. Therefore, we named these collected samples "pre-emergence" or "pre-flight period." For stub collection, we divided each plot into two equal sections. From the first half of each remaining plot area, we uprooted all the stubs and wrapped these inside a uniquely labelled paper bag. Thereafter, we randomly selected at least 25 stubs that contained hibernaculae with diapaused larvae from each plot and recorded the status, dead or alive, of the overwintered larvae after dissection. We selected 25 stubs to maintain uniform sample size across all cultivars and sites. Additionally, we categorized the dead larvae by mortality causes, including parasitism, pathogens, or unknown factors.

Next, in July (Amsterdam 2016: 10 July 2017; Amsterdam 2017: 12 July 2018; Big Sandy 2017: 20 July 2018), we collected the remaining stubs from each plot which we considered “post-emergence” samples. Then, we again randomly separated 25 stubs per plot, dissected them and recorded the status as either emerged or dead. For assessing potential emergence, we additionally considered whether the top part of the stub, the frass plug, had only a single emergence hole and did not have any indicators of larval or pupal mortality, such as a cadaver or more correctly, fragments of a cadaver, or a parasitoid cocoon. Otherwise, we recorded the life stage as dead by the appropriate cause of mortality.

Causes of Mortality

As described, we collected samples for 3 stages of *C. cinctus* development that occur within a stem: summer samples (pre-diapause period), overwintered (pre-flight period), and post emergence (at the end of the flight period). After determining the status, we categorized the death of an individual by one of the following mortality causes: cannibalism, plant defense, parasitism, pathogens, and unknown factors.

Within obligate cannibalism events, we categorized two groups. The first was egg cannibalism when we found a stem with multiple eggs or a larva and an egg. The second was larval cannibalism if a stem contained more than two larvae. In both cases, a single larva eventually consumes all others, both eggs and larvae, in that stem. Only a single larva per infested stem proceeds to cut the stem and hibernate if the larva survives other causes of mortality (Wallace and McNeal 1996, Holmes 1954, Weiss and Morrill 1992, Weaver et al. 2005).

We categorized plant defense as a cause of death if we found a stem contained dead neonates or the stem lining had only couple of millimeters of feeding scars at the larval emergence site. Additionally, if we saw only feeding scars, tiny amounts of frass or a small fragment of a dead neonate in the stem, then we also categorized the cause of death as plant defense.

We combined the mortality due to parasitoids and predators under the category of parasitism because we only found two stems with larvae of the clerid predator, *Phyllobaenus dubius* Wolcott (Coleoptera: Cleridae), described in Morrill et al. (2001), at the site near Amsterdam in 2016. Thus, otherwise for this paper, “parasitism” represents a stem containing either a parasitoid larva, a parasitoid cocoon or a diagnostic emergence hole in the stem wall. For the rate of parasitism, we categorized the larvae into two groups based on their relative size: early growth stages or stages vulnerable to parasitism. Not all larval stages are vulnerable to parasitism. Thus, an early stage before parasitism was possible occurred from the 2nd to 4th week of sampling and the records for the period with stages vulnerable to parasitism occurred from the 5th to 9th week of sampling, as well as being evident in stub samples taken for the pre- and post-flight periods.

If the larval cadaver was covered with white or pink hyphae or was characteristically pink (Wenda-Piesik et al. 2009), we assumed the cause of death was entomopathogenic fungi and we categorized it as “pathogens.” If the larva mined through multiple internodes but was found dead from other than the above-mentioned mortality causes, it was classified as having died due to "unknown factors."

Construction of Multiple Decrement Life Tables

For the multiple decrement life table analysis, we used abridged life tables to calculate mortality causes and relevant mortality proportions by those causes in the presence of other mortality causes (Carey 1993). We produced the multiple decrement life tables by cultivar and study site using the spreadsheet program M-DEC (Davis et al. 2011). After accounting for mortality causes from summer samples, pre-emergence stubs, and post emergence stubs, we calculated the percentage of mortality for each cause of mortality in the presence of other mortality causes, and subsequently the irreplaceable mortality. Irreplaceable mortality is the proportion of mortality that cannot be replaced by another cause of mortality in the absence of the particular cause of interest, for each specific cause of mortality and was determined using the methods described by Carey (1993), Peterson et al. (2009, 2011), and Davis et al. (2011). In this technique, the variables are defined as: x = life stage index, l_x = the number of individuals alive at each x, k_x = the number at beginning of each x, d_x = the total number of deaths in each stage, al_x = the fraction of the cohort living at the beginning of the stage (starting at 1.0 for the first state and calculated by al_{x-1} - ad_{x-1}), ad_{ix} = fraction of death attributable to one cause, ad_x = fractions of all deaths from all the causes (ad_{1x} + ad_{2x} + … ad_{5x}), aq_x = stage specific probability of dying from all listed causes (d_x/k_x)

Similarly, the probability for cause of death in the absence of other causes can be estimated using a quadratic solution. Elimination-of-cause analysis relies on the probability of surviving each source of mortality (px) and its complement ($1 - qx$) where $(1 - q_1) \times \ldots \times (1 - q_n)$ is the chance of jointly surviving a set of mortality causes and its complement, $1 - [(1 - q_1) \times \ldots \times (1 - q_n)]$, is the chance of jointly dying from a set of mortality cause. To estimate mortality in the absence of one or more causes, two simultaneous equations with two unknowns are used. For example, by expression q_1 (e.g. cannibalism) in terms of q_2 (e.g. all other mortality causes), D_1 and D_2 (the faction of all individuals observed that died of cause 1 and cause 2), this yields the quadratic equation:

$aq_2^2 + bq_2 + c = 0$, where $a = D_1$, $b = -(D_1 + D_2)$, and $c = D_2(D_1 + D_2)$.

The value of q_2 can be calculated by substituting a, b, and c in the quadratic formula. Similarly, to calculate irreplaceable mortality for any cause of mortality, for instance irreplaceable mortality due to cannibalism, we subtracted total mortality caused by all estimated mortality causes (cannibalism, parasitism, plant defense, pathogen, and unknown) from the total mortality (parasitism, plant defense, pathogen, and unknown) except the mortality from cannibalism (Davis et al. 2011, Peterson et al. 2011).

Data Analyses

We compared the percentage mortality of each mortality cause in the presence of other mortality causes by cultivars and by sites. In addition, we compared percentage of irreplaceable mortality caused by each mortality cause by cultivars and by sites. We used linear and nonlinear mixed effects model (nlme: Pinherio et al. 2020) with percentage mortality attributed to each cause as the response variable, while cultivars, sites and their interaction were fixed effects. Year was considered as a random factor for the analysis. All the percentage data were square-root transformed to meet the assumption of homogeneity of variance, but untransformed means and the associated standard errors are presented. First, we included a site x cultivar interaction for the analysis and later dropped it if $p > 0.05$ for interaction. Post-hoc analysis was done whenever $p \leq 0.05$ by using Tukey HSD (multcomp: Hothorn et al. 2017). Tukey HSD was used to reduce error due to unequal sample sizes across cultivars. We used the R package ‘scales’ (Wickham and Seidel 2019) to prepare a heat map to visualize life table. All data were analyzed in Comprehensive R Archive Network (CRAN) version 3.5.3 (RCoreTeam 2019) and visualization by using ggplot2 (Wickham 2016).

Results

Multiple decrement life tables were produced for each study site and each cultivar (Supplementary Table 1; Fig. 5). Cannibalism, plant defense, and unknown factors were the three main causes of mortality for eggs through pre-diapause larvae, whereas unknown factors, pathogen, and parasitism were main causes of mortality for overwintering larvae (Table 1, Fig. 5).

The probability of death from a specified cause in the presence of combinations of other causes by all stages is shown in Table 2. For instance, cannibalism alone would kill 71% of population. The combination of cannibalism, unknown factors, and plant defense would kill 92% of the population, while the combination of all t5 listed causes of mortality would kill 93% of the population. Thus, the effect of mortality due to parasitism or pathogens for the population in the presence of other 3 causes was negligible.

Larval mortality from plant defense was significantly different by site and cultivar ($F_{14,35} = 3.09$, $P = 0.003$). In addition, 'Hockett' had the lowest larval mortality due to plant defense compared to the other cultivars at all sites (Fig. 1).

Mortality from cannibalism was significantly different for site × cultivar ($F_{14, 35} = 4.12$, $P = 0.004$). The variation in mortality due to cannibalism was greatest for near Amsterdam in 2016 when compared with near Amsterdam in 2017 and near Big Sandy in 2017 (Fig. 2).

Parasitism and pathogens caused less than 20% mortality individually, while unknown factors caused approximately 30% (Fig. 3). All sites had a similar pattern of mortality within each cause of mortality in all cultivars (Parasitism: $F_{14, 35} = 1.833$, $P = 0.073$; Pathogens: $F_{14, 35} = 0.637$, $P = 0.815$; Unknown factors: $F_{14, 35} = 1.314$, $P = 0.248$).

There was no site effect on irreplaceable mortality within the specified cause of mortality: cannibalism ($F_{14, 35} = 0.97$, $P = 0.493$), pathogens ($F_{14, 35} = 0.97$, $P = 0.494$), plant defense ($F_{14, 35} = 0.74$, $P = 0.715$), and unknown factors ($F_{14, 35} =$

0.96, *P* = 0.502). The irreplaceable mortality due to cannibalism in 'Hockett' was higher than the other cultivars (Table 2). Similarly, irreplaceable mortality due to pathogens was the lowest compared to other causes of mortality (Table 2).

Irreplaceable mortality due to parasitism was less than 4% but was significantly different by cultivar and by site ($F_{14, 35} = 2.19$, $P = 0.029$) (Fig. 4). The lowest irreplaceable mortality due to parasitism was found near Big Sandy in 2017.

Discussion

Our findings indicate that plant defense, cannibalism, and unknown factors were the three major causes of mortality for *C. cinctus* developing in barley (Table 1). Furthermore, irreplaceable mortality due to parasitism, pathogen, and unknown factors was lower than irreplaceable mortality due to cannibalism and plant defense (Table 2 and Fig 4). Although irreplaceable mortality due to plant defense and cannibalism was similar to previous reports for wheat (Buteler et al. 2015, Peterson et al. 2011), the mortality due to parasitism and pathogens was quite low in our study compared with previous observations made in wheat.

Irreplaceable mortality due to pathogens was lowest across all cultivars at < 1% (Table 2). Similarly, Buteler et al. (2015) observed that pathogens contributed a small fraction (< 5%) to the mortality of *C. cinctus* in wheat. We did not assess which pathogen species killed larvae. Studies show that entomopathogenic fungi (Vidal and Jaber 2015, Portman et al. 2018) and certain *Fusarium* spp. (Wenda-Piesik et al. 2009) can colonize plant tissue without developing highly apparent symptoms in the plants, but these fungi can kill diapausing larvae (Vidal and Jaber 2015, Wenda-Piesik et al. 2009). Thus, our results suggest that barley cultivars may also harbor entomopathogenic fungi, but the level of mortality was negligible.

The overall parasitism rate was < 20% in the presence of other causes of mortality across all cultivars and sites (Table 1). Similarly, the irreplaceable mortality due to parasitism was < 4% (Fig 4). Conversely, several other studies have reported that *C. cinctus* mortality due to parasitism was as much as 60% (Farstad and Platt 1946, Buteler et al. 2015, Peterson et al. 2011, Cárcamo et al. 2016). Using an elimination-of-cause analysis (Peterson et al. 2011), the mortality from parasitism would most likely have been largely replaced by other mortality causes if parasitism was not present. Like our results, the irreplaceable mortality due to parasitism was < 5% (Peterson et al. 2011, Buteler et al. 2015). Discrepancies in the rate of parasitism are associated with cultivar traits. For instance, Buteler et al. (2015) found a greater rate of parasitism in *C. cinctus* in a susceptible hollow stem wheat cultivar compared to a resistant solid stem wheat cultivar. The cultivars included in our study had a wide range of resistance levels against *C. cinctus* and that could explain the varying rates of parasitism by cultivars, with cultivars with greater levels of plant defense generally having less parasitism.

Parasitoid attraction to hosts is mediated by volatile compounds released by injured plants. These volatile compounds are called herbivore-induced plant volatiles and plants use these as defense responses to the herbivores (Dicke et al. 2009), invoking both direct and indirect defense. In our study, we observed a lower parasitism rate when compared with parasitism rates in several previous studies in wheat. However, it is premature to determine whether the lower parasitism rates that occurred in our study were due to lower parasitoid populations, or if parasitoid adults did not find a sufficient number of the larval hosts because of a greater proportion of larval mortality due to plant defense and cannibalism. Similarly, we cannot know if barley cultivars did not release enough specific volatile compounds, or if the parasitoids themselves could not clearly recognize volatiles released by the infested barley plants.

Parasitoids generally exploit their visual and olfactory cues as well as optimized innate memory to detect their host (Turlings et al. 1993). Additionally, odor cues that present on their pupal cocoons are important when developing host seeking behaviors (Herard et al. 1988) Thus, parasitoid adults might not fully recognize cues from their host, *C. cinctus* larvae, in infested barley. This is because use of barley by host *C. cinctus* is relatively new when compared with wheat at our study locations (Varella et al. 2018) and thus these specialist idiobiont parasitoids, with *C. cinctus* as their only known host, might have a problem locating large larvae in barley stems.

Our results show that mortality due to plant defense caused the second-highest percentage of mortality (Table 2), while irreplaceable mortality due to plant defense was greatest. For example, in the cultivar ‘Craft’, the percentage of irreplaceable mortality due to plant defense was 4-fold higher than the irreplaceable mortality due to cannibalism, 12-fold higher than irreplaceable mortality due to unknown factors, and almost 20-fold higher than irreplaceable mortality due to parasitism and pathogen (Table 2 and Fig 4). Thus, ‘Craft’ has stronger antibiosis traits compared with other cultivars; however, it was beyond the scope of our study to identify causation in those traits. We suggest that both the physical strength of stem tissue and biochemical mechanisms, which can collectively be considered antibiosis, play important roles.

In wheat, a higher proportion of larval mortality occurs in pith-filled solid stems than in hollow stems (Sherman et al. 2010, Talbert et al. 2014). Even though all barley cultivars in this study have hollow stems, our results reveal the percentage of mortality due to plant defense was higher than the mortality in any solid-stem wheat cultivar. Therefore, we suggest that larval mortality in barley is likely caused by traits other than those associated with stem architecture.

Our results indicate that cannibalism caused the greatest mortality in the presence of other mortality causes (Fig 2) and high levels of irreplaceable mortality (Table 2). The mean irreplaceable mortality due to cannibalism was 5.58 ± 1.29% in ‘Craft’ and 29.14 ± 4.19% in ‘Hockett’ (Table 2). Buteler et al.

(2015) observed that the irreplaceable mortality due to cannibalism was as much as 20% in wheat but the mortality due to cannibalism was greater in hollow stem compared to solid stem wheat cultivars. In terms of cannibalism, comparisons from hollow stem vs solid stems in wheat are similar to comparing more susceptible ‘Hockett’ vs less susceptible ‘Craft’ in barley. Neonate mortality rates in both solid stem wheat or ‘Craft’ barley is greater than the mortality in hollow stem wheat or ‘Hockett’ barley. In both wheat and barley, mortality in *C. cinctus* due to plant defense and cannibalism are somewhat inimical in stems. It is obvious that plant defenses kill neonates, while cannibalism occurs only after attaining increased larval size and potentially mining another internode, to then subsequently encounter an egg or another larva in the same stem. Thus, we found not only a greater rate of irreplaceable mortality due to cannibalism in ‘Hockett’ than in ‘Craft,’ but also found levels of plant defense and cannibalism are in a delicate balance while together yielding most of the cumulative mortality.

Cannibalism was apparent as a major cause of mortality, but typically allows for survival of one individual in a cohort of multiple immatures within a single stem. This usually results in a mature larva surviving to cut the stem in preparation for overwintering, which is agronomically undesirable (Bekkerman and Weaver 2018). However, from the perspective of population ecology cannibalism seems to be an important cause of mortality when considering recruitment and it possibly has fitness implications in the host that contains toxic compounds or lacks essential nutrients. The fitness can be by gaining additional nutritional resources or by reducing the rate of parasitism to larvae (Richardson et al. 2010, Weaver et al. 2005).

Cannibalism is well known to provide additional nutritional resources for survivors. For instance, *Harmonia axyridis* Pallas shortens development time and increases survivorship more by consuming conspecific larvae than by consuming prey aphid species that sequester plant compounds as a defense mechanism (Snyder et al. 2000). By consuming conspecific larvae, *H. axyridis* avoids the potential negative effect of plant toxins (Snyder et al. 2000). Several previous reports (Farstad and Platt 1946, Varella et al. 2018) and the current study have indicated that a large proportion of neonate mortality indicates that either the stem tissues contain antibiotic toxins, or possibly the tissues lack some essential nutrients required for neonate growth and development. But by consuming conspecific larvae and eggs, each cannibal avoids both tissue toxins or diet deficiency and acquires additional nutrient resources. Further, due to extra nutritional resources, cannibalizing individuals would be more vigorous and could also consume vulnerable parasitized hosts, effectively eliminating the larval ectoparasitoids (Holmes et al. 1963, Weaver et al. 2005). Cannibalism reduces larval host density available for parasitism and can ultimately reduce the rate of parasitism (Wang and Danne 2014, Richardson et al. 2010). Cannibalism also reduces injury to stem tissue by eliminating other feeding individuals, thus limiting tritrophic communication to parasitoids that could immediately favor the infested stem (Buteler et al. 2008) Thus, cannibalism can increase the fitness of survivors and populations in this system (Polis 1981).

Cannibalism is obligate in *C. cinctus* when stems contain multiple eggs (Wallace and McNeal 1996; Holmes 1954, Buteler et al. 2015). Why a cohort of *C. cinctus* females deposits multiple eggs in a stem over a flight period of several weeks is not known, but the following could be potential reasons. First, females prefer to lay eggs in hosts that release greater amounts of attractive volatiles such as *β*-ocimene, (*Z*)-3-hexenyl acetate, and (*Z*)-3-hexenol acetate (Buteler and Weaver 2012, Weaver et al. 2009) and therefore may indiscriminately lay eggs in a host even though conspecific females have already laid eggs (Ainslie 1920, Buteler et al. 2009). Second, the females are short-lived, typically surviving about 7 days, which may provide an overarching imperative to lay eggs in any available host instead of dying with a greater complement of eggs while searching for an optimal host. For instance, in the absence of primary hosts for oviposition, *C. cinctus* can lay eggs in flax (Farstad 1944) and in wheat straw (Holmes and Peterson 1960). The behavior of accepting a lower quality hosts in the absence of higher quality hosts can be accompanied by higher levels of cannibalism on less suitable hosts in phytophagous species (Kakimoto et al. 2003). However, in our study more cannibalism occurred in cultivars with less evidence of plant defense which also received more eggs; these cultivars are arguably more, rather than less suitable as hosts. Regardless of which of these reasons cause females to lay multiple eggs in a stem, our results strongly suggest cannibalism plays a considerable role in influencing the population dynamics of *C. cinctus* in barley.

Multiple eggs in a stem is not by a single ovipositing female (Buteler et al. 2009, Varella et al. 2017), but by more than one female because of a failure to discriminate previous infestation (Buteler et al. 2009). This suggests that neither self-provisioning nor opportunistic oviposition facilitates the occurrence of cannibalism in larval *C. cinctus*, as might be expected from studies on species that oviposit in clutches, some of which may have evolved parental guarding (Dickinson 1992, Sherratt et al. 1999, Lopez-Ortega and Williams 2018). For example, ‘Craft,’ ‘Champion,’ and ‘Celebration’ had fewer stems with multiple eggs so that they had a lower percentage of mortality due to cannibalism compared to ‘Hockett.’ Fewer eggs per stem were likely because these cultivars were less attractive to foraging gravid females or because behaviors while exploring the stem, somewhat similar to those observed for solid-stem wheat (Varella et al. 2017), inhibit deposition of eggs when probing with the ovipositor.

Individual females do not sacrifice their own egg by laying multiple eggs in a stem to avoid cannibalism. Cannibalism poses risks to survivors of being infected by species-specific pathogens from their kin and potential mortality due to fighting (Pfenning et al. 1998). We hypothesize that having multiple eggs from different females in a stem is mediated primarily by host plant volatiles and not by cues released from already oviposited eggs (Buteler et al. 2009), yet this strategy favors increased cannibalism.

We conducted our research at two disparate locations. These locations have different agroclimatic conditions (e.g. the duration of crop growth was 115 days near Amsterdam in 2016 and only 99 days near Big Sandy in 2017). Therefore, we observed relative differences in larval mortality, as well as the expression of host-plant performance related to larval mortality, within cultivars across our study sites. However, the overall pattern we observed across the cultivars remains clear, where we see that there is a major tradeoff between plant defense and cannibalism as the major causes of irreplaceable mortality (Fig 5).

Finally, our overall results suggest that cannibalism and plant defense were the two major causes of mortality for pre-diapause larvae across study sites. Further, irreplaceable mortality due to these two factors has a significant impact that reduces the size of pre-diapause larval populations of *C. cinctus*, which drives the population growth the following year. Both of these causes of mortality are mediated by host plant traits, either relative preference (Weaver et al. 2009) or antibiosis (Fig 5). Thus, capitalizing on cultivar traits that cause both a greater proportion of antibiotic neonate mortality and those that result in obligate cannibalism when attracting more females to oviposit (Buteler et al. 2010, Buteler and Weaver 2012) are critical considerations to reduce economic losses caused by this species.

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Table 3. 1: Multiple decremental life tables of wheat stem sawfly, *Cephus cinctus*, in barley

Category x	Alive l_x	Fraction dying aq_x	Fraction of living al_x	Fraction of total dying ad_x	Parasitism aq_{1x}	Plant defense aq_{2x}	Cannibalism aq_{3x}	Unknown aq_{4x}	Pathogen aq_{5x}
Egg	8968	0.619	1	0.619	0	0	0.619	0	0
Larva I	18447	0.478	0.381	0.182	0	0.102	0.072	0.008	0
Larva III	17254	0.554	0.199	0.110	0.010	0.063	0.009	0.027	0
Larva IV	1546	0.169	0.089	0.015	0.002	0	0	0.012	0.0007
Larva V	1499	0.1368	0.074	0.010	0.002	0	0	0.006	0.0013
Adult	1291	0	0.064	0.064					
Total				1	0.015	0.165	0.701	0.054	0.002

Larva I: pre-parasitism period; Larva III: parasitism period; Larva IV: overwintered larva (pre-flight period); Larva V: overwintered larva (post-flight period).

aq_x = fraction of death caused by all the given mortality causes in the stage x given that the individual is alive at the beginning of stage x; al_x = fraction of survivor at stage x out of original cohort of all; ad_x = fraction of deaths in stage x from all the mortality causes; aq_{ix} = fraction of death from cause i in stage x in the presence of all other mortality causes given that the individual is live at the beginning of state x.

Table 3. 2: Irreplaceable mortality by cause of mortality and cultivar

Cultivar	Irreplaceable mortality percent (mean ± SE*)			
	Plant defense	Cannibalism	Unknown factors	Pathogen
Celebration	18.74 ± 2.69	8.96 ± 1.78	5.81 ± 1.79	0.11 ± 0.07
Champion	17.02 ± 3.33	9.41 ± 2.17	2.24 ± 0.25	0.45 ± 0.23
Craft	21.65 ± 7.05	5.59 ± 1.92	1.72 ± 0.46	0.04 ± 0.03
Haxby	17.27 ± 6.08	16.10 ± 3.87	2.75 ± 0.96	0.51 ± 0.37
Haybet	12.26 ± 4.35	18.38 ± 2.56	5.32 ± 1.40	0.10 ± 0.07
Hockett	6.42 ± 2.60	29.15 ± 4.19	4.92 ± 1.06	0.40 ± 0.19
Lavina	15.61 ± 3.72	12.80 ± 2.96	6.44 ± 1.22	0.28 ± 0.18
Tradition	22.29 ± 6.35	11.13 ± 3.82	6.22 ± 1.80	0.86 ± 0.25
DF	7,49	7,49	7,49	7,49
F-value	2.44	11.35	2.69	1.16
p-value	0.037	<0.001	0.024	0.347

*SE is standard error of the mean.

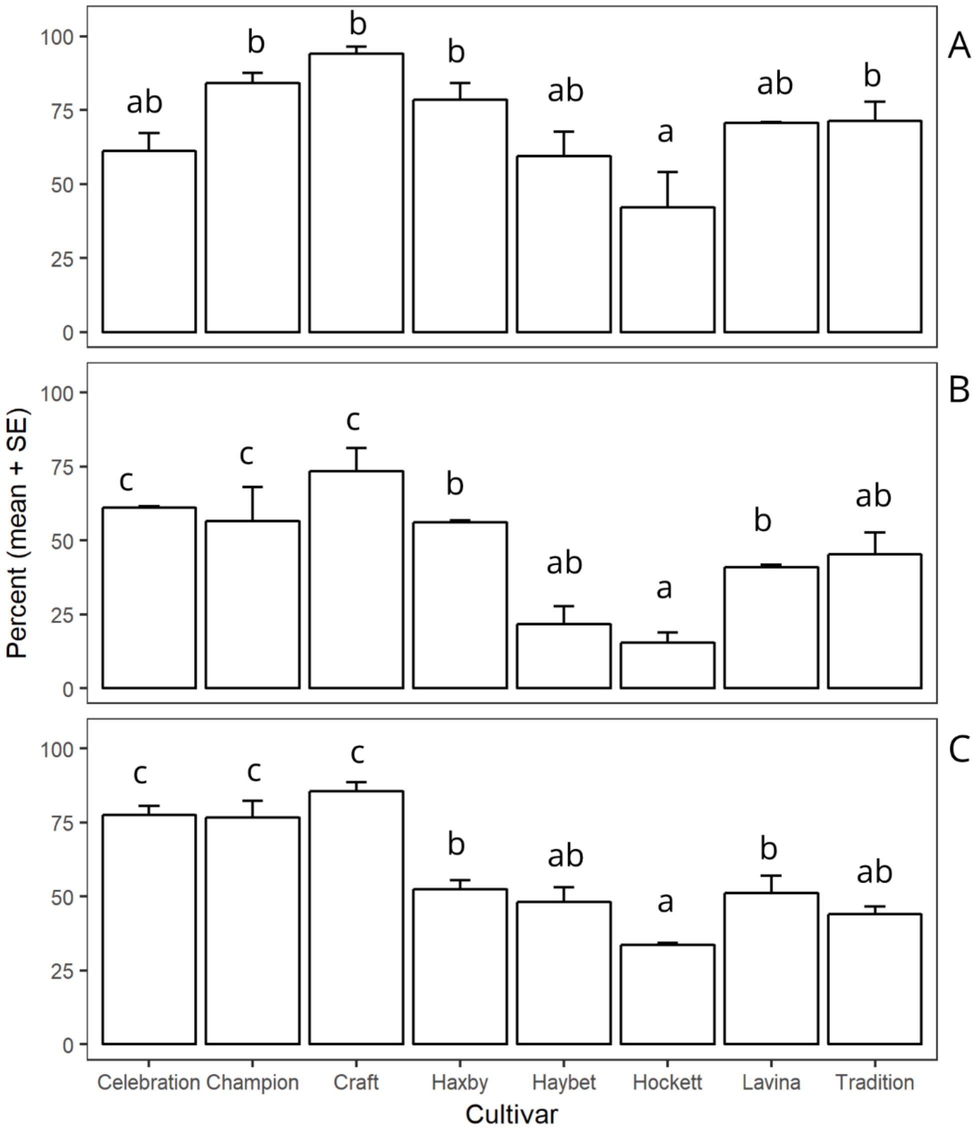

Figure 3. 1: Mean mortality percentages due to plant defense by site and cultivar. Bars within the site followed by different letters are significantly different (α = 0.05). Panel A: Amsterdam 2016 ($F_{7,14} = 6.25$, $P = 0.001$); Panel B: Amsterdam 2017 ($F_{7,7} = 14.80$, $P = 0.001$); Panel C: Big Sandy 2017 ($F_{7,14} = 25.05$, $P < 0.001$). SE is standard error of the mean

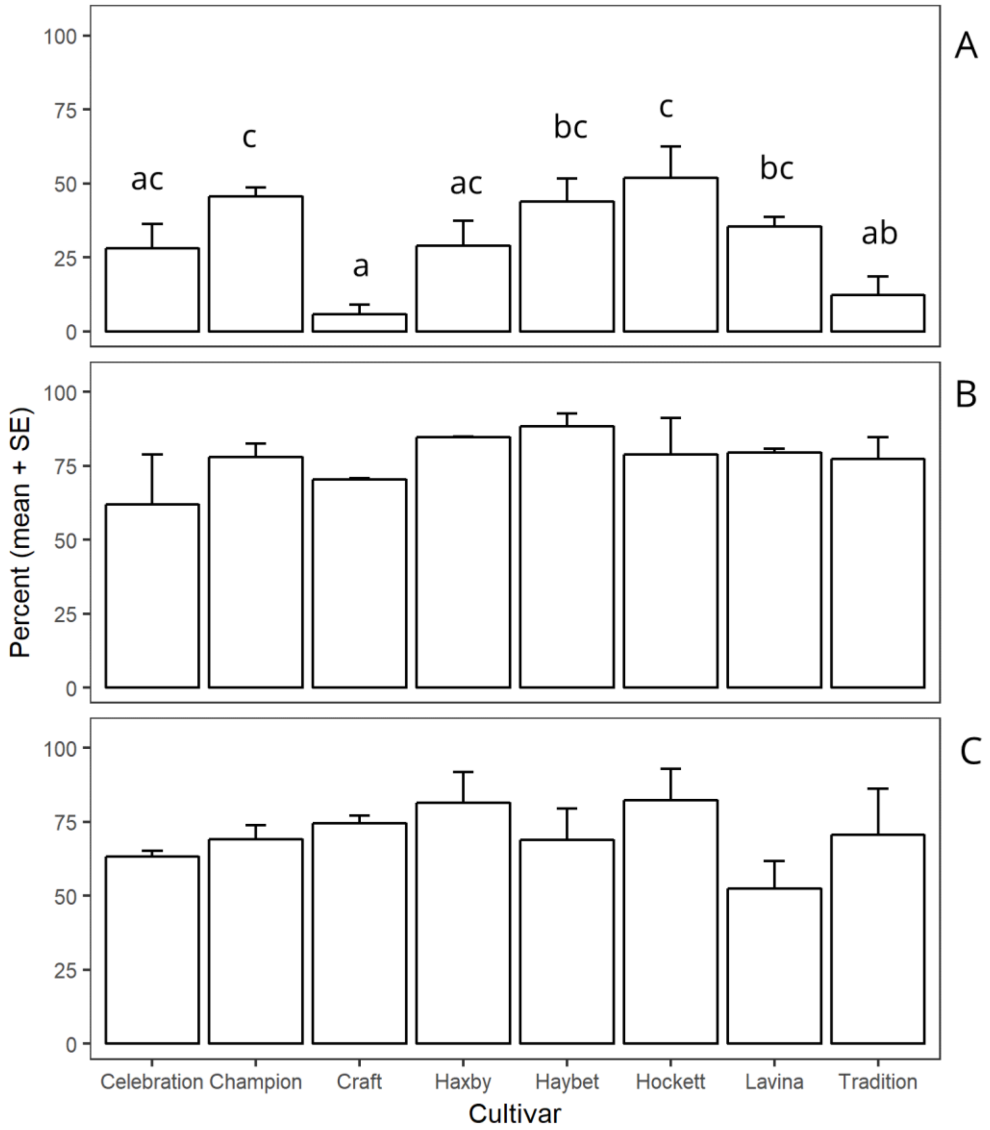

Figure 3. 2: Mean mortality percentages due to cannibalism by site and cultivar. Bars within the site followed by different letters are significantly different (α = 0.05) and the bars without letters within the site are not different (α = 0.05). Panel A: Amsterdam 2016 ($F_{7, 14}$ = 6.11, *P* = 0.002); Panel B: Amsterdam 2017 ($F_{7, 7}$ = 0.95, *P* = 0.523); and Panel C: Big Sandy 2017 ($F_{7, 14}$ = 1.008, *P* = 0.465). SE is standard error of the mean.

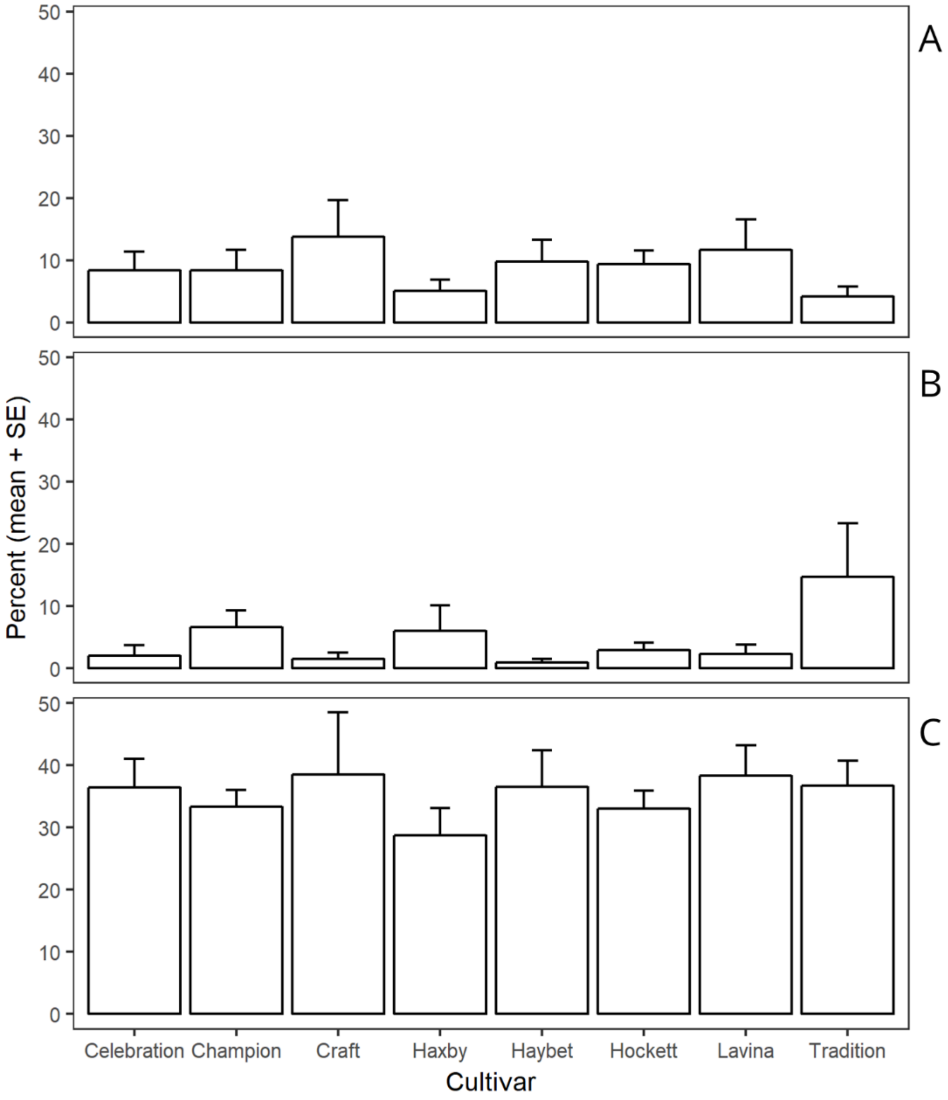

Figure 3. 3: Mean mortality percentages by parasitism, pathogen, and unknown factors by site and cultivar. Bars without letter within the mortality causes are not different (α = 0.05). Panel A: Parasitism (Cultivar: $F_{7, 35} = 1.40$, $P = 0.233$); Panel B: Pathogen (Cultivar: $F_{7, 35} = 1.47$, $P = 0.208$); Panel C: Unknown factors (Cultivar: $F_{7, 35} = 1.31$, $P = 0.248$). SE is standard error of the mean.

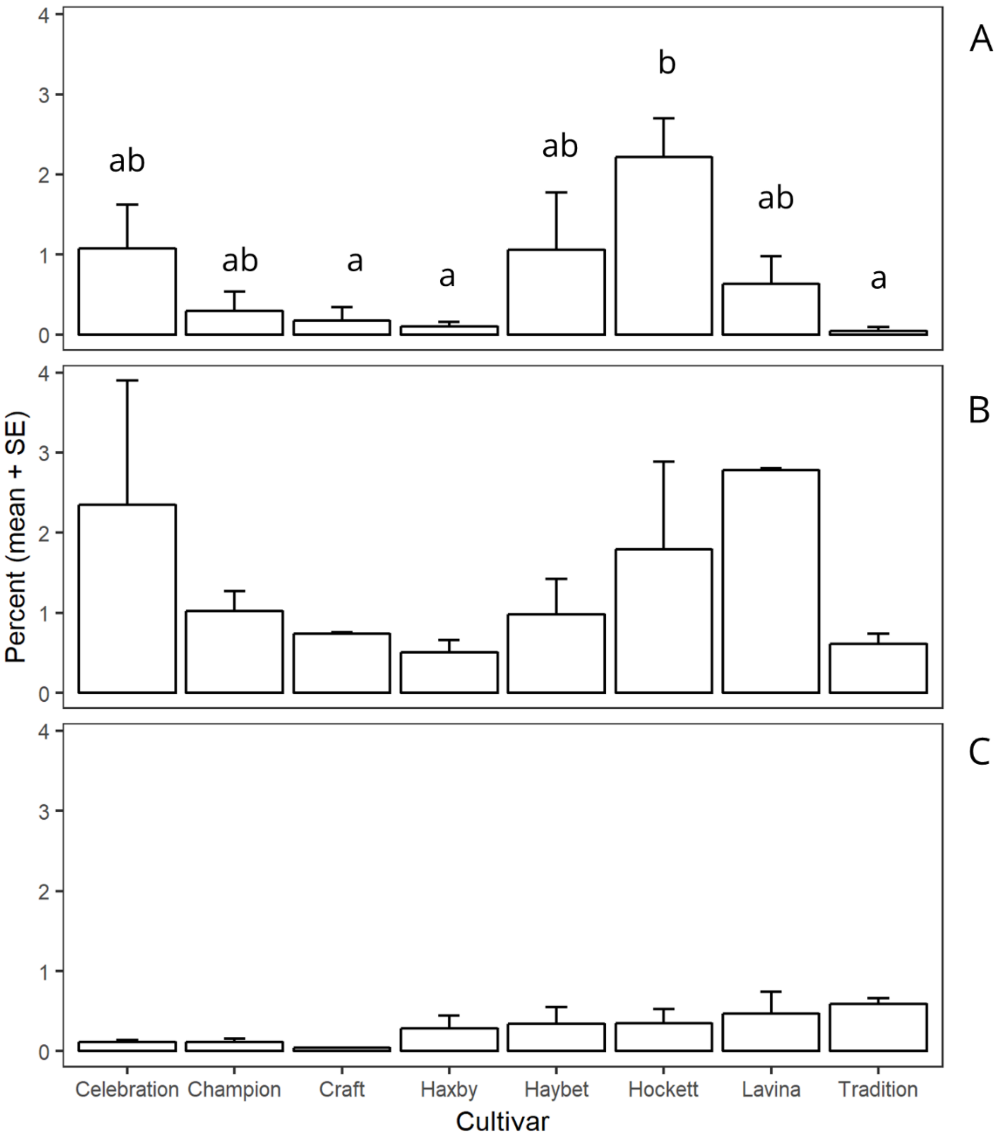

Figure 3. 4: Mean percentages of irreplaceable mortality due to parasitism by site and cultivar. Bars within the site followed by different letters are significantly different (α = 0.05) and the bars without letters within the site are not different (α = 0.05). Panel A: Amsterdam 2016 ($F_{7,14} = 3.96$, $P = 0.013$); Panel B: Amsterdam 2017 ($F_{7,7} = 2.30$, $P = 0.146$); Panel C: Big Sandy 2017 ($F_{7,14} = 1.86$, $P = 0.152$). SE is standard error of the mean.

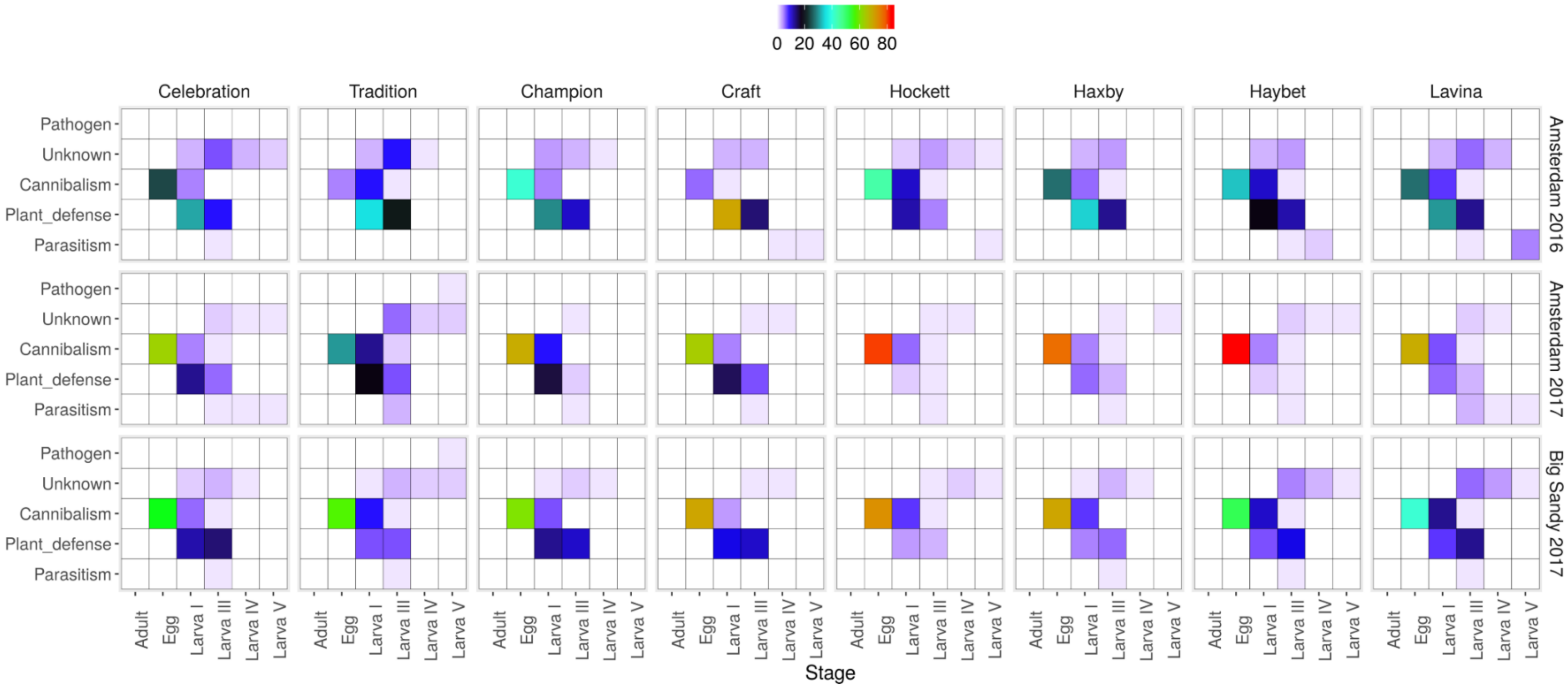

Figure 3.5: Heat map displaying the percentage of mortality by each cause of mortality at each developmental stage *Cephus cinctus* in barley cultivars that were grown in Montana. Larva I: pre-parasitism period; Larva III: parasitism period; Larva IV: overwintered larva (pre-flight period); Larva V: overwintered larva (post-flight period).

CHAPTER SIX

HOST SELECTION AND OVIPOSITION BEHAVIORS OF *CEPHUS CINCTUS* (HYMENOPTERA: CEPHIDAE) IN BARLEY

Contribution of Authors and Co-Authors

Manuscript in Chapter 6.

Author: Buddhi B. Achhami

Contributions: conducted experiment study, analyzed data, prepared the manuscript.

Co-Author: Gadi V. P. Reddy

Contributions: assisted in preparation of the manuscript.

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Co-Author: Robert K. D. Peterson

Contributions: assisted in preparation of the manuscript.

Co-Author: David K. Weaver

Contributions: obtained funding for the project, supervised the work, assisted in preparation of the manuscript.

Manuscript Information Page

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Abstract

Wheat stem sawfly, *Cephus cinctus* Norton, females use complex behaviors for host selection and oviposition in wheat. The female of *C. cinctus* not only prefers but also lays a greater number of eggs in more susceptible hollow stem wheat cultivars compared to resistant solid stem cultivars. Neonate mortality caused by antibiosis is greater in solid stem wheat cultivars than in hollow stem cultivars. In barley, neonate mortality due to antibiosis across cultivars is even greater than in solid stem wheat cultivars. Does this greater proportion of neonate mortality in barley affect the host choice and oviposition behaviors of *C. cinctus* in a manner different from what is known for wheat hosts? To evaluate this, we carried out greenhouse experiments comparing two barley cultivars— 'Hockett' and 'Craft'— to understand host choice and oviposition behaviors on barley stems. A previous field study using 8 cultivars found these cultivars differed most for antibiotic and antixenotic outcomes. We found that *C. cinctus* females preferred volatiles from the cultivar 'Hockett' over 'Craft' in Y-tube olfactometer tests. Subsequently, in caged choice experiments, frequencies of oviposition behaviors such as walking and abdominal tapping were higher in 'Hockett' than in 'Craft' at the Zadoks 49 plant growth stage, while the frequencies of these behaviors were similar on stems at the earlier Zadoks 34 growth stage. The number of eggs per stem was greater in 'Hockett' at both plant stages. Volatile profiles indicate that the amount of linalool present was greater in aerations from more resistant 'Craft' than in aerations from 'Hockett', but only at the early growth stage of Zadoks 34. In contrast the amount of the known attractant (*Z*)-3 hexenyl acetate in aerations was greater in more susceptible 'Hockett' at both growth stages. Olfactometer assays, frequencies of oviposition behaviors and the number of eggs per stem indicate that females can use multiple modalities to make oviposition decisions favoring the more suitable host in barley. These results suggest we can manipulate host plant traits expressed in developing barley to potentially divert or attract *C. cinctus* to specific cultivars to ultimately reduce future losses caused by this species in barley.

Key words: volatiles, Y-tube olfactometer, oviposition behaviors, eggs

Introduction

Insect host selection behaviors are complex and are repeated sequentially in a fixed order (Atkins 1980). The behaviors are governed by a number of factors, such as host plant defenses, environmental constraints, plus the offspring survival rate (Gripenberg et al. 2010), adult longevity and capacity for dispersal (Larsson and Ekbom 1995), among others. These behaviors are first initiated to discriminate between non-host and host plants, and subsequently to distinguish the most suitable plants within the range of host plants (Schoonhoven et al. 2005). To identify a suitable host, the insect first evaluates a blend of volatile compounds that are emitted by the host plants (Cha et al. 2011) and subsequently evaluates the plant architecture (Rudgers and Whitney 2006). In polyphagous insects, the host selection process is less refined when compared to host selection processes in oligophagous insects (Gripenberg et al. 2010).

An important example of an oligophagous insect species is the wheat stem sawfly, *Cephus cinctus* Norton. This species causes serious economic losses in wheat (Beres et al. 2011a), and more recently, barley (Varella et al. 2018) crops grown on the Northern Great Plains of the USA and the Canadian Prairie provinces. The economic loss is caused by larval feeding injuries in the stem. Further, once the crop reaches maturity, the larva descends to the root crown to make a hibernaculum for overwintering (Criddle 1923). Before making a hibernaculum, the larva makes a circular notch that encircles the stem internally and makes the stem susceptible to lodging in response to wind and gravity (Ainslie 1929, Weiss and Morrill 1992). Lodged stems are not easily collected by combine harvesters and this causes major losses (Holmes and Peterson 1965, Beres et al. 2007, Bekkerman and Weaver 2018). Approximate annual economic loss caused by this species in Montana is estimated between US $50-80 million (Fulbright et al. 2017) with annual losses in North America estimated at US $350 million (Beres et al. 2011a, 2011b).

Several IPM tools can be used to reduce *C. cinctus* losses, but the effectiveness of these tools is variable and limited. For instance, insecticide applications are generally ineffective (Knodel et al. 2009), while several tillage operations— more than one is required to expose overwintered larvae— increase soil erosion and nutrient losses in the field (Morrill et al. 1993) and negatively impact specialist parasitoids (Runyon et al. 2002). The parasitoid species tend to frequently be locally effective but have limited overall impact (Morrill et al. 1998, Weaver et al. 2004, 2005, Portman et al. 2018). A foundation for management is afforded by the use of resistant solid-stem wheat cultivars (Beres et al. 2011b, Varella et al. 2015), but these can vary in effectiveness in response to environmental conditions (Holmes 1984, Beres et al. 2017). The constrained success of these approaches has led to a search for alternative tools to be used in IPM (Morrill et al. 2001, Weaver et al. 2004, Beres et al. 2009).

Alternative management can be developed by manipulating host selection and oviposition behaviors of this species. As a native insect (Lesieur et al. 2016), *C. cinctus* was present across a broad landscape before wheat was cultivated. The list of host grasses for this species was recently updated and includes numerous native and introduced species (Cockrell et al. 2018). This species has a unique biology where the larvae cannot leave a host stem; rather adults emerge from cut stubs after metamorphosis early in the next growing season. Obligate cannibalism occurs when multiple eggs are placed within a stem (Wallace and McNeal 1966, Buteler et al. 2015, Achhami et al. submitted: Chapter 5). One or more females deposit multiple eggs within a stem because females oviposit indiscriminately in stems that have already been infested by ovipositing conspecifics (Buteler et al. 2009). These features of *C. cinctus* biology illustrate the importance of understanding host selection and oviposition behaviors to formulate new management tools.

Host selection and oviposition decisions do not necessarily increase larval survival in *C. cinctus*. If the stem tissues are not palatable due to toxic compounds or lack of essential nutrients to the larva, the larva will die. Previous studies have shown that *C. cinctus* females will oviposit in stems where larvae have lower survival rates. For example, larval survival rates in solid stem wheat cultivars (Varella et al. 2015, Talbert et al. 2014), downy brome (Perez-Mendoza et al. 2006), and barley (Varella et al. 2018) are lower than survival rates in susceptible hollow stem wheat cultivars. Females occasionally oviposit eggs in stems of oat species even though all larvae die before reaching their maturity (Criddle 1922, Weaver et al. 2004).

Females can also avoid selecting a less suitable host when appropriate host attributes are correctly detected. More eggs are deposited in stems of the susceptible hollow stem wheat cultivar ‘Reeder’ than in stems of the resistant wheat cultivar ‘Conan’ (Weaver et al. 2009). Additionally, females prefer to deposit a greater number of eggs in near-isogenic wheat lines that were derived from susceptible parents ‘Reeder’ and ‘Scholar’ than in isolines derived from the resistant parent ‘Conan’ (Varella et al. 2016).

Yet, multiple eggs within suitable hosts reduces larval survival by obligate cannibalism (Buteler et al. 2015, Achhami et al. submitted: Chapter 5). Thus, even selecting a good host is not perfectly reflected in the survival of offspring. Under the preference-performance hypothesis, a female prefers a host plant that supports a higher rate of offspring survival (Jaenike 1978). However, at the same time, the female opts to choose an optimal host that provides sufficient food to her offspring. Good host plant decisions influence the overall performance of adult offspring. Females prefer to deposit eggs in larger and thicker stems (Perez-Mendoza et al. 2006); as a result the emerging adults from the host plant with larger stems have greater body size and longer lifespan compared with the adults’ body size and life span that were emerged from smaller host stems (Morrill and Weaver 2000, Morrill et al. 2000, Perez-Mendoza et al. 2006). Thus, host traits are important for larval survival and emerging adult life span and body size.

To select a host, the female of *C. cinctus* displays a series of behaviors that are grouped into two categories—pre-alighting and post-alighting behaviors. Pre-alighting behaviors are mediated by visual and long-distance olfactory cues that are perceived from the host plants (Piesik et al. 2008, Weaver et al. 2009, Gress et al. 2013), while post-alighting behaviors are mainly due to contact cues, including those perceived by the ovipositor (Buteler et al. 2009, Robertson et al. 2018).

These behaviors have been previously described using wheat cultivars. However, even compared to solid stem wheat cultivars that are resistant to *C. cinctus*, barley is far from an ideal host for *C. cinctus* due to larval antibiosis (Farstad 1946, Varella et al. 2018). Thus, like for wheat, do *C. cinctus* females display similar host selection and oviposition behaviors, subsequently depositing varying number of eggs in barley cultivars that represent the higher and lower levels of antibiosis traits? To answer this question, we initiated a study in the greenhouse using two barley cultivars, ‘Craft’ and ‘Hockett’. More susceptible ‘Hockett’ has a greater number of eggs per infested stem while ‘Craft’ has both a lower number of eggs and greater larval mortality due to antibiosis (Achhami et al. submitted: Chapter 3). Thus, we categorized ‘Hockett’ as a susceptible cultivar and ‘Craft’ as a more resistant cultivar. This study will improve our understanding of *C. cinctus* host plant choice and oviposition behaviors within barley cultivars, which will improve our ability to manipulate *C. cinctus* populations and reduce economic losses in the field.

Materials and Methods

Insect Preparation

For experiment, we acquired adult *C. cinctus* from wheat stubs that contained overwintering diapause larvae. Stubs were collected from a wheat field near Amsterdam, MT, USA (45°45’29.85” N, 111°22’49.32” W) and were maintained in cold storage (0−4°C) for 3−6 months to allow them to complete diapause. For adult emergence, the stored stubs were transferred into plastic Tupperware® boxes (Tupperware Corporation; Orlando, FL, USA) (70 × 35 × 20 cm) and held at room temperature (22−27°C) for 4−5 weeks. Newly emerged adults were held in 2-liter Ball® Mason glass jars (Jarden Home Brands, Daleville, IN, USA) that contained moistened filter paper and several wooden skewers to allow the adults to perch, rest, and climb until used for experiments. We used 24–48-h old adults for the experiments.

Plant Culture

Two barley cultivars developed by Montana State University were used in these experiments: ‘Hockett’ (Montana State University, 2011) and ‘Craft’ (PI 646158: developed by Montana State University). These were grown as single plants in tapered plastic cone-tainers (6.35 cm diameter top × 25.4 cm deep) in a greenhouse at the Montana State University Plant Growth Center (MSU-PGC) in Bozeman, MT USA. We used MSU-PGC soil mix (equal parts of sterilized Bozeman silt loam soil and washed concrete sand with Canadian sphagnum peat moss incorporated) and Sunshine Mix#1 (Canadian sphagnum peat moss, perlite, vermiculite, and Dolomite lime: Sun Gro Horticulture, Bellevue, WA, USA) in a 1:1 ratio as the medium for plant growth. Both natural light and artificial light (GE Multi-Vapor Lamps model MVR 1000/C/U, GE Lighting, General Electric Company, Cleveland, OH) were used, with a photoperiod of 14:10 (L: D) h, 22 ± 2^{o}C and 20–40% RH. Plants were watered daily and fertilized twice a week with Peters General Purpose Fertilizer (J.R. Peters, Allentown, PA) at 100 ppm in aqueous solution.

Y-tube Olfactometer

Our Y-tube olfactometer bioassay used a procedure similar to one first illustrated in Daisy et al. (2002) with modifications as described in Piesik et al. (2008) and Varella et al. (2016). The bioassay was used to evaluate whether *C. cinctus* females recognized and were attracted to volatiles emanating from live plants. We tested the relative attraction of *C. cinctus* females to the volatile compounds present in the airstreams passing over the two barley cultivars. Humidified air was delivered through a charcoal filter and then split into two streams using a threaded 24/410 (inner diameter 24-mm) cap with a Teflon liner coupled to a 0.64 cm Swagelok union to deliver air to a pair of glass chambers (40-mm diameter and 800-mm long). Each glass chamber enclosed a single barley plant in a pot. An enhanced spectrum LED grow light (Sunshine Systems Grow UFO Light SS−Gu90-w) was used to approximate field light levels. We sealed the base of the plant stem with a Teflon sleeve to prevent unfiltered air from entering the system. Next, Teflon tubing delivered the air from the odor source chambers to each arm of the Y-tube. Stimulus odors were conveyed to each arm of the Y-tube via glass tubes. For consistent air flow, we used an airflow of 0.1 L/min. Tests were conducted in a 28-mm diameter × 300-mm long Corning glass tubing that branched at 20 cm, had an interior angle of the "Y" of 120^o, and the diverging arms extended for 4 cm in each direction before becoming parallel to each other. The length of parallel arms measured 10 cm. We used a fiber optic illuminator (T-Q/FOI-1, TechniQuip Corp, EL Segundo, CA, USA) at the center of the Y-tube arms. For bioassays, we used a pairing of 'Hockett' and 'Craft,' as test stimuli. We used plants that were at the Zadoks 35 growth stage. This point in plant development is early in stem elongation, which is synchronized with the initiation of *C. cinctus* oviposition in the field. The planting date for the plants that were used in Y-tube olfactometer observation are listed in supplementary Table 1a.

We released individual females at the basal unbranched section of Y-tube and placed a wire (about 10 cm long) from the release point to the bifurcation to facilitate female movement upwind toward the junction of Y-tube. Each released female was observed for 5 min or until it entered one of the two arms of the Y-tube olfactometer. We repeated this process for 10 trials. For each trial we tested 17–20 females, using a new Y-tube for each female.

Volatile Collection and Quantification

We collected and quantified volatiles from the barley plants as first described in Piesik et al. (2006). Briefly, plant volatiles were collected by pulling air through a volatile collection trap for six hours between 1000 and 1600 hours. Plants of each cultivar were enclosed in a 40-mm diameter and 800-mm length glass volatile collection chamber (Analytical Research Systems, Inc., Gainesville, Florida, USA). The glass chamber was attached to a volatile collection port and was open on the other end to enclose the plant. Glass volatile collection traps (6.35-mm OD x 76-mm long; Sigma Scientific, Micanopy, FL) containing 30 mg of HayeSeq-Q adsorbent (Alltech Associates, Deerfield, IL) were inserted into each volatile collector port. In each glass chamber, purified and humidified air was supplied at a rate of 1.0 liter/min. Airflow was maintained by a regulated vacuum pump at the same flow rate. We used a Teflon guillotine (Analytical Research Systems, Gainesville, FL) to encircle the base of the plant stems; it was taped to the exterior surface of the glass chamber to prevent outside air entering into the system.

To elute the traps, we used 200 μl dichloromethane and collected the aliquot in a glass insert within a 1.5 ml screw-top glass vial using gentle pressure from purified nitrogen gas. Thereafter, we added 10 μl of the internal standard, nonyl acetate

(Sigma-Aldrich, Milwaukee, WI), in a dichloromethane solution. The collected samples were analyzed by gas chromatography-mass spectrometry (GC-MS: Agilent 6890 instrument; Agilent Technologies, Santa Clara, CA, USA) fitted with a fused silica-column (30 m × 0.25-mm) with a 0.25 μm DB-5 stationary phase. The oven was held at 50^{o}C for 4 min after injection into a 250^{o}C port and increased at 5^{o}C per min to 160^{o}C, followed by a 25^{o}C per min ramp to 280^{o}C for 3 min, with the carrier gas maintained at a flow rate of 1.2 liters/min. The samples were injected onto the column in pulsed-splitless mode that had an initial pressure of 0.84 kg/cm for 1 min. A constant temperature of 300^{o}C was maintained on the transfer line to the Mass Selective Detector (MSD). Compound detection in the eluted samples was performed by an Agilent 5973 MSD (150^{o}C) in electron-impact ionization mode scanning masses 10–300. The identities of volatile compounds were determined from the comparison to the mass spectra and retention times of authentic standards. We recorded all compounds that had a mass spectra peak area more than 1 million in the total ion chromatogram because the purity of smaller peaks was irresolvable. Subsequently, the selected compounds were identified by comparing mass spectra and retention time of authentic standard for a similar set up on this instrument, and by using the National Institute of Standards and Technology (NIST) library. The quantity of the compounds is presented as ng per gram per hour.

On each day of volatile collection, a set of six plants of each cultivar were randomly arranged within the 12-chamber volatile collection system. We conducted all collections with plants at Zadoks 34 before beginning those using plants at Zadoks 49. We repeated the collections four or five times for plants at Zadoks 34 and 49, respectively. The planting dates for the plants that were used for collecting volatiles are listed in supplementary Table 1c.

Oviposition Behaviors

We recorded *C. cinctus* oviposition behavior as described by Buteler et al. (2009) and Varella et al. (2017). For oviposition behaviors, one 'Hockett' plant and one 'Craft' plant were placed in a 46.5 × 46.5 × 91 cm cage with 530-µm mesh openings (BioQuip products, Rancho Dominguez, California, USA). In each cage with a designated pair of plants, we released 10 females and 5 males of *C. cinctus* and allowed them to mate and then oviposit. The experiments were conducted for three hours between 1000 and 1300 hours in the greenhouse. Planting dates for the plants that were used in this experiment are presented in supplementary Table 1b.

After releasing *C. cinctus* inside the cage, we recorded the frequencies and duration of each behavior for the females on both plants. For analysis, we summarized these frequencies into four categories: resting (did not move but sometimes groomed antennae), walking on the leaves and stems, abdominal tapping (several light strikes of the ovipositor on the stem), and ovipositor insertion.

At the end of the experiment, we dissected the stems and recorded the number of eggs in each stem of each cultivar and compared the number of ovipositor insertions to the number of eggs found. In addition, we compared both frequency and duration of each type of event between the two cultivars within a similar developmental stage (Zadoks 34 or 49). We used 10 pairs of plants that were at Zadoks 34 and 6 pairs of plants that were at Zadoks 49.

Oviposition Choice

Choice tests were performed to study *C. cinctus* oviposition preferences between two cultivars, ‘Craft’ and ‘Hockett’, in the greenhouse Screen cages (46.5 × 46.5 × 91 cm) with 530 μm mesh openings (BioQuip Products, Rancho Dominguez, CA) were used for this study. Each cage contained one plant of each cultivar at their similar developmental stage at Zadoks 34 or 49. Ten females and five males were released into each cage and allowed them to mate and to oviposit for three days. Thereafter, we immediately removed the plants from the cage and dissected the stems to count the number of eggs. In total, we used 25 pairs of plants at Zadoks 34 and 16 pairs of plants from Zadoks 49.

Data Analyses

For the oviposition choice-test trials, the number of eggs per plant for each cultivar at each developmental stage were analyzed using linear and nonlinear mixed effects models (nlme) (Pinheiro et al. 2019). Replicate was used as a random factor, and stem height and stem diameters were used as covariates in the model. The response variable “eggs per plant” was transformed using the log (x +2) before analysis to achieve normality of the residuals, but untransformed data are presented. Furthermore, mean stem height, and stem diameter between two cultivars and between two developmental scales were analyzed with a t-test and a Wilcoxon rank sum test respectively.

We used a factorial multivariate analysis of variance (MANOVA) to determine differences among the volatile compounds emitted by cultivars at two developmental scales. Photosynthetically active radiation (PAR), measured every hour, was acquired from the local university weather station. These data were used as a covariate in the model. The Wilks’ lambda test statistic was used to determine the significant main effect in the MANOVA. One-way ANOVAs were conducted to further analyze the differences between the cultivars within plant stage for each compound. Amounts of volatiles were transformed using the log (x+2) for normality, but untransformed data are presented.

Durations of specific oviposition behaviors (i.e., walking, abdominal tapping, and ovipositor insertion) were analyzed using linear and nonlinear mixed effects models (nlme) (Pinheiro et al. 2019). Fixed effects included cultivars and plant stage, while day of experiment was a random factor. The duration data were transformed using a Box–Cox transformation (Box and Cox 1964), but untransformed data are presented. The frequencies of each oviposition behavior were subjected to a Chi-square test to distinguish between the cultivars and between the two plant stages. Subsequently, results from Y-tube olfactometer assays were analyzed using Chi-squared test for small sample sizes (Sokal and Rohlf 1995). All analyses were performed in R version 3.6.2 (2019).

Results

Host Selection Behavioral Assays in Y-tube Olfactometer

In the Y-tube bioassays, females preferred volatiles from ‘Hockett’ over those from ‘Craft’ (χ^2 = 5.23, df = 1, *P* = 0.022: Total number of females used = 183, non-responding females = 11). The time required (in seconds) for females to decide on a preferred stimulus between the Y-tube arms for ‘Hockett’ (120.42 ± 8.66) and for ‘Craft’ (139.83 ± 13.42) was not significantly different (Kruskal-Wallis χ^2 = 1.47, df =1, *P* = 0.23).

Barley Plant Biomass and Plant Volatile Compounds

Aboveground plant biomass between the two barley cultivars was not significantly different at either developmental stage (Zadoks 34: Table 1a; Zadoks 49: Table 1b). Therefore, we compared the quantity of volatile compounds in terms of plant weight (g) as ng /g/h at each plant stage. At Zadoks scale 34, we found greater amount of linalool in ‘Craft’ volatile profiles, whereas (*Z*)-3-hexenyl acetate was more abundant in the volatile profiles from ‘Hockett’ (Table 1a). At Zadoks 49, the amounts of decanal, (*Z*)-3-hexenyl acetate, *β*- phellandrene, pentadecane, and heptadecane were different between the two cultivars (Table 1b).

Oviposition Behaviors

At both Zadoks 34 and Zadoks 49, stem diameter was greater in ‘Craft’ than in ‘Hockett’ (Zadoks 34: Wilcoxon rank sum value = 2005.5, $P < 0.001$; Zadoks 49: Wilcoxon rank sum value = 2755.5, $P < 0.001$), but the stem height was not significantly different between these cultivars at either growth stage (Zadoks 34: Wilcoxon rank sum value = 1102, $P = 0.2434$; Zadoks 49: Wilcoxon rank sum value = 1998.5, $P = 0.2673$) (Table 2).

We recorded both frequency and duration of four oviposition behaviors: (1) resting on leaves or stems, (2) walking on leaves or stems, (3) abdominal tapping on stems, and (4) ovipositor insertion, in each cultivar at each Zadoks stage. However, we removed the resting behavior from our analysis because half of the females ($n = 7$) were inactive (only displayed resting behavior) during the entire observation period.

The frequency of the remaining three oviposition behaviors (walking, abdominal tapping, and ovipositor insertion) was not significantly different between ‘Hockett’ versus ‘Craft’ stems at Zadoks 34 (Table 3). But walking and abdominal tapping frequencies were significantly greater on ‘Hockett’ stems than on ‘Craft’ on stems at Zadoks 49 (Table 3).

The duration of both walking and ovipositor insertion events were not statistically different between the two cultivars (walking: $F_{1,34} = 0.34$, $P = 0.46$; ovipositor insertion: $F_{1,34} = 0.38$, $P = 0.53$) (Table 4). Also, there was no difference in those two behaviors (walking: $F_{1,34} = 1.24$, $p = 0.27$; ovipositor insertion: $F_{1,34} = 1.08$, $P = 0.30$) between the Zadoks stages (Table 4). The duration of abdominal tapping events was not significantly different between the two cultivars ($F_{1,34} = 1.21$, $P = 0.27$) at Zadoks 34, but was significantly longer on plants at Zadoks 49 than at Zadoks 34 ($F_{1,34} = 4.19$, $P = 0.04$) (Table 4).

Host Plant Preference for Oviposition

The number of eggs laid per stem was significantly greater in 'Hockett' than in 'Craft' at both growth stages (Zadoks 34: $F_{1,92} = 10.13$, $P = 0.002$; Zadoks 49: $F_{1,113} = 6.50$, $P = 0.01$) (Table 2). Mean stem height was statistically similar in both cultivars within each growth stage (Zadoks 34: Wilcoxon rank sum value = 1102, $P = 0.24$; Zadoks 49: Wilcoxon rank sum value = 1998.5 $P = 0.27$) (Table 2). Mean stem diameter was greater in 'Craft' than in 'Hockett' within each growth stage (Zadoks 34: Wilcoxon rank sum value = 2005.5 $P < 0.001$; Zadoks 49: Wilcoxon rank sum value = 2755.5 $P < 0.001$) (Table 2). The number of tillers were not different by cultivars within each growth stage (Zadoks 34: $t_{20} = 0.67$, $P = 0.51$; Zadoks 49: $t_{18} = 0.65$, $P = 0.52$) (Table 2).

Discussion

Host plant choices and oviposition behaviors were complex in *C. cinctus* females exploring at both growth stages of these two barley cultivars. As expected from findings using wheat, these behaviors are influenced by host plant phenological traits—stem diameter, volatile compound amounts, and plant growth stage—in the barley cultivars 'Hockett' and 'Craft.' *C. cinctus* females preferred 'Hockett' over 'Craft' based on the results from Y-tube olfactometer bioassays, frequencies of oviposition behaviors (walking and abdominal tapping), and number of eggs per stem in choice trials.

Our Y-tube olfactometer results showed that while stimuli from 'Hockett' were preferred over those from 'Craft.' This result aligns with the previous study in Y-tube assays has shown that more *C. cinctus* females choose stimuli released from the susceptible wheat cultivar 'Reeder' compared with stimuli released from a resistant wheat cultivar 'Conan' (Weaver et al. 2009, Varella et al. 2016).

In our study, we found higher levels of the attractive compound, (*Z*)-3- hexenyl acetate, in the volatile profiles from more susceptible 'Hockett' than in those from more resistant 'Craft' plants at both growth stages (Zadoks 34 and 49) (Table 1). The attractiveness of (*Z*)-3-hexenyl acetate was observed in several studies using wheat (Weaver et al. 2009, Buteler et al. 2010). In addition, a susceptible wheat cultivar 'Reeder' emits more (*Z*)-3-hexenyl acetate than a resistant wheat cultivar 'Conan'

(Weaver et al. 2009). In our study, ‘Hockett’ released more (*Z*)-3-hexenyl acetate than was released by ‘Craft’ as a result, the females selected ‘Hockett’ over ‘Craft’ when presented a choice between the two cultivars.

Conversely, we measured a higher level of linalool in ‘Craft’ than in ‘Hockett,’ thus the concentration of this monoterpene plays a role in orienting *C. cinctus* females away from ‘Craft’ at close range. Linalool is well known as a defense compound in plants by either direct toxicity (Weaver et al. 1991, Davoudi et al. 2011) or repellency (Muller et al. 2009, Dekker et al. 2011) as well as an attractant for natural enemies of pests (Kessler and Baldwin 2001, Du et al. 1998). Although further study is needed to quantify the behavioral activity of linalool, our results support the attractiveness of (*Z*)-3-hexenyl acetate and the potential deterrent effect of linalool play a key role to attract females toward ‘Hockett’ than in ‘Craft.’

Gravid *C. cinctus* females land on host stems, which indicates that the female initially accepted the plant as a potential host. After landing, the female displays series of oviposition behaviors—walking, abdominal tapping, and ovipositor insertion. In our study the females did not show a difference in the duration of these oviposition behaviors between ‘Hockett’ and ‘Craft’ within each developmental scale (Zadoks 34 and 49). However, we recorded higher frequencies of walking and abdominal tapping on ‘Hockett’ than on ‘Craft’ on older plants (Zadoks scale 49). Walking on a host indicates that the female is searching for oviposition sites, while abdominal tapping is sampling for an oviposition site on a host stem (Buteler et al.

2009). Displaying higher frequencies of walking and abdominal tapping indicates that the host remains acceptable to females.

Conversely, we found a similar frequency in both cultivars on smaller plants (Zadoks 34) (Table 4). At Zadoks stage 34, only the main stem has the two separate nodes suitable for oviposition, although a plant will eventually have 3–4 elongated stems in tillers. We observed statistically similar oviposition behaviors both in durations and in frequencies in these two cultivars, which could be limited by the number of suitable tillers.

The oviposition rate— the number of eggs per stem—was higher in ‘Hockett,’ than in ‘Craft’ at both developmental stages (Zadoks 34 and 49). Several previous studies reported that ‘Hockett’ is preferred over ‘Craft’ (Varella et al. 2018, Achhami et al. submitted: Chapter 3). Oviposition in a preferred cultivar may indicate that the cultivar is supportive to the larval survival. For instance, females deposit more eggs in stems of the susceptible wheat cultivar ‘Reeder’ than in the resistant cultivar ‘Conan’ (Weaver et al. 2009). Additionally, near isogenic lines derived from susceptible parents (‘Reeder’ and ‘Scholar’) receive more eggs than isolines derived from the resistant parent ‘Conan’ (Varella et al. 2017). It is too early in this behavioral research on barley to conclude that females foreshadow offspring survival rates and deposit more eggs in a susceptible cultivar because cannibalism must occur when more than one egg is in a stem, resulting in only a single survivor (Buteler et al. 2015, Achhami et al. submitted: Chapter 5).

C. cinctus selects a host by exploiting host plant's visual and olfactory cues. For visual cues, longer stems are preferred (Buteler et al. 2009) and concomitant olfactory cues are equally important to attract and potentially stimulate oviposition (Buteler et al. 2009, Buteler and Weaver 2012; Gress et al. 2013; Robertson et al. 2018). We found greater stem diameter in 'Craft' than in 'Hockett' at both Zadoks 34 and 49. The contradictory result— not selecting a host that has larger stems using dimeter as a criteria— is perhaps due to the condition that choosing a larger stem is more applicable within the same cultivar than across cultivars (Buteler et al. 2009).

In addition to host plant traits, maternal characteristics could also influence host selection and oviposition. These characteristics are primarily driven by the short female lifespan which may result in depositing eggs in any available host. As they age, females may accept any available stem as suitable regardless of subsequent offspring survival rate. For example, eggs are routinely deposited in solid stem wheat (Varella et al. 2015, Talbert et al. 2014), downy brome (Perez-Mendoza et al. 2006; Keren et al. 2015), barley (Varella et al. 2018), and oat species (Criddle 1922; Weaver et al. 2004), despite reduced or even zero survival. A similar pattern of apparent disregard to offspring survival rates is reported in the fall armyworm, *Spodoptera frugiperda* J. E. Smith, where females oviposit more in Balsas teosinte (*Zea mays* ssp. *parviglumis* Iltis & Doebley) than in maize (*Zea mays* L.) even though larval mortality is greater in teosinte (Bernal et al. 2015). Further, female

Spodoptera littoralis Boisduval preferred to oviposit on host plants where the larval parasitism rate is lower than on the developmentally favored host plant where parasitism is higher (Sadek et al. 2010). It is possible that selecting a less preferable host is a strategy to reduce parasitism rates on *C. cinctus* offspring by limiting the number of large larvae available for parasitism. Eventually females may choose to lay eggs in any available substrate, even one as unsuitable as wheat straw (Holmes and Peterson, 1960), rather than dying without laying eggs if preferred hosts are not available.

Our study indicates that barley volatiles and stem morphological features act together in host plant selections made by female *C. cinctus*. We conducted these studies under greenhouse conditions, which could be different from field conditions that influence the plant volatile compound production and emission rates that we found in our study, particularly photosynthetically available radiation and soil moisture gradients (Gouinguené et al. 2001; Gouinguené and Turlings 2002). Additionally, further study is needed to decipher the significance of the detected volatile compounds as components of a blend, in relation to *C. cinctus* foraging behavior. However, *C. cinctus* females ultimately made a clear choice for oviposition favoring ‘Hockett’ over ‘Craft.’ Thus, we can potentially manipulate varying levels of cultivar preference in pest management by developing attractive cultivars with limited larval survival to plant as a lethal diversion away from more susceptible cultivars.

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Table 4. 1 Mean amount (± SE) of volatile compounds emitted by two barley cultivars at Zadoks 34

Cultivar	Zadoks scale	Biomass	Compound ng/g/h							
			(*Z*)-3-Hexenyl acetate	Linalool	Nonanal	Terpineol	Decanal	Elemene	*β*-Caryophyllene	α-Caryophyllene
Craft	34	9.04 ± 0.38	0.024 ± 0.005	0.960 ± 0.18	0.248 ± 0.05	0.193 ± 0.036	0.114 ± 0.017	0.068 ± 0.028	0.935 ± 0.40	0.337 ± 0.086
Hockett	34	9.41 ± 0.27	0.071 ± 0.023	0.345 ± 0.07	0.32 ± 0.105	0.138 ± 0.029	0.093 ± 0.023	0.033 ± 0.01	0.764 ± 0.42	0.467 ± 0.118
	df	1, 82	1, 82	1, 82	1, 82	1, 82	1, 82	1, 82	1, 82	1, 82
	F-value	0.13	10.26	8.05	0.078	1.93	1.47	0.63	0.001	0.88
	P-value	0.97	0.001	0.005	0.78	0.17	0.23	0.43	0.97	0.35

Table 4. 2 Mean amount (± SE) of volatile compounds emitted by two barley cultivars at Zadoks 49

Cultivar	Zadoks scale	Biomass	Compound ng/g/h												
			(*Z*)-3-Hexenyl acetate	Linalool	Nonanal	Terpineol	Decanal	Elemene	*β*-Caryophyllene	α-Caryophyllene	β-Phellandrene	Sylvestrene	Alfa-Copaene	Pentadecane	Heptadecane
Craft	49	19.92 ± 0.548	0.039 ± 0.008	0.097 ± 0.02	0.153 ± 0.05	0.047 ±0.013	0.077 ± 0.008	0.023 ± 0.005	0.344 ± 0.068	0.062 ± 0.012	0.023 ± 0.006	0.06 ± 0.014	0.032 ± 0.005	0.071 ± 0.011	0.05 ± 0.011
Hockett	49	20.03 ± 0.66	0.128 ± 0.033	0.054 ± 0.008	0.103 ± 0.01	0.023 ± 0.008	0.057 ± 0.004	0.037 ± 0.009	0.59 ± 0.112	0.086 ± 0.022	0.008 ± 0.002	0.054 ± 0.009	0.052 ± 0.007	0.019 ± 0.008	0.019 ± 0.006
	df	1, 34	1, 34	1, 34	1, 34	1, 34	1, 34	1, 34	1, 34	1, 34	1, 34	1, 34	1, 34	1, 34	1, 34
	F-value	0.01	6.79	3.13	0.98	2.86	5.04	1.64	3.22	0.24	6.13	0.24	1.001	14.22	5.68
	P-value	0.9	0.013	0.86	0.33	0.09	0.03	0.2	0.08	0.62	0.01	0.62	0.32	0.03	0.02

Table 4. 3 Stem height (mean ± SE), stem diameter (mean ± SE), and number of *Cephus cinctus* eggs per stem (mean ± SE) in oviposition choice tests using two barley cultivars at two developmental scale.

Cultivar	Zadoks scale	Height (cm)	Diameter (mm)	Number of tillers	Eggs
Craft	34	20.64 ±1.17a	2.64 ± 0.07a	4.54 ± 0.28 a	1.00 ± 0.25a
Hockett	34	22.90 ± 1.27a	2.14 ± 0.04b	4.81 ± 0.29 a	1.98 ± 0.36b
Craft	49	37.35 ± 1.88a	2.64 ± 0.07a	5.7 ± 0.67 a	0.16 ± 0.09a
Hockett	49	34.26 ± 1.84a	2.18 ± 0.04b	6.3 ± 0.63 a	0.48 ± 0.11b

Comparisons are made between rows of parameters within each Zadoks scale. Rows with different letters within a column of corresponding Zadoks scale indicate significant difference (*P* <0.05).

Table 4. 4 Summary statistics of *Cephus cinctus* oviposition behaviors frequencies for two barley cultivars

Cultivar	Zadoks scale	Behaviors								
		Walking			Abdominal tapping			Ovipositor insertion		
		Observed	Chi-square	*P*-value	Observed	Chi-square	*P*-value	Observed	Chi-square	*P*-value
Craft	34	121	1.53	0.22	19	0.22	0.64	13	0.53	0.47
Hockett	34	141			22			17		
Craft	49	153	19.26	**<0.001**	58	24.53	**<0.001**	21	1.92	0.17
Hockett	49	240			125			31		

Bold value indicates difference between cultivars within the Zadoks scale (*P* at the 0.05 level of significance).

Table 4. 5 Mean (± SE) duration in minute of *Cephus cinctus* oviposition behaviors in two barley cultivars

Cultivar	Zadoks scale	Walking	Abdominal tapping	Ovipositor insertion
Craft	34	11.62 ± 5.23a	0.72 ± 0.46a	1.006 ± 0.09a
Hockett	34	11.53 ± 6.23a	1.001 ± 0.58a	1.06 ± 0.51a
Craft	49	14.83 ± 7.03a	2.28 ± 1.47a	1.49 ± 0.78a
Hockett	49	23.47 ± 9.98a	4.96 ± 2.28a	2.51 ± 1.15a

Comparisons were made between the cultivars within Zadoks scale categories. Rows with different letters within a column with corresponding Zadoks scale indicate a significant difference (*P* at the 0.05 level of significance).

CHAPTER SEVEN

CONCLUSIONS

The goal of this study was to better develop barley as a viable option to manage WSS in North America by exploring barley antixenosis, antibiosis, and tolerance traits against WSS. Thus, we can incorporate these trait (s) into developing barley cultivars, and subsequently reduce the economic losses caused by this species.

In Chapter 3, I assessed the WSS infestation rate by counting the number of eggs, frass, and larvae per stem. Additionally, I assessed the rate of stem cutting. Based on the infestation rate, ‘Hockett’ was the most preferred cultivar. The lowest rate of stem cutting was 6.51± 0.68% (mean ± SE) in ‘Craft’ and the highest rate was 24.52 ± 1.21% in ‘Hockett’. To assessed tolerance traits, I compared grain yield within a cultivar among three categories of stem infestation status: 1) uninfested, 2) infested with dead larva(e), 3) and infested with a surviving larva that cut the ripening stem. The greater grain yield in the infested stems with dead larvae than in cut and uninfested stems in all cultivars except in 'Celebration’ could be due to tolerance traits in those cultivars.

In Chapter 4, I explored the pre-diapause (summer) larval survival rate in relation to precipitation, temperature, and growing degree days of barley. Larval survival was the lowest in ‘Craft’. Conversely, in ‘Hockett’, larval survival was not only the highest but also had more than 50% of larvae remaining alive until the crop ripened. At crop ripening, the live larvae proceed to diapause. Pre-diapause larval survival influences the WSS population the next growing season because of the univoltine life cycle. Thus, ‘Hockett’ supported a greater proportion of larvae that proceeded to diapause and subsequently increased WSS population growth in next spring.

In Chapter 5, I constructed multiple decrement life tables using all eight barley cultivars. I categorized the causes of mortality into five groups: plant defense, cannibalism, pathogens, parasitoids, and unknown factors. And, I also calculated the proportion of mortality for each given cause of mortality in the presence of other causes of mortality, as well as the irreplaceable mortality. The results revealed that plant defense and cannibalism were two major causes of mortality, while pathogens and parasitoids caused the lowest proportion of mortality. But the proportion of mortality by each cause varied by cultivar. For instance, in ‘Craft’ plant defense and in ‘Hockett’ cannibalism caused the greatest proportion of mortality.

In Chapter 6, In the greenhouse, I observed oviposition and host choice behaviors of WSS in two cultivars: ‘Hockett’ and ‘Craft’. I used plants that were at two developmental stages, Zadoks 34 and Zadoks 49. Further, I compared plant volatiles collected from these two cultivars. The Y-tube olfactometer results revealed that WSS females preferred volatile stimuli from ‘Hockett’ over those from ‘Craft’. The duration and frequencies of oviposition behaviors: walking, abdominal tapping, and ovipositor insertions were similar between these two cultivars at Zadoks scale 34. But the frequencies of these behaviors were significantly greater in ‘Hockett’ than in ‘Craft’ at Zadoks scale 49. By comparing the amount of plant volatiles, I found a significantly greater amount of linalool in ‘Craft’ while there was more (*Z*)-3-hexnyl acetate in ‘Hockett’. These results suggest host selection behaviors for WSS on barley plants are complex and are influenced by developmental stage and volatile compounds emitted by the plants at these stages.

I conducted these field studies in two locations of Montana, Big Sandy and Amsterdam, in two different years. Although both locations are rainfed, agroecologically both locations are different. Further, even for the two proximal sites near Amsterdam in 2016 and in 2017, the crop growth duration was different due to reseeding in 2017. Therefore, some of the cultivar reactions to WSS were dissimilar, such as the rate of stem cutting and resulting grain yields within a cultivar. However, my results revealed that the barley cultivars possess varying levels of antibiosis, antixenosis, and tolerance traits against WSS. Also, the results provide strong background information to use in the development of barley cultivars that can produce higher yields in spite of WSS injury.

For instance, antixenosis traits influence oviposition preference. If the traits that are favorable to gravid females lead to more egg deposition; this subsequently causes more mortality due to larval-egg cannibalism or larval cannibalism. If the traits are unfavorable to gravid females this leads to less egg deposition and a lower amount of stem infestation.

Similarly, antibiosis traits cause a higher proportion of neonate mortality in stems. There is still a limited understanding of antibiosis mechanisms that kill larvae. For example, it is not known whether the antibiosis trait limits essential nutrients for larval growth and development or results from the toxic effect of plant compounds on the developing larvae.

Further, tolerance traits ensure the potential grain yield despite the presence of larval feeding injuries in stems. Like for antibiosis traits, these tolerance traits in response to WSS larval feeding injuries are yet to be explored in barley.

In the short term, by using my results, we can categorize the barley cultivars according to their performance. For instance, a group of cultivars that attract a higher number of gravid females and also cause greater neonate mortality in stems can be recommend to growers after assessing the WSS population sizes in their fields. These categorized cultivars can be implemented to formulate integrated WSS management tools such as the deployment of trap crops.

In the long run, in depth research on these traits (antixenosis, antibiosis, and tolerance) using modern technologies such as metabolomics and transcriptomics can help to decipher the links between traits that attract gravid females and the traits that cause greater neonate mortality, as well as the traits for potential compensatory grain yield despite larval feeding in stems. Thereafter, we can isolate the desirable traits and use them to develop resistant barley cultivars. These new cultivars will either be WSS tolerant or WSS resistant and will ultimately reduce the economic losses caused by this species in North America.

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APPENDICES

APPENDIX A

CHAPTER 3 SUPPLEMENTAL INFORMATION

A.1: Number of Tunneled Nodes in Infested Stem but Dead Larvae and Infested and Stems Cutting by Mature Larvae

Cultivar	Tunneled node Mean ± SE	
	Infested stems but dead larvae	Infested and stems cutting by mature larvae
Celebration	2.8 ± 0.15	3.3 ± 0.15
Champion	2.4 ± 0.08	3.4 ± 0.1
Craft	2.1 ± 0.11	3.4 ± 0.15
Haxby	2.3 ± 0.09	3.6 ± 0.1
Haybet	2.7 ± 0.07	2.8 ± 0.09
Hockett	3.1 ± 0.1	3.6 ± 0.09
Lavina	2.2 ± 0.08	3.1 ± 0.1
Tradition	2.5 ± 0.13	3.5 ± 0.2
Mean	2.5 ± 0.03	3.3 ± 0.04

A2: Comparison of regression coefficient diameter and stem infestation status for grain yield in barley of 3 year *sites grown in Montana

Location × Year	Cultivar	Infestation status	Estimated coefficient ± SE	Infestation status
Amsterdam 2016	Celebration	Uninfested	0.43 ± 0.05	Uninfested - Infested but dead larva
		Infested but dead larva	0.59 ± 0.08	Uninfested - Infested and cut stem
		Infested and cut stem	0.07 ± 0.15	Infested but dead larva - Infested and cut stem
	Champion	Uninfested	0.34 ± 0.02	Uninfested - Infested but dead larva
		Infested but dead larva	0.22 ± 0.03	Uninfested - Infested and cut stem
		Infested and cut stem	0.26 ± 0.06	Infested but dead larva - Infested and cut stem
	Craft	Uninfested	0.21 ± 0.02	Uninfested - Infested but dead larva
		Infested but dead larva	0.14 ± 0.06	Uninfested - Infested and cut stem
		Infested and cut stem	0.04 ± 0.11	Infested but dead larva - Infested and cut stem
	Haxby	Uninfested	0.2 ± 0.02	Uninfested - Infested but dead larva
		Infested but dead larva	0.12 ± 0.02	Uninfested - Infested and cut stem
		Infested and cut stem	0.21 ± 0.04	Infested but dead larva - Infested and cut stem
	Haybet	Uninfested	0.08 ± 0.03	Uninfested - Infested but dead larva
		Infested but dead larva	0.16 ± 0.05	Uninfested - Infested and cut stem
		Infested and cut stem	0.24 ± 0.05	Infested but dead larva - Infested and cut stem
	Hockett	Uninfested	0.36 ± 0.05	Uninfested - Infested but dead larva
		Infested but dead larva	0.2 ± 0.06	Uninfested - Infested and cut stem
		Infested and cut stem	0.25 ± 0.06	Infested but dead larva - Infested and cut stem
	Lavina	Uninfested	0.09 ± 0.02	Uninfested - Infested but dead larva
		Infested but dead larva	0.13 ± 0.03	Uninfested - Infested and cut stem
		Infested and cut stem	0.08 ± 0.04	Infested but dead larva - Infested and cut stem
	Tradition	Uninfested	0.48 ± 0.04	Uninfested - Infested but dead larva

Location × Year	Cultivar	Infestation status	Estimated coefficient ± SE	Infestation status
		Infested but dead larva	0.6 ± 0.13	Uninfested - Infested and cut stem
		Infested and cut stem	0.99 ± 0.31	Infested but dead larva - Infested and cut stem
Amsterdam 2017				
	Celebration	Uninfested	0.73 ± 0.09	Uninfested - Infested but dead larva
		Infested but dead larva	0.23 ± 0.16	Uninfested - Infested and cut stem
		Infested and cut stem	0.75 ± 0.60	Infested but dead larva - Infested and cut stem
	Champion	Uninfested	0.36 ± 0.07	Uninfested - Infested but dead larva
		Infested but dead larva	0.38 ± 0.04	Uninfested - Infested and cut stem
		Infested and cut stem	0.13 ± 0.2	Infested but dead larva - Infested and cut stem
	Craft	Uninfested	0.4 ± 0.04	Uninfested - Infested but dead larva
		Infested but dead larva	0.28 ± 0.03	Uninfested - Infested and cut stem
		Infested and cut stem	0.3 ± 0.08	Infested but dead larva - Infested and cut stem
	Haxby	Uninfested	0.15 ± 0.04	Uninfested - Infested but dead larva
		Infested but dead larva	0.26 ± 0.06	Uninfested - Infested and cut stem
		Infested and cut stem	0.11 ± 0.12	Infested but dead larva - Infested and cut stem
	Haybet	Uninfested	0.17 ± 0.09	Uninfested - Infested but dead larva
		Infested but dead larva	0.2 ± 0.03	Uninfested - Infested and cut stem
		Infested and cut stem	0.05 ± 0.06	Infested but dead larva - Infested and cut stem
	Hockett	Uninfested	0.35 ± 0.06	Uninfested - Infested but dead larva
		Infested but dead larva	0.29 ± 0.07	Uninfested - Infested and cut stem
		Infested and cut stem	0.06 ± 0.07	Infested but dead larva - Infested and cut stem
	Lavina	Uninfested	0.27 ± 0.04	Uninfested - Infested but dead larva
		Infested but dead larva	0.33 ± 0.04	Uninfested - Infested and cut stem
		Infested and cut stem	0.12 ± 0.11	Infested but dead larva - Infested and cut stem

Location × Year	Cultivar	Infestation status	Estimated coefficient ± SE	Infestation status
Big Sandy 2017	Tradition	Uninfested	0.21 ± 0.11	Uninfested - Infested but dead larva
		Infested but dead larva	0.81 ± 0.12	Uninfested - Infested and cut stem
		Infested and cut stem	0.62 ± 0.18	Infested but dead larva - Infested and cut stem
	Celebration	Uninfested	0.66 ± 0.1	Uninfested - Infested but dead larva
		Infested but dead larva	0.53 ± 0.15	Uninfested - Infested and cut stem
		Infested and cut stem	0.77 ± 1.07	Infested but dead larva - Infested and cut stem
	Champion	Uninfested	0.34 ± 0.04	Uninfested - Infested but dead larva
		Infested but dead larva	0.25 ± 0.05	Uninfested - Infested and cut stem
		Infested and cut stem	0.03 ± 0.12	Infested but dead larva - Infested and cut stem
	Craft	Uninfested	0.42 ± 0.03	Uninfested - Infested but dead larva
		Infested but dead larva	0.38 ± 0.19	Uninfested - Infested and cut stem
		Infested and cut stem	0.32 ± 0.08	Infested but dead larva - Infested and cut stem
	Haxby	Uninfested	0.36 ± 0.03	Uninfested - Infested but dead larva
		Infested but dead larva	0.52 ± 0.07	Uninfested - Infested and cut stem
		Infested and cut stem	0.15 ± 0.11	Infested but dead larva - Infested and cut stem
	Haybet	Uninfested	0.2 ± 0.03	Uninfested - Infested but dead larva
		Infested but dead larva	0.17 ± 0.07	Uninfested - Infested and cut stem
		Infested and cut stem	0.17 ± 0.10	Infested but dead larva - Infested and cut stem
	Hockett	Uninfested	0.40 ± 0.04	Uninfested - Infested but dead larva
		Infested but dead larva	0.52 ± 0.07	Uninfested - Infested and cut stem
		Infested and cut stem	0.21 ± 0.10	Infested but dead larva - Infested and cut stem
	Lavina	Uninfested	0.3 ± 0.02	Uninfested - Infested but dead larva
		Infested but dead larva	0.3 ± 0.04	Uninfested - Infested and cut stem

Location × Year	Cultivar	Infestation status	Estimated coefficient ± SE	Infestation status
		Infested and cut stem	0.2 ± 0.08	Infested but dead larva - Infested and cut stem
	Tradition	Uninfested	0.21 ± 0.12	Uninfested - Infested but dead larva
		Infested but dead larva	0.81 ± 0.12	Uninfested - Infested and cut stem
		Infested and cut stem	0.63 ± 0.18	Infested but dead larva - Infested and cut stem

Figure A3: Correlation Between Seed Number and Grain Weight

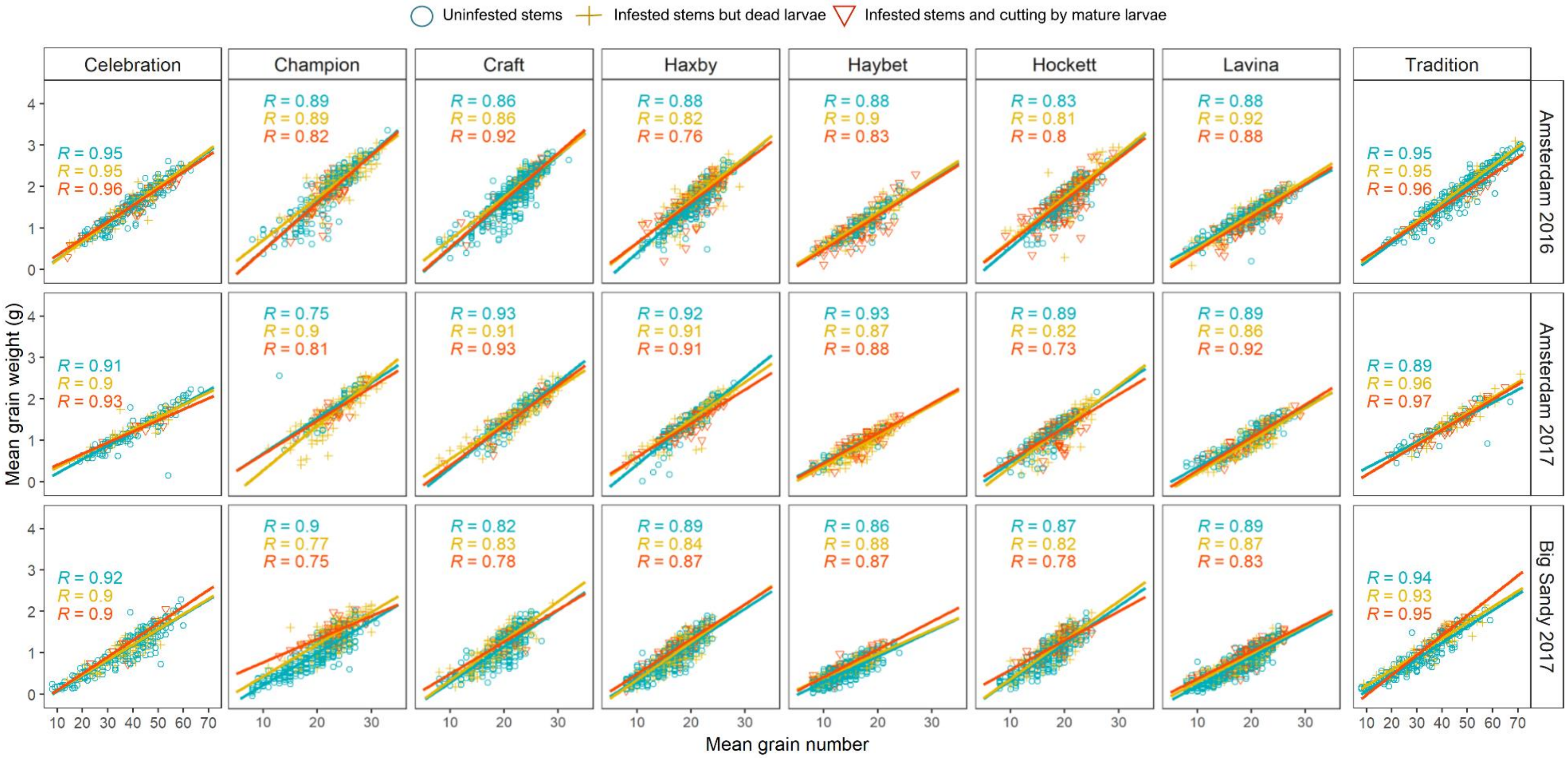

Figure A3: Correlation of seed number and grain weight by stem infestation status (uninfested, infested but dead larva, and infested and cut stems) by cultivar and site across 3 site × years in Montana. R value indicate the correlation coefficient in respective cultivar and are arranged by from top to bottom; first uninfested stems, second infested stems and dead larvae, and third infested and stem cutting by mature larvae.

APPENDIX B

CHAPTER 4 SUPPLEMENTAL INFORMATION

B1: Weather Data Amsterdam 2016

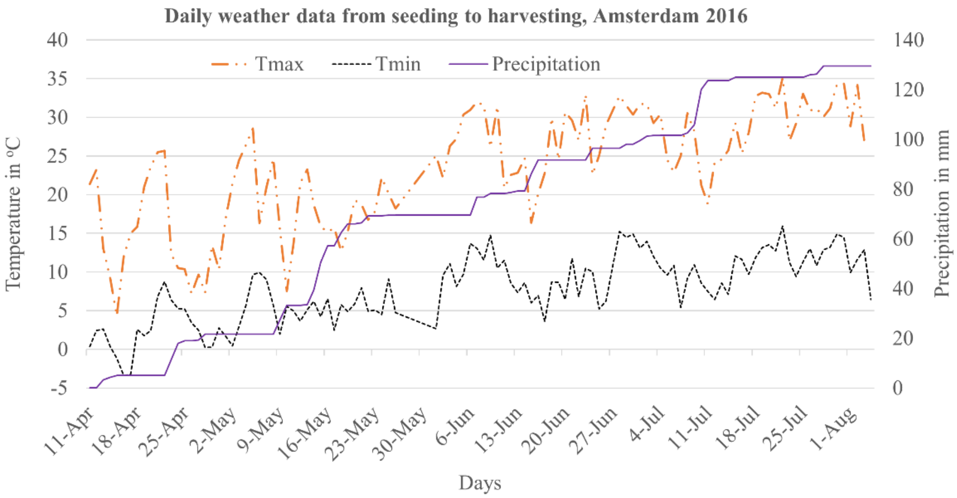

Figure B1: Daily weather data from seeding to harvesting, Amsterdam 2016

B2: Weather Data Amsterdam 2017

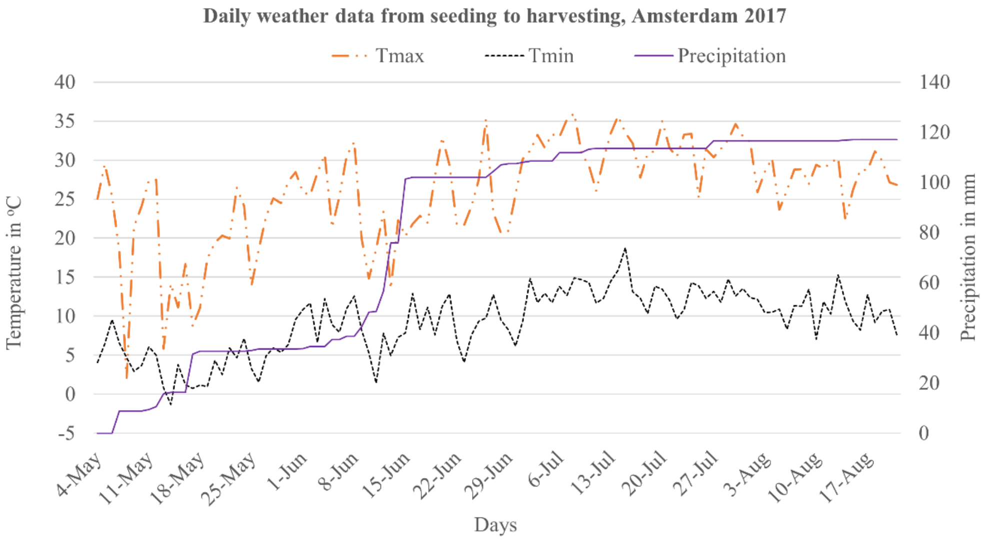

Figure B2: Daily weather data from seeding to harvesting, Amsterdam 2017

B3: Weather Data Big Sandy 2017

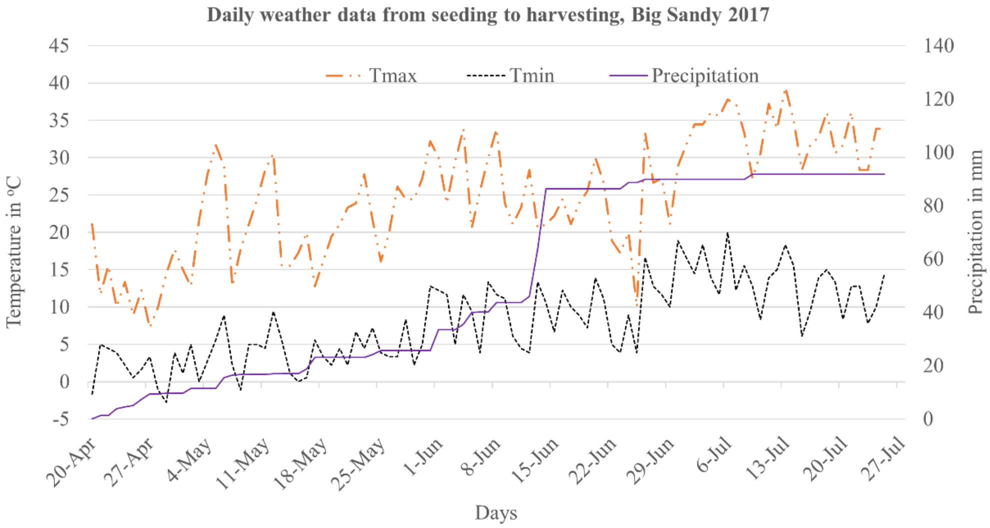

Figure B3: Daily weather data from seeding to harvesting, Big Sandy 2017

B4: PCA Degree days, Total Precipitation and Survival Proportion

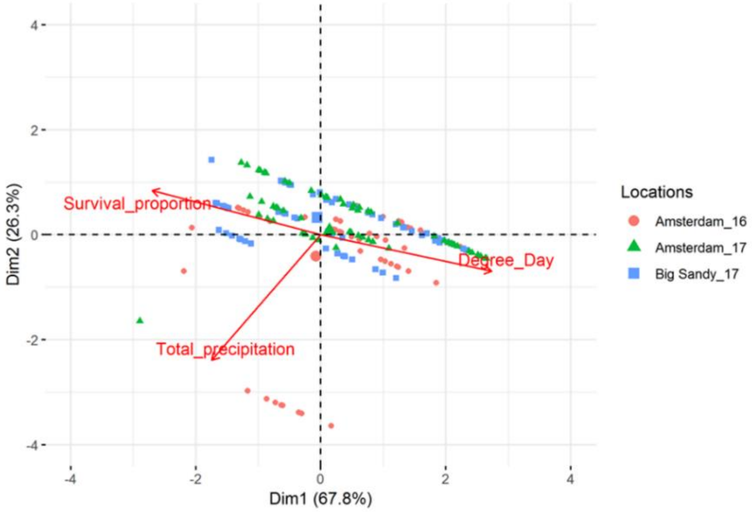

Figure B4: Principal component analysis (PCA), comparing the relationship between the total precipitation and degree-days on larval survival proportions in three locations. Each point represents weekly survival in one cultivar at a corresponding location.

APPENDIX C

CHAPTER 5 SUPPLEMENTAL INFORMATION

C1: Multiple decrement life tables of wheat stem sawfly, Cephus cinctus, in barley cultivars that were grown at Amsterdam 2016 and 2017, and Big Sandy 2017 in Montana

Location	Cultivar	Category, x	Fraction Dying, aqx	Fraction of living,	Fraction of	Parasitism, aq1x	Plant defense, aq2x	Cannibalism, aq3x	Unknown, aq4x	Pathogen,
Amsterdam 2016	Celebration	Egg	0.24	1.00	0.24	0.00	0.00	0.24	0.00	0.00
		Larva I	0.51	0.76	0.39	0.00	0.31	0.05	0.03	0.00
		Larva III	0.49	0.37	0.18	0.01	0.09	0.00	0.07	0.00
		Larva IV	0.18	0.19	0.03	0.00	0.00	0.00	0.03	0.00
		Larva V	0.16	0.15	0.02	0.00	0.00	0.00	0.02	0.00
		Adult	0.00	0.13	0.13	0.00	0.00	0.00	0.00	0.00
		Total				0.02	0.40	0.30	0.15	0.00
	Champion	Egg	0.41	1.00	0.41	0.00	0.00	0.41	0.00	0.00
		Larva I	0.63	0.59	0.37	0.00	0.29	0.05	0.04	0.00
		Larva III	0.67	0.22	0.15	0.00	0.11	0.00	0.03	0.00
		Larva IV	0.14	0.07	0.01	0.00	0.00	0.00	0.01	0.00
		Larva V	0.04	0.06	0.00	0.00	0.00	0.00	0.00	0.00
		Adult	0.00	0.06	0.06	0.00	0.00	0.00	0.00	0.00
		Total				0.00	0.40	0.46	0.08	0.00
	Craft	Egg	0.06	1.00	0.06	0.00	0.00	0.06	0.00	0.00
		Larva I	0.78	0.94	0.74	0.00	0.70	0.01	0.03	0.00
		Larva III	0.79	0.21	0.16	0.00	0.14	0.00	0.03	0.00
		Larva IV	0.29	0.04	0.01	0.01	0.00	0.00	0.00	0.00
		Larva V	0.20	0.03	0.01	0.01	0.00	0.00	0.00	0.00
		Adult	0.00	0.02	0.02	0.00	0.00	0.00	0.00	0.00
		Total				0.02	0.84	0.06	0.06	0.00
	Haxby	Egg	0.27	1.00	0.27	0.00	0.00	0.27	0.00	0.00
		Larva I	0.59	0.73	0.43	0.00	0.34	0.06	0.03	0.00
		Larva III	0.60	0.30	0.18	0.00	0.13	0.01	0.04	0.00

Location	Cultivar	Category, x	Fraction Dying, aqx	Fraction of living,	Fraction of	Parasitism, aq1x	Plant defense, aq2x	Cannibalism, aq3x	Unknown, aq4x	Pathogen,
		Larva IV	0.03	0.12	0.00	0.00	0.00	0.00	0.00	0.00
		Larva V	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00
		Adult	0.00	0.12	0.12	0.00	0.00	0.00	0.00	0.00
		Total				0.00	0.47	0.34	0.07	0.00
	Haybet	Egg	0.33	1.00	0.33	0.00	0.00	0.33	0.00	0.00
		Larva I	0.47	0.67	0.32	0.00	0.18	0.11	0.03	0.00
		Larva III	0.50	0.36	0.18	0.01	0.12	0.01	0.04	0.00
		Larva IV	0.09	0.18	0.02	0.02	0.00	0.00	0.00	0.00
		Larva V	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00
		Adult	0.00	0.16	0.16	0.00	0.00	0.00	0.00	0.00
		Total				0.02	0.30	0.44	0.07	0.00
	Hockett	Egg	0.45	1.00	0.45	0.00	0.00	0.45	0.00	0.00
		Larva I	0.47	0.55	0.26	0.00	0.12	0.11	0.02	0.00
		Larva III	0.33	0.29	0.10	0.00	0.05	0.01	0.04	0.00
		Larva IV	0.12	0.20	0.02	0.00	0.00	0.00	0.02	0.00
		Larva V	0.12	0.17	0.02	0.01	0.00	0.00	0.01	0.00
		Adult	0.00	0.15	0.15	0.00	0.00	0.00	0.00	0.00
		Total				0.02	0.17	0.57	0.09	0.00
	Lavina	Egg	0.27	1.00	0.27	0.00	0.00	0.27	0.00	0.00
		Larva I	0.54	0.73	0.40	0.00	0.30	0.08	0.03	0.00
		Larva III	0.60	0.34	0.20	0.01	0.13	0.01	0.06	0.00
		Larva IV	0.20	0.13	0.03	0.00	0.00	0.00	0.03	0.00
		Larva V	0.50	0.11	0.05	0.05	0.00	0.00	0.00	0.00
		Adult	0.00	0.05	0.05	0.00	0.00	0.00	0.00	0.00
		Total				0.06	0.43	0.35	0.11	0.00
	Tradition	Egg	0.05	1.00	0.05	0.00	0.00	0.05	0.00	0.00
		Larva I	0.49	0.95	0.46	0.00	0.35	0.09	0.03	0.00

Location	Cultivar	Category, x	Fraction Dying, aqx	Fraction of living,	Fraction of	Parasitism, aq1x	Plant defense, aq2x	Cannibalism, aq3x	Unknown, aq4x	Pathogen,
		Larva III	0.61	0.48	0.30	0.00	0.20	0.01	0.09	0.00
		Larva IV	0.08	0.19	0.01	0.00	0.00	0.00	0.01	0.00
		Larva V	0.02	0.17	0.00	0.00	0.00	0.00	0.00	0.00
		Adult	0.00	0.17	0.17	0.00	0.00	0.00	0.00	0.00
		Total				0.00	0.55	0.14	0.13	0.00
Amsterdam 2017	Celebration	Egg	0.63	1.00	0.63	0.00	0.00	0.63	0.00	0.00
		Larva I	0.49	0.37	0.18	0.00	0.13	0.05	0.00	0.00
		Larva III	0.50	0.19	0.09	0.01	0.06	0.01	0.02	0.00
		Larva IV	0.18	0.10	0.02	0.01	0.00	0.00	0.01	0.00
		Larva V	0.17	0.08	0.01	0.01	0.00	0.00	0.01	0.00
		Adult	0.00	0.06	0.06	0.00	0.00	0.00	0.00	0.00
		Total				0.02	0.19	0.68	0.04	0.00
	Champion	Egg	0.69	1.00	0.69	0.00	0.00	0.69	0.00	0.00
		Larva I	0.82	0.31	0.25	0.00	0.16	0.09	0.00	0.00
		Larva III	0.56	0.06	0.03	0.01	0.02	0.00	0.01	0.00
		Larva IV	0.13	0.02	0.00	0.00	0.00	0.00	0.00	0.00
		Larva V	0.26	0.02	0.01	0.00	0.00	0.00	0.00	0.00
		Adult	0.00	0.02	0.02	0.00	0.00	0.00	0.00	0.00
		Total				0.01	0.17	0.79	0.02	0.00
	Craft	Egg	0.64	1.00	0.64	0.00	0.00	0.64	0.00	0.00
		Larva I	0.57	0.36	0.20	0.00	0.15	0.05	0.00	0.00
		Larva III	0.62	0.15	0.09	0.01	0.07	0.00	0.01	0.00
		Larva IV	0.17	0.06	0.01	0.00	0.00	0.00	0.01	0.00
		Larva V	0.10	0.05	0.00	0.00	0.00	0.00	0.00	0.00
		Adult	0.00	0.04	0.04	0.00	0.00	0.00	0.00	0.00

Location	Cultivar	Category, x	Fraction Dying, aqx	Fraction of living,	Fraction of	Parasitism, aq1x	Plant defense, aq2x	Cannibalism, aq3x	Unknown, aq4x	Pathogen,
		Total				0.01	0.22	0.70	0.03	0.00
	Haxby	Egg	0.77	1.00	0.77	0.00	0.00	0.77	0.00	0.00
		Larva I	0.50	0.23	0.11	0.00	0.06	0.05	0.00	0.00
		Larva III	0.54	0.11	0.06	0.01	0.03	0.01	0.01	0.00
		Larva IV	0.10	0.05	0.01	0.00	0.00	0.00	0.00	0.00
		Larva V	0.19	0.05	0.01	0.00	0.00	0.00	0.01	0.00
		Adult	0.00	0.04	0.04	0.00	0.00	0.00	0.00	0.00
		Total				0.01	0.09	0.83	0.02	0.00
	Haybet	Egg	0.83	1.00	0.83	0.00	0.00	0.83	0.00	0.00
		Larva I	0.42	0.17	0.07	0.00	0.02	0.05	0.00	0.00
		Larva III	0.53	0.10	0.05	0.01	0.01	0.01	0.02	0.00
		Larva IV	0.20	0.05	0.01	0.00	0.00	0.00	0.01	0.00
		Larva V	0.23	0.04	0.01	0.00	0.00	0.00	0.01	0.00
		Adult	0.00	0.03	0.03	0.00	0.00	0.00	0.00	0.00
		Total				0.02	0.03	0.88	0.04	0.00
	Hockett	Egg	0.81	1.00	0.81	0.00	0.00	0.81	0.00	0.00
		Larva I	0.41	0.19	0.08	0.00	0.02	0.06	0.00	0.00
		Larva III	0.41	0.11	0.05	0.01	0.01	0.01	0.01	0.00
		Larva IV	0.08	0.07	0.01	0.00	0.00	0.00	0.01	0.00
		Larva V	0.11	0.06	0.01	0.00	0.00	0.00	0.00	0.00
		Adult	0.00	0.05	0.05	0.00	0.00	0.00	0.00	0.00
		Total				0.01	0.02	0.88	0.02	0.00
	Lavina	Egg	0.69	1.00	0.69	0.00	0.00	0.69	0.00	0.00
		Larva I	0.45	0.31	0.14	0.00	0.06	0.07	0.00	0.00
		Larva III	0.51	0.17	0.09	0.03	0.03	0.01	0.02	0.00
		Larva IV	0.26	0.08	0.02	0.01	0.00	0.00	0.01	0.00
		Larva V	0.16	0.06	0.01	0.01	0.00	0.00	0.00	0.00

Location	Cultivar	Category, x	Fraction Dying, aqx	Fraction of living,	Fraction of	Parasitism, aq1x	Plant defense, aq2x	Cannibalism, aq3x	Unknown, aq4x	Pathogen,
		Adult	0.00	0.05	0.05	0.00	0.00	0.00	0.00	0.00
		Total				0.04	0.09	0.78	0.04	0.00
	Tradition	Egg	0.30	1.00	0.30	0.00	0.00	0.30	0.00	0.00
		Larva I	0.45	0.70	0.32	0.00	0.18	0.13	0.00	0.00
		Larva III	0.47	0.38	0.18	0.03	0.07	0.02	0.06	0.00
		Larva IV	0.13	0.20	0.03	0.00	0.00	0.00	0.02	0.00
		Larva V	0.13	0.17	0.02	0.00	0.00	0.00	0.02	0.01
		Adult	0.00	0.15	0.15	0.00	0.00	0.00	0.00	0.00
		Total				0.04	0.25	0.46	0.10	0.01
Big Sandy	Celebration	Egg	0.55	1.00	0.55	0.00	0.00	0.55	0.00	0.00
		Larva I	0.45	0.45	0.20	0.00	0.12	0.06	0.02	0.00
		Larva III	0.73	0.24	0.18	0.01	0.14	0.01	0.03	0.00
		Larva IV	0.19	0.07	0.01	0.00	0.00	0.00	0.01	0.00
		Larva V	0.10	0.05	0.01	0.00	0.00	0.00	0.00	0.00
		Adult	0.00	0.05	0.05	0.00	0.00	0.00	0.00	0.00
		Total				0.01	0.26	0.62	0.06	0.00
	Champion	Egg	0.60	1.00	0.60	0.00	0.00	0.60	0.00	0.00
		Larva I	0.53	0.40	0.21	0.00	0.13	0.07	0.01	0.00
		Larva III	0.71	0.19	0.13	0.00	0.11	0.00	0.02	0.00
		Larva IV	0.17	0.06	0.01	0.00	0.00	0.00	0.01	0.00
		Larva V	0.14	0.05	0.01	0.00	0.00	0.00	0.00	0.00
		Adult	0.00	0.04	0.04	0.00	0.00	0.00	0.00	0.00
		Total				0.00	0.24	0.68	0.04	0.00
	Craft	Egg	0.70	1.00	0.70	0.00	0.00	0.70	0.00	0.00
		Larva I	0.47	0.30	0.14	0.00	0.10	0.04	0.00	0.00
		Larva III	0.81	0.16	0.13	0.00	0.11	0.00	0.01	0.00

Location	Cultivar	Category, x	Fraction Dying, aqx	Fraction of living,	Fraction of	Parasitism, aq1x	Plant defense, aq2x	Cannibalism, aq3x	Unknown, aq4x	Pathogen,
		Larva IV	0.19	0.03	0.01	0.00	0.00	0.00	0.01	0.00
		Larva V	0.24	0.02	0.01	0.00	0.00	0.00	0.00	0.00
		Adult	0.00	0.02	0.02	0.00	0.00	0.00	0.00	0.00
		Total				0.00	0.21	0.74	0.03	0.00
	Haxby	Egg	0.70	1.00	0.70	0.00	0.00	0.70	0.00	0.00
		Larva I	0.44	0.30	0.13	0.00	0.05	0.08	0.01	0.00
		Larva III	0.59	0.17	0.10	0.01	0.06	0.00	0.03	0.00
		Larva IV	0.11	0.07	0.01	0.00	0.00	0.00	0.01	0.00
		Larva V	0.10	0.06	0.01	0.00	0.00	0.00	0.00	0.00
		Adult	0.00	0.06	0.06	0.00	0.00	0.00	0.00	0.00
		Total				0.01	0.11	0.78	0.05	0.00
	Haybet	Egg	0.52	1.00	0.52	0.00	0.00	0.52	0.00	0.00
		Larva I	0.38	0.48	0.18	0.00	0.07	0.11	0.00	0.00
		Larva III	0.57	0.29	0.17	0.01	0.10	0.01	0.05	0.00
		Larva IV	0.22	0.13	0.03	0.00	0.00	0.00	0.03	0.00
		Larva V	0.14	0.10	0.01	0.00	0.00	0.00	0.01	0.00
		Adult	0.00	0.08	0.08	0.00	0.00	0.00	0.00	0.00
		Total				0.01	0.16	0.65	0.09	0.00
	Hockett	Egg	0.73	1.00	0.73	0.00	0.00	0.73	0.00	0.00
		Larva I	0.42	0.27	0.12	0.00	0.04	0.08	0.00	0.00
		Larva III	0.33	0.16	0.05	0.00	0.03	0.01	0.01	0.00
		Larva IV	0.18	0.11	0.02	0.00	0.00	0.00	0.02	0.00
		Larva V	0.09	0.09	0.01	0.00	0.00	0.00	0.01	0.00
		Adult	0.00	0.08	0.08	0.00	0.00	0.00	0.00	0.00
		Total				0.00	0.07	0.82	0.03	0.00
	Lavina	Egg	0.41	1.00	0.41	0.00	0.00	0.41	0.00	0.00
		Larva I	0.37	0.59	0.22	0.00	0.08	0.13	0.00	0.00
		Larva III	0.56	0.38	0.21	0.01	0.13	0.01	0.06	0.00

Location	Cultivar	Category, x	Fraction Dying, aqx	Fraction of living,	Fraction of	Parasitism, aq1x	Plant defense, aq2x	Cannibalism, aq3x	Unknown, aq4x	Pathogen,
		Larva IV	0.25	0.17	0.04	0.00	0.00	0.00	0.04	0.00
		Larva V	0.13	0.12	0.02	0.00	0.00	0.00	0.01	0.00
		Adult	0.00	0.11	0.11	0.00	0.00	0.00	0.00	0.00
		Total				0.01	0.22	0.55	0.11	0.00
	Tradition	Egg	0.57	1.00	0.57	0.00	0.00	0.57	0.00	0.00
		Larva I	0.40	0.43	0.17	0.00	0.07	0.09	0.01	0.00
		Larva III	0.50	0.26	0.13	0.01	0.07	0.01	0.03	0.00
		Larva IV	0.13	0.13	0.02	0.00	0.00	0.00	0.02	0.00
		Larva V	0.21	0.11	0.02	0.00	0.00	0.00	0.02	0.01
		Adult	0.00	0.09	0.09	0.00	0.00	0.00	0.00	0.00
		Total				0.01	0.14	0.67	0.08	0.01

Larva I: pre-parasitism period; Larva III: parasitism period; Larva IV: overwintered larva (pre-flight period); Larva V: overwintered larva (Post-flight period).

aq_x = fraction of death caused by all the given mortality causes in the stage x given that the individual is alive at the beginning of stage x; al_x = fraction of survivor at stage x out of original cohort of all; ad_x = fraction of deaths in stage x from all the mortality causes; aq_{ix} = fraction of death from cause i in stage x in the presence of all other mortality causes given that the individual is live at the beginning of state x.

C2: Estimated total mortality in C. cinctus for different cause-specific combinations

Mortality causes	Number of causes	Estimated total mortality
Parasitism	1	0.104
Plant defense	1	0.573
Cannibalism	1	0.717
Unknown factors	1	0.355
Pathogen	1	0.027
Parasitism + Plant defense	2	0.617
Parasitism + Cannibalism	2	0.746
Parasitism + Unknown factors	2	0.422
Plant defense + Cannibalism	2	0.879
Plant defense + Unknown factors	2	0.724
Cannibalism + Unknown factors	2	0.817
Parasitism + Pathogen	2	0.128
Plant defense + Pathogen	2	0.584
Cannibalism + Pathogen	2	0.724
Unknown factors + Pathogen	2	0.372
Parasitism + Plant defense + Cannibalism	3	0.892
Parasitism + Plant defense + Unknown factors	3	0.753
Parasitism + Cannibalism + Unknown factors	3	0.836
Plant defense + Cannibalism + Unknown factors	3	0.922
Parasitism+ Plant defense + Pathogen	3	0.627
Parasitism+ Cannibalism + Pathogen	3	0.753
Parasitism+ Unknown factors + Pathogen	3	0.438
Plant defense + Cannibalism + Pathogen	3	0.882
Plant defense + Unknown factors + Pathogen	3	0.732
Cannibalism + Unknown factors + Pathogen	3	0.822
Parasitism + Plant defense + Cannibalism + Unknown factors	4	0.930
Parasitism + Plant defense + Cannibalism + Pathogen	4	0.894
Parasitism + Plant defense + Unknown factors + Pathogen	4	0.760
Parasitism + Cannibalism + Unknown factors + Pathogen	4	0.841
Plant defense + Cannibalism + Unknown factors + Pathogen	4	0.924
Parasitism + Plant defense + Cannibalism + Unknown factors + Pathogen	5	0.932

APPENDIX D

CHAPTER 6 SUPPLEMENTAL INFORMATION

D1: Barley plantings and Y-tube olfactometer observation date

SN	Planting date	Observation date	Zadoks scale	Observation frequencies
1	6/6/2018	7/6/2018	34	1
2	6/13/2018	7/17/2018	34	1
3	6/26/2018	7/26/2018	34	1
4	7/13/2018	7/13/2018	34	1
5	7/9/2018	8/26/2018	34	1
6	9/30/2018	10/30/2018	34	1
7	10/1/2018	11/1/2018	34	2
8	10/20/2018	11/20/2018	34	2

D2: Barley plantings and oviposition choice behaviors observation date

SN	Planting date	Observation date	Zadoks scale	Number of observations
1	5/23/2018	6/20/2018	34	1
2	6/13/2018	7/11/2018	34	1
3	6/6/2015	7/6/2018	34	1
4	7/4/2018	8/3/2018	34	2
5	7/12/2018	8/13/2018	34	2
6	7/19/2018	8/19/2018	34	2
7	9/12/2018	10/15/2018	34	1
8	6/13/2018	7/24/2018	49	1
9	7/12/2018	8/22/2018	49	2
10	7/26/2018	9/5/2018	49	2
11	9/5/2018	10/17/2018	49	2
12	9/12/2018	10/24/2018	49	2

D3: Barley plantings and volatile compounds collection date

SN	Planting date	Collection date	Zadoks scale	Collection frequencies
1	7/19/2018	8/16/2018	34	1
		8/17/2018	34	1
2	7/23/2018	8/23/2018	34	1
		8/24/2018	34	1
3	9/12/2018	10/12/2018	34	1
		10/14/2018	34	1
4	11/25/2018	12/24/2018	34	1
		12/25/2018	34	1
		12/26/2018	34	1
		12/27/2018	34	1
		12/29/2018	34	1
5	7/19/2018	8/21/2018	49	1
		8/22/2018	49	1
6	9/5/2018	10/19/2018	49	1
7	9/20/2018	11/3/2018	49	1
		11/4/2018	49	1
8	11/25/2018	12/24/2018	35	1
		12/25/2018	35	1
		12/26/2018	35	1
		12/27/2018	35	1
		12/29/2018	35	1