DISSERTATION

WHEAT STEM SAWFLY OVIPOSITION PREFERENCE AND SURVIVORSHIP ON WINTER WHEAT AND DOWNY BROME

Submitted by

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ABSTRACT

WHEAT STEM SAWFLY OVIPOSITION PREFERENCE AND SURVIVORSHIP ON WINTER WHEAT AND DOWNY BROME

The wheat stem sawfly *Cephus cinctus* Norton (Hymenoptera: Cephidae) is a major economic pest of wheat in the United States can decrease the yield and grain quality. Wheat stem sawfly infests both cultivated and uncultivated host plants. It is important to understand the oviposition behavior and host selection of the female wheat stem sawfly.

Wheat stem sawfly selects the most suitable host for oviposition. The host selection behavior and oviposition preference of wheat stem sawfly for downy brome (*Bromus tectorum* L.) grass and winter wheat (*Triticum aestivum* L.) in Colorado was studied to assess whether downy brome is affecting the presence and infestation levels of wheat stem sawfly in winter wheat in northeastern Colorado. Infestation rates and larval development of the wheat stem sawfly were studied for two years (2013 and 2014) at seven commercial winter wheat fields and eight nearby downy brome sites in northeastern Colorado. Stem samples of each plant species were randomly collected per site weekly and sweep samples of sawfly adults were taken after adult emergence. Infestation rate in winter wheat was 12 to nearly 14 times higher than in downy brome throughout the two year survey. Larval mortality was twice as high in downy brome than in winter wheat over the two year survey. More adults were collected in May from winter wheat than from downy brome in the two year survey. Wheat stem sawfly females preferred to oviposit in wheat plants over downy brome.

Female oviposition behavior influences by different host quality cues such stem height and diameter, volatile production, and growth stage. Host selection and oviposition preference by wheat stem sawfly females were studied with a combination of greenhouse choice and no-choice tests using a susceptible (hollow stem) winter wheat variety 'Byrd', a resistant (solid stem) winter wheat variety 'Bearpaw', and downy brome. Female sawflies in the no-choice tests laid similar numbers of eggs in Byrd and Bearpaw plants irrespective of growth stage, which were approximately 2.5 times the number of eggs laid on downy brome. Similarly, when given a choice, females laid similar numbers of eggs on Byrd and Bearpaw, but nearly twice as many eggs on either of these two cultivars than on downy brome. Females preferred to oviposit in larger diameter plant stems. However, stem height did not affect female preference for plants at growth stage Zadoks 49, but females prefered taller stems at growth stages Zadoks 32 and 60. Larval survivorship was lowest in the solid stem Bearpaw and was highest in downy brome and the hollow-stem Byrd. Hollow stem wheat had the highest larval survivorship.

Female wheat stem sawflies use chemical cues from plants to identify a suitable host for oviposition. A Y-tube bioassay was developed to evaluate female wheat stem sawfly behavior in response to an airflow that passed over winter wheat cultivars and downy brome. Choice and no-choice tests were conducted with three host plants: wheat cultivars Byrd and Bearpaw and downy brome at three different growth stages: Zadoks 32, 49, and 60. Adult females were attracted to wheat cultivars Byrd and Bearpaw over downy brome at all growth stages. Downy brome was least preferred (31.82%) by female wheat stem sawfly in choice test of Bearpaw vs. downy brome at Zadoks 32 while it was equally preferred (49.21%) in the choice test of 'Byrd vs. downy brome' at growth stage of Zadoks 60. The female's response speed did not differ when given a choice between the two wheat cultivars Byrd and Bearpaw. However, females were faster in making a choice (100% ≈ 86% females made fast choice) toward each of wheat cultivars over downy brome in the choice tests of Bearpaw vs. downy brome and Byrd vs. downy brome at all growth stages.

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CHAPTER 1- INTRODUCTION

The wheat stem sawfly *Cephus cinctus* Norton (Hymenoptera: Cephidae) is a pest of major economic importance to wheat in the United States. Yield in wheat infested by wheat stem sawfly can be decreased by more than 25%, resulting in annual losses of almost US$350 million in the US and Canada (Beres *et al.* 2011). The wheat stem sawfly has damaged spring wheat primarily in the northern plains but is becoming an increasing problem on winter wheat in more southern wheat growing regions.

While the wheat stem sawfly was first reported in Colorado in 1872 feeding on native grasses such as western wheat grass, basin wildrye, and other wheat grasses in the genus *Agropyron,* infestation and damage to winter wheat was not reported in Colorado until 2010 (Irell & Peairs 2011). Sawflies also survive on a number of wild grasses, including species of *Agropyron*, *Bromus*, *Elymus* and *Elytrigia*, in addition to other cereal crops such as barley and rye (Ainslie 1929, Shanower & Hoelmer 2004).

Cephus cinctus has become the most serious insect pest of wheat in the western US and Canada. It was reported in 1895 infesting wheat in Manitoba. The wheat stem sawfly was found on wheat at Bozeman, Montana, in 1900 (Montana Agricultural Experiment Station and Montana Extension Service 1946). In 1902, *C. cinctus* larvae were found in different grass species in the Northwest Territories (Ainslie 1920). In 1907, this pest was found in wheat at Minot, North Dakota (Ainslie 1920). In 1910, the wheat stem sawfly damage was reported in Montana at Bainville (Montana Agricultural Experiment Station and Montana Extension Service 1946). In 1911, Ainslie found *C. cinctus* feeding in native grasses in some western states (Ainslie 1920). The first recorded severe infestation and damage to wheat occurred in 1922 in western Canada

(Criddle 1923). In 1926, damage and losses were reported in Canada (King 1929). The economic importance of this sawfly started to increase in Montana and North Dakota from 1943 to 1946 (Callenbach & Reinhardt 1945), and in Canada in 1955 (Bird 1955).

Wheat Stem Sawfly Biology

The wheat stem sawfly is a univoltine, herbivorous wasp. Males emerge a few days before females in late May or early June. They become active when temperatures are above 10˚C. Usually, adults live about one week and emergence lasts about 3-4 weeks. The first females to emerge mate with males and lay fertilized eggs that produce daughters, while the later emerging females lay unfertilized eggs that produce sons presumably because males die earlier and thus are not available to mate. Females lay about 30-50 eggs over their adult lifespan (Ainslie 1920, 1929).

Adults fly only far enough to locate a suitable host plant, typically a maximum of several miles from their site of emergence. Wheat stem sawfly larvae develop and feed within stems, leaving it packed with frass. Although multiple eggs can be laid per stem, only one larva will survive to produce an adult because the larvae are cannibalistic (Criddle 1923, Holmes 1982). As the plants senesce in late summer, the larvae move to the base of the dry stems to diapause. The larvae make a "v"-shaped cut on the inside of the stem. Stems weakened in this manner may break before harvest ("lodge"), especially after wind events. The larvae overwinter in the stubble close to the soil. The larva creates a membrane-like case to protect it from harsh conditions such as excessive moisture or desiccation (Holmes & Peterson 1960). Diapause is broken as the temperature, moisture levels, and photoperiods increase in the spring after temperatures have remained below 10˚C for at least 90 days. The larvae initiate pupation when temperature and moisture conditions are suitable. The pupal stage lasts almost two weeks. The adult will chew through the frass plug and emerge from the stubble or grasses.

Management of Wheat Stem Sawfly

There are several methods to manage infestations of wheat stem sawfly, including plant resistance, cultural control, biological control, and insecticides. Resistant wheat varieties have a "solid-stem" trait that is correlated with mortality of wheat stem sawfly larvae. Cultural control practices such as delayed plantings, crop rotations, and tillage are also used to manage infestations. Development of resistant wheat cultivars and various tillage operations to destroy larvae and/or pupae in the stubble are considered to be general approaches to wheat stem sawfly management (Shanower & Hoelmer 2004). The use of pesticides to control sawflies, on the other hand, is not considered effective because stem-boring larvae are generally inaccessible to available insecticides. The wheat stem sawfly has been the target of biological control programs in Canada and the United States, with variable levels of success (Shanower & Hoelmer 2004).

Biological Control and Parasitoids

Nine species of hymenopteran parasitoids have been recorded from *C. cinctus* (Morrill *et al.* 1998; Meers 2005) in western North America, of which the most important are *Bracon cephi* (Nelson & Farstad 1953, Meers 2005) and *B. lissogaster* (Meers 2005). Both Ainslie (1920) and Criddle (1923) reported parasitoid species attacking larvae in grass stems, but not in wheat stems. Of the nine recorded parasitoids of *C. cinctus*, only *B. cephi* and *B. lissogaster* have been found in *C. cinctus* populations attacking wheat (Morrill *et al.* 1998).

The wheat stem sawfly shifted rapidly from wild grasses to wheat, but the shift of parasitoids has been slower (Beres *et al.* 2011). *Bracon lissogaster* is now active in Montana and North Dakota (Meers 2005). The parasitoids *B. cephi* and *B. lissogaster* cause significant sawfly mortality. These species are difficult to separate because they have similar biology and impacts on wheat stem sawflies. The first parasitoid generation could cause significant reduction in infestation and, thus, in yield loss (Beres *et al.* 2011, Buteler *et al.* 2008). The success of the second generation depends on crop maturity and the timing of overwintering chamber formation (Beres *et al.* 2011, Holmes *et al.* 1963). The second generation can be successful if wheat does not mature until mid-August. An important aspect of wheat stem sawfly population dynamics is larval mortality caused by the parasitoid *B. cephi* (Holmes *et al.* 1963). The effects of different parasitoid species depend on their host's habitat. *Bracon lissogaster* prefers sawflies feeding in wild grasses over cultivated cereals. Recent studies have shown that *Bracon cephi* can survive in solid-stem wheat making biological control a valuable addition to the overall control strategy (Jones & Meers 2008). In 1956, wheat stem sawfly infestations were reduced by high rates of parasitism (Holmes 1982). In a survey by Shanower and Waters (2006) parasitism was low and did not approach the levels reported by Morrill *et al*. (1998). Many parasitoids such as *Bracon* spp. may respond to hosts in a density-dependent manner. More detailed behavioral and ecological studies are needed to better understand the impact of natural enemies on wheat stem sawfly population dynamics (Shanower & Waters 2006).

Cultural Control

Cultural control of the wheat stem sawfly includes various practices such as burning stubble, tillage, and crop rotation. However, some of the cultural practices have negative effects such as fall tillage of wheat stubble. Even though tillage has caused significant levels of overwinter mortality, fall tillage has not resulted in a significant reduction in sawfly infestation the following spring because tillage did not destroy all sawflies and still some adults emerged (Morrill 1992, Morrill *et al.* 1993). Also, tillage reduces the number of parasitoids that overwinter in the upper part of the stubble (Morrill *et al.* 1998). Burning infested stubble could reduce both sawfly and parasitoid numbers. Burning is not a recommended practice because retaining stubble has several advantages for soil fertility and productivity as well as preventing soil erosion. Also, swathing practices may reduce the number of heads left on the ground because of wheat stem sawfly infestation, but it may not reduce the number of sawfly that have already moved down in the stems (Holmes 1977).

Crop rotation could be useful to reduce sawfly populations if nearby fields are not planted to wheat or grasses (Callenbach & Hansmeier 1945, Knodel *et al*. 2010). Farmers can plant crops that are resistant to wheat stem sawfly such as oats or any broadleaf crops. Delayed seeding of spring wheat can result in plants that are too young to attract ovipositing sawfly females. The other advantage of late maturing wheat is that it can reduce sawfly numbers in the following year because it promotes the production of two generations of the parasitoids. However, delayed seeding is not a good option because it causes losses in yield and grade of wheat (Morrill & Kushnak 1999). Trap crops such as rye grass that attract sawflies may cause reduction in sawfly population size and then the plants would be harvested before the larvae move to the base of the plants (Beres *et al*. 2009).

Insecticides

Several studies have been conducted to investigate the efficacy of insecticidal applications to control wheat stem sawfly. However, few results of these studies have been published because most of them were not effective in controlling sawflies. Chemical control is not a successful approach against wheat stem sawfly. The eggs, larvae, and pupae are protected inside the plant stem from foliar insecticides. Also, the timing is difficult when spraying adults because they emerge in different times so, newly emerged adults could fly to sprayed field and infest plants. The prolonged emergence time reduces of the effectiveness of spraying adults. In addition, early spray would kill only males. Later applications would reduce female numbers but only after most eggs have been deposited in the plant. The insecticide applications could have negative impacts on the parasitoids because the first generation of *B. cephi* would be in flight. Application of heptachlor insecticide with the seed caused significant mortality in larval stage of the wheat stem sawfly in wheat stems (Holmes & Peterson 1963). However, this insecticide is no longer available. In summary, applying insecticides is quite costly for a low-value, large-acreage crop such as wheat, it is relatively ineffective in controlling wheat stem sawfly, and is potentially damaging to beneficial parasitoid populations.

Resistant Wheat Cultivars

Solid-stem wheat varieties have been shown to be effective in reducing damage caused by the wheat stem sawfly. *Cephus cinctus* developing in solid-stemmed cultivars frequently suffer increased rates of egg and larval mortality (Beres *et al.* 2011). Mortality of eggs and larvae among solid-stem cultivars was highest in 'Golden Ball' (versus 'Rescue'). In oat, eggs survived but all larvae died (Holmes & Peterson 1961, 1962). Fitness (measured in terms of female size and fecundity) was greater when sawflies fed on hollow-stemmed cultivars than on solid-stemmed cultivars (Morrill *et al*. 2000, Cárcamo *et al.* 2005). In a study of the long-term effects of wheat cultivar on *C. cinctus*, populations decreased in the solid-stemmed cultivar 'Rescue' over a 5-year period (Holmes & Peterson 1957).

The use of solid-stemmed wheat cultivars has a long history as a control option for wheat stem sawfly in the northern Great Plains and the Prairie Provinces of Canada. In 1946, the first solid-stemmed cultivar was released, followed by numerous others (Berzonsky *et al.* 2003). Resistant cultivars of solid-stemmed spring and winter wheat developed to date all derive their resistance from the line S-615 (Beres *et al.* 2011). In Montana, cultivars of solid-stemmed spring wheat are available, including 'Fortuna', 'Lew', and 'Choteau' (Beres *et al.* 2011). However, some sawfly may survive and cause damage to solid stem varieties. Solid-stemmed wheat varieties vary in grain yields and solidness level, however, most solid stem wheat cultivars receive less cutting by wheat stem sawfly than susceptible cultivars.

In three solid-stemmed cultivars (Bearpaw, Genou, and Rampart) in Montana where sawflies damage was significant, Bearpaw grain yield was greater than Genou and Rampart while Rampart was more resistant to cutting (6%) by wheat stem sawfly than Bearpaw (9%) and Genou (20%). Overall, all three resistant wheat varieties performed better than susceptible varieties under sawfly infestation in Montana (Carlson 2013).

The solid stemmed wheat cultivar 'Bearpaw' was developed and released in 2011 by the Montana Agricultural Experiment Station. Bearpaw was derived from a combination of five F1 crosses with a common parent, DMS/'Rampart'//'Pronghorn'/3/'Rampart' crossed to 'Rampart', 'NuPlains', and three Montana unreleased hollow experimental lines. Bearpaw has an average of

(21.8) solidness score (rated on a 5-25 scale), which is similar to Rampart (21.5) (Carlson 2013)

Programs of wheat breeding in areas such as Colorado where the sawfly is a recent pest of wheat are beginning to focus on integration of the solid- stem trait into adapted winter wheat varieties by using both conventional selection and linked DNA markers (REFS).

Host Plants

The wheat stem sawfly attacks both cultivated and non-cultivated host plants. Originally, the wheat stem sawfly was reported in hollow-stemmed wild grasses such as downy brome in the United States and Canada. The wheat stem sawfly survives on a number of non-cultivated grasses, including species of *Agropyron*, *Bromus*, *Elymus* and *Elytrigia*, in addition to cereal crops (Criddle 1917, Ainslie 1920, Criddle 1922). The wheat stem sawfly may choose different hollow stem hosts such downy brome (*Bromus tectorum* L. also known as cheatgrass), smooth bromegrass (*Bromus inermis* Leyss.), and crested wheatgrass (*Agropyron cristatum* L.). However, the effect of alternative grass hosts on wheat stem sawfly population dynamics has not been studied. Early reports suggested that wheat stem sawflies might use non-cultivated grasses as alternative hosts (Criddle 1917). Understanding the role and effect of these non-cultivated hosts in wheat stem sawfly outbreaks could inform the development of management strategies for wheat stem sawfly. Therefore, it is important to compare the relationship between wheat stem sawfly populations in non-cultivated hosts (wild grasses) and those in cultivated hosts (wheat).

Downy brome is a winter annual grass, the ecology which is similar to wheat (Morrow & Stahlman 1984). Downy brome was first introduced into North America from Mediterranean Europe in the 19th century (Mack 1981) and has since become one of the most widespread weedy grasses in wheat fields in northeastern Colorado.

Host plant selection by wheat stem sawfly females is affected by several factors such as plant growth stage, stem diameter, and volatile compounds released by plant (Seamans 1945). Females prefer to oviposit in less mature stems and in larger stems (Perez-Mendoza *et al*. 2006). The wheat stem sawfly host selection is a process involving the discovery of multiple, potential host plants and the use of acceptance cues to locate and identify suitable host plants. Volatile compounds can provide cues to identify suitable stems for oviposition (Weaver *et al*. 2009). Host plant quality can be described in terms of nitrogen, carbon, and defensive compounds that affect insect herbivore performance (development and survivorship). Wheat stem sawfly behavior is affected by several host plant volatiles such as the green-leaf volatiles (Z)-3- hexenyl acetate and (Z)-3-hexenol, the terpene β-ocimene, and 6-methyl-5-hepten-2-one (Piesik *et al*. 2008). The amount of the volatile compounds such as (Z)-3-hexenyl acetate makes certain wheat cultivars preferred by wheat stem sawfly over others (Weaver *et al.* 2009).

The Preference–Performance Hypothesis

The preference–performance hypothesis predicts that the females of herbivorous insects search and choose the host plant where they will lay their eggs (offspring) based on the fitness returns resulting from these choices. Females should select the host plants that are beneficial to the development and survival of their offspring. Since the offspring are generally not able to move between plants, they spend most of their immature stages on the individual host plant that was selected by their mothers. This hypothesis is also known as the “mother knows best” or the “optimal oviposition” hypothesis (Scheirs *et al*. 2000, Mayhew 2001, Clark *et al*. 2011).

The oviposition preference for host plant species and offspring performance (e.g., growth, survival, and reproduction of offspring on those hosts) has been a major focus in the theory of insect-plant interactions (Thompson 1988). Female maximize their fitness in two ways. In some cases, females prefer to oviposit on the host plants that increase offspring survival, as expected by the "mother knows best" hypothesis (Mayhew 2001). However, females may also increase their own longevity by laying eggs on a given host plant, even though this behavior conflicts with offspring survival – the "optimal bad motherhood" hypothesis (Mayhew 2001). When larvae and adults feed on different host plants, it is expected that the hosts that increase the growth and survival of offspring are not the same host plants that increase adult longevity and fecundity. Such parent–offspring conflicts (Scheirs *et al*. 2000, García-Robledo & Horvitz 2012) arise when novel host plants decrease survival of larvae but increase adult longevity. For example, the leaf-mining fly *Chromatomyia nigra* has been studied to understand the adaption to novel host plants which decrease offspring survivorship but increase adult longevity (Scheirs *et al*. 2000). Searching behavior may influence the evolution of the preference-performance relationship. In some species, females have limited time to search for oviposition sites that may differ as a function of host-plant quality and distribution within habitat, potentially affecting the correlation of preference-performance. For example, more time spent searching and a narrow feeding niche or host range may cause in a strong preference-performance relationship if the additional search time increases the likelihood of finding a host plant suitable for one's offspring (Tilmon 2008). *Euura lasiolepis* has a positive preference-performance relationship because the spatial distribution arroyo willow does not result in searching limitations on the females. Arroyo willow trees, *Salix lasiolepis,* are distributed along streams and springs. Females of *E. lasiolepis* prefer to lay eggs on rapidly growing shoots where the offspring perform well in May and early June and there is enough time for the females to emerge and locate new host plants on which to oviposit eggs (Price & Craig 1984).

Environmental factors (e.g., temperature, humidity, elevation) can influence the preference-performance relationship in insect herbivores (Gripenberg *et al*. 2010). Host-plant preference can be influenced by seasonal changes. Some aphid species shift between different host-plants during sequential generations. Aphid females fly from the primary host plants where they emerged in spring to lay eggs during summer on the secondary host plants. The offspring mature quickly and in early fall, the young females fly back to the primary host plants to lay eggs. Seasonal factors such as photoperiod, plant condition, and temperature all may affect the behavioral switch (seasonal changes in host preference) in aphids. Finding the susceptible host plants (secondary host plants) positively affects offspring performance (Dixon 1971, Schoonhoven *et al*. 2005). Also, some bivoltine species such as females of *Muellerianella fairmairei* oviposit on grasses in spring while females of the second generation prefer to oviposit on rushes (Juncaceae). Seasonal factors affect the host plant quality and may cause females of the second-generation to switch to another host plant species (Schoonhoven *et al*. 2005). Host plant quality (volatiles and nutrients) may be affected by the seasonal changes, which may affect oviposition preference, as well.

Temperature can influence host-plant preference if it affects plant quality (chemicals or nutrients), which can affect the preference (feeding behavior) of an herbivore for one host over another. Colorado potato beetles given a choice between potato and woody nightshade preferred potato. However, when the beetles were given the same choice at 25°C or higher, they preferred the woody nightshade. Temperature may influence the host plant (plant chemicals) or insect behavior (Bongers 1970, Schoonhoven *et al*. 2005).

Also, host plant preference may be influenced by the presence of natural enemies. Some insect females avoid laying eggs on a host-plant in the presence of the natural enemies and may select other suboptimal host-plant where their offspring perform poorly. In a study of oviposition preferences and performance of the pine sawfly *Neodiprion sertifer* on Scots pine trees (*Pinus sylvestris*), females showed oviposition preference for trees where they were protected from natural enemies (enemy free space) over the trees (high resin acid trees) where the offspring performance was higher (Björkman *et al*. 1997). Enemy avoidance behavior can affect the female preference and the offspring are not able to move to other host plants so they forced to complete their life cycle on the host selected by their mother (Schoonhoven *et al*. 2005). Therefore, the females would choose the oviposition site where enemy-free space is available, which increases the resource complexity and the preference-performance relationship is negatively affected (Tilmon 2008).

A further complication for the preference-performance hypothesis arises from the fact that in most holometabolous insects, the resource requirements of immature stages and adults differ. For example, larvae specialize in feeding and growing while adults reproduce and are generally the stage that are involved in dispersal and colonization of suitable environments. Since the larvae need to grow and complete development, they have different food requirements than adults. Also, in most insect species, feeding in larval stages will determine adult fitness (García-Robledo & Horvitz 2012). In many herbivorous insects, host-plant preference may change across different developmental stages. For instance, gypsy moth larvae, *Lymantria dispar,* feed on different plant species as they develop based on the nutrients content of the host related to different diet requirements in each stage (Schoonhoven *et al*. 2005).

The relationship between female preference and offspring performance differs as a function of diet specialization. Insects that feed on one type of food would be predicted to make better decisions than insects with a mixture of foods, because decision-making for females becomes complicated with different choices of host plants (Gripenberg *et al*. 2010). Insect herbivores that feed on multiple food resources should exhibit a weaker relationship between preference and performance than herbivores that feed on only one type of food. This would be expected to make the process of finding suitable oviposition site more complex because females would have to evaluate the quality of multiple food resources (Tilmon 2008). For instance, the spittlebug *Aphrophora pectoralis* feeds on seven species of willow, *Salix lasiolepis,* so it likely experiences high resource complexity. Females need to evaluate and assess resource variation of three different aspects of potential host plants: shoot growth, number of other eggs already present, and willow species. Females have to evaluate many cues to determine these different aspects of the host. Although the resource complexity is high, the preference-performance relationship is positive (Craig & Ohgushi 2002).

In some species, females and their offspring feed on different host plants or resources, then females may maximize their fitness by ignoring the performance of their offspring (Mayhew 2001, Scheirs & De Bruyn 2002, Gripenberg *et al*. 2010). When feeding by immature insect herbivores is restricted to the same part of the host plant on which they were oviposited, we expect the relationship between oviposition preference and offspring performance to be stronger. The lack of offspring mobility in such cases emphasizes the importance of maternal oviposition decisions. We also expect strong selection for a positive correlation between oviposition preference and offspring performance in situations where females lay most of their eggs on one or a few plants as opposed to distributing their eggs over a larger number of host plants. If females made bad decisions in the first case, the risk of losing offspring would be much greater than in the other case where females lay only a few eggs per plant (Mangel 1987, Paukku & Kotiaho 2008, Gripenberg *et al*. 2010). Also, female fecundity would be less dependent on larval nutrition if the females have the ability to feed on host plants as adults. This suggests that feeding history or larval food resource would be less important to adult reproduction and longevity and that the relationship between the female preference and offspring performance would be weaker (Gripenberg *et al*. 2010). Therefore, in species where females do not feed as adults, the relationship between female preference and offspring performance would be expected to be weaker.

The sensory limitations of insects can affect the ability of the females to detect the quality cues of host plant when the oviposition occurs. This would weaken the relationship between preference and performance. Plant quality can change between the time of oviposition and maturation of the offspring and insect sensory can detect the future changes of plants if the plant does not completely change during growing season. For example, detecting future plant quality may be easier in woody plants than non-woody plants because the latter are expected to change more drastically during the growing season. Sensory systems of insect herbivores may change (e.g., change in chemoreceptors) when the host plant species range that females use for oviposition changes. Many insect herbivores show a preference for plants that grow fast and their sensory abilities may be able to detect plant growth cues (Tilmon 2008). The gall-inducing sawflies *Euura lasiolepis* oviposit on the arroyo willow, *Salix lasiolepis,* and the larvae complete development in the site chosen by their mother. Sensory limitations apparently do not constrain the female's ability of detecting the appropriate oviposition sites, which increases the correlation of preference and performance. Females can predict the host quality of the arroyo willow that will enhance the larval development because plant quality does not change during the larval development. Cues are available for females during oviposition allowing females to predict the appropriate host for their offspring (Craig *et al*. 1989).

Wheat stem sawfly oviposition preference is linked to larval performance. Similar links are found in galling sawflies in genus *Euura* and leaf folding sawflies in the genus *Phyllocolpa* in Japan (Ferrier & Price 2004, Price & Ohgushi 1995). Females prefer to oviposit on longer plants where their offspring have increased survival and fitness. Björkman *et al*. (1997) reported correlation between pine sawfly female (*Neodiprion sertifer*) preference and tree height, needle length, and high resin acid (diterpenoid) concentrations.

Wheat stem sawflies select plants with larger stems as oviposition sites where larvae will have greater survival and body weight (Perez-Mendoza et al. 2006). In many insect species, females have an oviposition preference for larger plants (Ferrier and Price 2004). In the study of *Apion curtirostre* weevils that attacking *Rumex acetosa,* Hopkins and Whittaker (1980) reported significant correlation between the stem diameter and larval survivorship. Dhileepan (2004) studied the interaction between stem-galling moth *Epiblema strenuana* Walker (Lepidoptera: Tortricidae) and its host *hysterophorus* L. (Asteraceae) and the pest preferred the vigorous plants that were taller, had higher biomass, and produced more flowers and branches.

To understand wheat stem sawfly oviposition behavior, it was necessary to investigate whether females prefer ovipositing in a specific host plant, while ignoring the other plants in their foraging environment. The wheat stem sawfly can infest most available host plants. Sawflies infest a wide range of non-cultivated grass hosts as mentioned earlier in this chapter. A key question is how sawflies make oviposition decisions and select one particular host over another in their habitat and how sawflies evaluate the appropriate oviposition site to oviposit their eggs that enhance offspring survivorship.

The aims of this dissertation were: 1) to study wheat stem sawfly on winter wheat and downy brome in northeastern Colorado by comparing wheat stem sawfly presence and survivorship (including overwintering) in wheat production fields and in nearby downy brome, 2) to determine the oviposition preference of wheat stem sawfly for a susceptible winter wheat variety (Byrd), a resistant winter wheat variety (Bearpaw), and downy brome, 3) to determine the influence of volatile compounds from these plants on wheat stem sawfly behavior in the Y-tube olfactometer, and 4) to study the performance and the fitness of wheat stem sawfly from winter wheat and downy brome and how plant quality can influence it. These results will increase our understanding of host selection behavior and oviposition preference for host plants in Colorado and assess whether downy brome is affecting the presence and infestation levels of wheat stem sawfly in winter wheat in northeastern Colorado.

REFERENCES CITED

Ainslie, C.N. (1920). The western grass-stem sawfly. USDA Technical Bulletin No. 841.

Ainslie, C.N. (1929). The western grass-stem sawfly: a pest of small grains. USDA Technical Bulletin No. 157.

Beres, B.L., Cárcamo, H. A., & Bremer, E. (2009). Evaluation of alternative planting strategies to reduce wheat stem sawfly (Hymenoptera: Cephidae) damage to spring wheat in the Northern Great Plains. Journal of Economic Entomology, 102: 2137–2145.

Beres, B.L., Dosdall, L.M., Weaver, D.K., Spaner, D.M. & Cárcamo, H.A. (2011). The biology and integrated management of wheat stem sawfly, *Cephus cinctus* (Hymenoptera: Cephidae), and the need for continuing research. The Canadian Entomologist. 143:105–125.

Berzonsky, W. A., Ding, H., Haley, S. D., Harris, M. O., Lamb, R. J., McKenzie, R. I. H., Ohm, H. W., Patterson, F. L., Peairs, F. B., Porter, D. R., Ratcliffe, R. H. & Shanower, T. G. (2003). Breeding wheat for resistance to insects. Plant Breeding Reviews 22: 221– 296.

Bird, R. D. (1955). Extension of faunal ranges in the prairie provinces. Entomological Society of Ontario, Annual Report, 86: 33-37.

Björkman, C., Larsson, S., & Bommarco, R. (1997). Oviposition preferences in pine sawflies: a trade-off between larval growth and defence against natural enemies. Oikos, 45-52.

Bongers, W. (1970). Aspects of host-plant relationship of the Colorado beetle. Mededelingen van de Landbouwhogeschool te Wageningen, 70(10).

Buteler, M., Weaver, D.K., & Miller, P. R. (2008). Wheat stem sawfly-infested plants benefit from parasitism of the herbivorous larvae. Agricultural and Forest Entomology, 10: 347–354; Corrigendum 11:123.

Callenbach, J. A., & Hansmeier, M. P. (1945). Wheat stem sawfly control. *Montana Extension Service in Agriculture and Home Economics, Circular*, (164).

Callenbach, J. A., & Reinhardt, J. F. (1945). Montana insect pests in 1943-1944. 15th Annual Report of State Entomologist. Montana Agricultural Experiment Station Publications. 425, 30 pp.

Cárcamo, H.A., Beres, B. L., Clarke, F., Byers, R. J., Mundel, H. H., May, K., & DePauw, R. (2005). Influence of plant host quality on fitness and sex ratio of the wheat stem sawfly (Hymenoptera: Cephidae). Environmental Entomology, 34: 1579–1592.

Carlson, G. R., Berg, J. E., Stougaard, R. N., Eckhoff, J. L., Lamb, P. F., Kephart, K. D., ... & Grey, W. E. (2013). Registration of 'Bearpaw' wheat. Journal of Plant Registrations, 7(2), 180-183.

Clark, K. E., Hartley, S. E., & Johnson, S. N. (2011). Does mother know best? The preference–performance hypothesis and parent–offspring conflict in aboveground–belowground herbivore life cycles. Ecological Entomology, 36(2), 117-124.

Craig, T.P., Itami, J.K. & Price, P.W. (1989) A strong relationship between oviposition preference and larval performance in a shoot-galling sawfly. Ecology, **70**, 1691–1699.

Craig, T. P., & Ohgushi, T. (2002). Preference and performance are correlated in the spittlebug *Aphrophora pectoralis* on four species of willow. Ecological Entomology, 27(5), 529-540.

Criddle, N. 1917. Further observations upon the habits of the western wheat stem sawfly in Manitoba and Saskatchewan. The Agricultural Gazette of Canada 4: 176-177.

Criddle, N. (1923). The life habits of *Cephus cinctus* Nort. in Manitoba. The Canadian Entomologist, 55: 1–4.

Criddle, N. 1922. The western wheat stem sawfly and its control. Dominion of Canada Department of Agriculture Pamphlet No. 6 New Series:1-8.

Dhileepan, K. (2004), The applicability of the plant vigor and resource regulation hypotheses in explaining *Epiblema* gall moth–*Parthenium* weed interactions. Entomologia Experimentalis et Applicata, 113: 63–70.

Dixon, A. F. G. (1971), The life-cycle and host preferences of the bird cherry-oat aphid, Rhopalosiphum padi L., and their bearing on the theories of host alternation in aphids. Annals of Applied Biology, 68: 135–147.

García-Robledo, C. & Horvitz, C. C. (2012). Parent–offspring conflicts, "optimal bad motherhood" and the "mother knows best" principles in insect herbivores colonizing novel host plants. Ecology and Evolution, 2: 1446–1457.

Gripenberg, S., Mayhew, P. J., Parnell, M., & Roslin, T. (2010). A meta-analysis of preference–performance relationships in phytophagous insects. Ecology Letters, 13: 383–393.

Holmes, N. D. (1977). The effect of the wheat stem sawfly, Cephus cinctus (Hymenoptera: Cephidae), on the yield and quality of wheat. *The Canadian Entomologist*, *109*(12), 1591-1598.

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

Holmes, N. D., Nelson, W. A., Peterson, L. K., & Farstad, C. W. (1963). Causes of variations in effectiveness of *Bracon cephi* (Gahan) (Hymenoptera: Braconidae) as a parasite of the wheat stem saw- fly. The Canadian Entomologist, 95: 113–126.

Holmes, N. D., & Peterson, L. K. (1957). Effect of continuous rearing in Rescue wheat on the survival of the wheat stem sawfly *Cephus cinctus* Nort. (Hymenoptera: Cephidae). The Canadian Entomologist, 89: 363–364.

Holmes, N. D., & Peterson, L. K. (1960). The influence of the host on oviposition by the wheat stem sawfly, *Cephus cinctus* Nort. (Hymenoptera: Cephidae). Canadian Journal of Plant Science, 40: 29–40.

Holmes, N. D., & Peterson, L. K. (1961). Resistance of spring wheats to the wheat stem sawfly, *Cephus cinctus* Nort. (Hymenoptera: Cephidae) I. Resistance to the egg. The Canadian Entomologist, 93: 250–260.

Holmes, N. D., & Peterson, L. K. (1962). Resistance of spring wheats to the wheat stem sawfly *Cephus cinctus* Nort. (Hymenoptera: Cephidae) II. Resistance to the larva. The Canadian Entomologist, 94: 348–365.

Holmes, N. D., & Peterson, L. K. (1963). Heptachlor as a systemic insecticide against the wheat stem sawfly, *Cephus cinctus* Nort. The Canadian Entomologist, *95*(08), 792-796.

Hopkins, M. J. G. and Whittaker, J. B. (1980), Interactions between *Apion* species (Coleoptera: Curculionidae) and Polygonaceae. I. *Apion curtirostre* Germ, and *Rumex acetosa* L. Ecological Entomology, 5: 227–239.

Irell, B., & Peairs, F. (2011). Wheat Stem Sawfly: A new pest of Colorado wheat. Colorado State University, U.S. Department of Agriculture.

Jones, J., & Meers, S. (2008). Wheat stem sawfly. Alberta Agriculture and Rural Development, Agdex 622-26.

King, K. M. (1929). Insects affecting field crops and gardens in Saskatchewan, 1922-1927. Scientific Agriculture, 9: 373-390.

Knodel, J., Shanower, T., & Beauzay, P. (2010). Integrated pest management of wheat stem sawfly in North Dakota. North Dakota State University and U.S. Department of Agriculture cooperating.

Mack, R. N. (1981). Invasions of *Bromus tectorum* L. into western North America: an ecological chronicle. Agro-Ecosystems 7: 145-165.

Mangel, M. (1987). Oviposition site selection and clutch size in insects. Journal of Mathematical Biology, 25(1), 1-22.

Mayhew, P. J. (2001). Herbivore host choice and optimal bad motherhood. Trends in Ecology &

Evolution, 16:165–167.

Meers, S. B. (2005). Impact of harvest operations on parasitism of the wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae). M.Sc. thesis, Montana State University, Bozeman, Montana.

Montana Agricultural Experiment Station and Montana Extension Service. (1946). The wheat stem sawfly in Montana. 10 pp.

Morrill, W. L., Gabor, J. W., Weaver, D. K., Kushnak, G. D., & Irish, N. J. (2000). Effect of host plant quality on the sex ratio and fitness of female wheat stem sawflies (Hymenoptera: Cephidae). Environmental Entomology, 29: 195–199.

Morrill, W. L., Kushnak, G. D., & Gabor, J. W. (1998). Parasitism of the wheat stem sawfly (Hymenoptera: Cephidae) in Montana. Biological Control, 12: 159–163.

Nelson, W.A., & Farstad, C.W. (1953). Biology of *Bracon cephi* (Gahan) (Hymenoptera: Braconidae), an important native parasite of the wheat stem sawfly, *Cephus cinctus* Nort. (Hymenoptera: Cephidae), in Western Canada. The Canadian Entomologist, 85: 103–107.

Perez-Mendoza, J., Weaver, D. K., & Morrill, W. L. (2006). Infestation of wheat and downy brome grass by wheat stem sawfly and subsequent larval performance. Environmental Entomology, 35(5), 1279-1285.

Paukku, S., & Kotiaho, J. S. (2008). Female oviposition decisions and their impact on progeny life-history traits. Journal of Insect Behavior, 21(6), 505-520.

Piesik, D., Weaver, D. K., Runyon, J. B., Buteler, M., Peck, G. E., & Morrill, W. L. (2008). Behavioural responses of wheat stem sawflies to wheat volatiles. Agricultural and Forest Entomology, 10: 245–253.

Price, P. W., & Craig, T. P. (1984). Life history, phenology, and survivorship of a stem-galling sawfly, *Euura lasiolepis* (Hymenoptera: Tenthredinidae), on the arroyo willow, *Salix lasiolepis*, in northern Arizona. Annals of the Entomological Society of America, 77(6), 712-719.

Scheirs, J., De Bruyn, L., & Verhagen, R. (2000). Optimization of adult performance determines host choice in a grass miner. Proceedings of the Royal Society of London. Series B: Biological Sciences, 267(1457), 2065-2069.

Scheirs, J. & De Bruyn, L. (2002). Integrating optimal foraging and optimal oviposition theory in plant–insect research. Oikos, *96*(1), 187-191.

Schoonhoven, L. M., Van Loon, J. J., & Dicke, M. (2005). Insect-plant biology (No. Ed.

Shanower, T. G., & Hoelmer, K. A. (2004). Biological control of wheat stem sawflies: past and future. Journal of Agricultural and Urban Entomology, 21: 197–221.

Shanower, T. G. & Waters, D. K. (2006). A survey of five stem-feeding insect pests of wheat in the Northern Great Plains. Journal of Entomological Science, 41:40–48.

Thompson, J. N. (1988). Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. Entomologia Experimentalis et Applicata, 47(1), 3-14.

Tilmon, K. J. (Ed.). (2008). Specialization, speciation, and radiation: the evolutionary biology of herbivorous insects. Univ of California Press.

Weaver, D. K., Buteler, M., Hofland, M. L., Runyon, J. B., Nansen, C., Talbert, L. E., & Carlson, G. R. (2009). Cultivar preferences of ovipositing wheat stem sawflies as influenced by the amount of volatile attractant. Journal of Economic Entomology, 102: 1009–1017.

CHAPTER 2-WHEAT STEM SAWFLY *CEPHUS CINCTUS* NORTON IN WINTER WHEAT AND DOWNY BROME IN NORTHEASTERN COLORADO

Summary

Infestation rates and larval development of the wheat stem sawfly *Cephus cinctus* Norton (Hymenoptera: Cephidae) were studied for two years (2013 and 2014) at seven commercial winter wheat (*Triticum aestivum* L.) fields and eight nearby downy brome (*Bromus tectorum* L.) sites in northeastern Colorado. Fifty randomly-selected stems of each plant species were collected per site weekly from late spring through autumn and monthly from winter through early spring. Sweep samples of sawfly adults were taken shortly after adult emergence in May. Infestation rate in winter wheat was 12 to nearly 14 times higher than in downy brome throughout the two year survey. Larval mortality was twice as high in downy brome than in winter wheat over the two year survey. Throughout collecting and processing samples, we observed that most larval mortality was due to desiccation in the thinner stems of downy brome. More adults were collected in May from winter wheat than from downy brome in the two year survey. Wheat stem sawfly females preferred to oviposit in wheat plants over downy brome. These results suggest that while downy brome is a poorer quality hostplant for larval development, it could be an important alternate host plant that reduces the survival and infestation of the wheat stem sawfly in wheat when it functions as a trap plant. Further study of wheat stem sawfly population dynamics and establishment in downy brome is needed to test this possibility.

Introduction

The wheat stem sawfly was first reported in Colorado in 1872 feeding on native grasses such as western wheat grass, basin wildrye, and other wheat grasses in the genera *Agropyron*, *Bromus*, *Elymus,* and *Elytrigia* (Ainslie 1929; Shanower & Hoelmer 2004). The wheat stem sawfly *Cephus cinctus* Norton (Hymenoptera: Cephidae) is native to North America and became a pest when it adapted to wheat as acreage devoted to wheat cultivation increased. Wheat stem sawfly also survives in other cereal crops such as barley and rye (Ainslie 1929; Shanower & Hoelmer 2004). Yield in wheat infested by wheat stem sawfly can be decreased by >25%, resulting in annual losses of almost US$350 million in the US and Canada (Beres *et al.* 2011, 2012). The wheat stem sawfly has also damaged spring wheat in the northern plains.

The European sawflies *Cephus pygmaeus* (L) and *Trachelus tabidus* (F) are found in some countries in Middle East and North Africa such as Turkey, Iran, Iraq; Cyprus, Algeria, Lebanon, Palestine, Egypt and Morocco (Rashwani 1981). *Cephus cinctus* has become the most serious insect pest of wheat in the western US and Canada. In North America, the damage caused by the wheat stem sawfly *C. cinctus* is currently greatest in the following states and provinces: Montana, North Dakota, Alberta, Saskatchewan, and Manitoba (Shanower & Hoelmer 2004). The wheat stem sawfly was first reported as a pest of cereal grains in the Prairie Provinces of Canada in the late 1890s (Weiss & Morrill 1992). It was reported in 1895 on wheat in Manitoba. The wheat stem sawfly was found on wheat near Bozeman, Montana, in 1900 (Montana Agricultural Experiment Station and Montana Extension Service 1946). In 1902, *C. cinctus* larvae were found in different grass species in the Northwest Territories (Ainslie 1920). In 1907, sawflies were found in wheat at Minot, North Dakota (Ainslie 1920). In 1910, the wheat stem sawfly damage was reported in Montana at Bainville (Montana Agricultural Experiment Station and Montana Extension Service 1946). In 1911, Ainslie found *C. cinctus* feeding in native grasses in some western states (Ainslie 1920). The first recorded severe infestation and damage to wheat occurred in 1922 in western Canada (Criddle 1923). In 1926, damage and losses were reported in Canada (King 1929). The economic importance of this sawfly started to increase in Montana and North Dakota from 1943 to 1946 (Callenbach and Reinhardt 1945), and in Canada in 1955 (Bird 1955). Infestation and damage to winter wheat was not reported in Colorado until 2010 (Irell & Peairs 2011).

The wheat stem sawfly *C. cinctus* was first reported in Colorado in 1872 feeding on native grasses such as western wheat grass, basin wildrye, and other wheat grasses in the genus *Agropyron.,* Iinfestation and damage to winter wheat was not reported in Colorado until 2010 (Irell and Peairs 2011). Sawflies also survive on a number of native concultivated grasses, including species of *Agropyron*, *Bromus*, *Elymus* and *Elytrigia*, in addition to other cereal crops such as barley and rye (Ainslie 1929; Shanower and Hoelmer 2004).

The life histories of this and the other sawflies species previously mentioned are very similar. Sawfly females oviposit into the growing stems of host plants in late May or early June. The larvae feed and move down within the stem. Females lay about 30-50 eggs over their adult lifespan. While multiple eggs may be laid per stem, only a single larva survives to maturity because larvae are cannibalistic (Ainslie 1920, 1929). Larvae move down to the base of the stem as plants senesce. Larvae chew a “V”-shaped cut inside of the stem. The stem cavity is plugged with frass below the cut and above the larva. Stems weakened in this manner may break before harvest (“lodge”), especially during wind events. The larva creates a membrane-like case to protect it from harsh conditions such as excessive moisture or desiccation (Holmes and Peterson 1960). The larvae enter diapause and overwinter in the stubble close to the soil.

Larval diapause is broken as temperature, moisture levels, and photoperiods increase in the spring. The prepupal and pupal stages last almost two weeks, depending on the temperatures. The adult will chew through the frass plug and emerge from the stubble. Adults emerge in May over a 3-4 week period and live about one week to ten days. The first females to emerge mate with males and lay fertilized eggs that produce daughters, while the later emerging females lay unfertilized eggs that produce sons presumably because males die earlier and thus are not available. Adults fly only far enough to locate a suitable host plant, but may fly up to a maximum of several miles from their site of emergence (Ainslie 1920, 1929).

Winter wheat and downy brome, *Bromus tectorum,* have similar ecology and growth stages as both are annual winter grasses (Morrow & Stahlman 1984). In the 19th century, downy brome was accidentally introduced into North America from Mediterranean Europe (Mack 1981). In Montana, wheat stem sawfly has been reported to infest both winter wheat and downy brome. In northeastern Colorado, downy brome is often found near winter wheat fields. The objectives of this study were 1) to study the infestation of wheat stem sawfly *C. cinctus* on winter wheat and downy brome in northeastern Colorado and 2) to compare wheat stem sawfly presence and survivorship (overwintering) in wheat production fields and in nearby downy brome. These results will increase our understanding of host selection behavior and oviposition preference for these grasses in Colorado and assess whether downy brome is affecting the presence and infestation levels of wheat stem sawfly in winter wheat in northeastern Colorado.

Materials and Methods

Field Sampling

Seven commercial wheat fields and eight nearby downy brome sites in northeastern Colorado along Hwy 14 east of Fort Collins were selected in 2013 and 2014 (Figure 2.1) (Table 2.1). Susceptible hollow-stemmed winter wheat cultivars are grown in wheat fields including 'Byrd' and 'Hatcher'. In wheat fields, growers mostly used no-till practices. I randomly collected 50 stem samples from each site weekly beginning in spring (mid-May) through the summer and 50 stubble samples from each site monthly in fall and winter. Sweep samples (100 sweeps per site) were taken during the sawfly flight period from late May through early June. Samples were placed in pre-labeled plastic zippered storage bags. The bags were stored in a cooler with ice packs during transport to the laboratory at the Colorado State University where they were held in a refrigerator until plant samples could be dissected. Mature stems or stubble were dissected with an X-ACTO knife and examined for the presence of sawfly larvae. The number of eggs, larvae, and pupae were recorded in each stem. Stems containing frass (evidence of larval sawfly feeding) but no larvae, as well as stems containing one of the sawfly developmental stages were considered to be infested. Larval establishment was defined by feeding within the stem, even if the larva died before reaching maturity. A stem containing frass but no dead larvae as well as stems containing dead larvae were recorded as instances of larval mortality. Larval survival was defined as the presence of a living larva within the stem at the time of sampling. Overall presence of wheat stem sawfly was compared in winter wheat and grasses.

Table 2.3. Commercial wheat fields and nearby downy brome sites selected for the fields survey of wheat stem sawfly *C. cinctus* in northeastern Colorado in 2013 and 2014.

Host Plants	Year	Sites*	Latitude	Longitude	Elevation (m)
Winter Wheat	2013	Merten1.a	40°35'37.05"N	103°53'4.17"W	1455
		Merten2.a	40°34'22.35"N	103°53'52.65"W	1471
		Dan1.a	40°47'22.74"N	103°29'49.92"W	1346
		Dan2.a	40°47'23.64"N	103°29'23.09"W	1352
		Scott1.a	40°59'15.54"N	104°21'17.17"W	1636
		Wickstrom1.a	40°30'49.79"N	104° 4'14.95"W	1492
		Wickstrom2.a	40°30'51.90"N	104° 4'13.32"W	1494
	2014	Merten1.b	40°35'39.05"N	103°52'49.51"W	1457
		Merten2.b	40°34'15.89"N	103°53'50.28"W	1471
		Dan1.b	40°47'21.59"N	103°29'48.59"W	1346
		Dan2.b	40°47'20.43"N	103°29'28.79"W	1350
		Scott1.b	40°59'16.52"N	104°21'14.59"W	1635
		Wickstrom1.b	40°31'29.21"N	104° 4'20.14"W	1495
		Wickstrom2.b	40°34'3.58"N	104° 4'5.33"W	1486
Downy brome	2013 and 2014	Merten3	40°35'44.29"N	103°52'49.86"W	1458
		Merten4	40°34'15.31"N	103°53'52.62"W	1471
		Dan3	40°45'37.37"N	103°31'1.34"W	1339
		Dan4	40°47'21.48"N	103°29'51.00"W	1346
		Dan5	40°47'25.14"N	103°29'14.86"W	1350
		Scott2	40°58'24.49"N	104°19'30.74"W	1625
		Scott3	40°59'15.27"N	104°21'10.98"W	1635
		Wickstrom3	40°30'49.36"N	104° 4'13.41"W	1493

*Different numbers refer to different sites within the grower's field
*Different letters refer to sites of wheat in different years (letter "a" for 2013 and "b" for 2014)

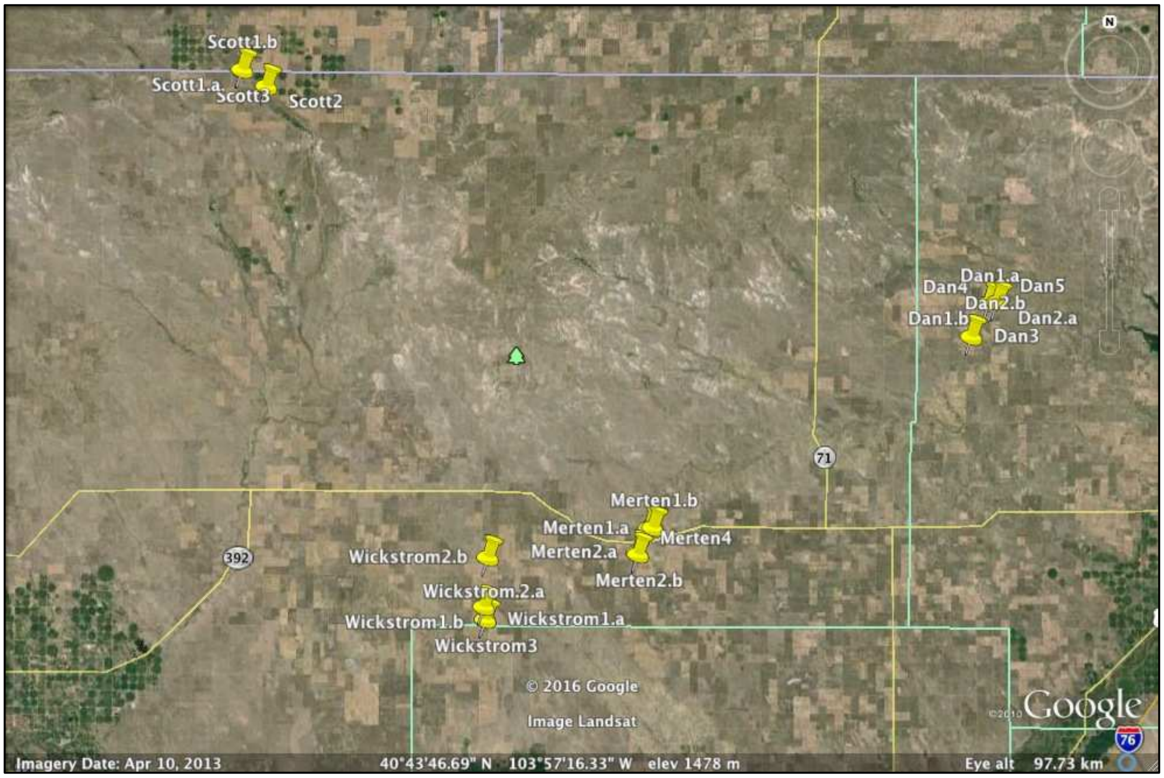

Figure 2.1. Commercial wheat fields and nearby downy brome sites selected for the fields survey of wheat stem sawfly *C. cinctus* in northeastern Colorado in 2013 and 2014.

Data Analysis

The effect of each factor (sample date, collection site, and host plant) on the infestation rate of the wheat stem sawfly were analyzed using separate one-way ANOVAs for each year (2013 and 2014). Larval mortality percentage on wheat and downy brome were assessed across dates for sites in each year. The t-test was used to compare the wheat stem sawfly infestation in wheat fields and downy brome across sites for each year of the survey separately. The effect of each factor (sample date, collection site, and host plant) on the number of sawfly adults collected in May by sweep samples was analyzed using a one-way ANOVA for 2013 and 2014.

Results

The collection date did not affect sawfly infestation in the two years of the survey (give test statistics here). However, site did have an effect on the wheat stem sawfly infestation in each of the two years of the survey; 2013: $F_{14, 232}$= 133.99, $P < 0.0001$; 2014: $F_{14, 229}$= 257.90, $P < 0.0001$ (Figure 2.2 and 2.3). Merten1.a had the highest level of wheat stem sawfly infestation among all wheat fields in 2013 while Wickstrom1.b had the highest infestation on wheat in 2014 (Tables 2.2 and 2.4). Downy brome had the highest wheat stem sawfly infestation at Wickstrom3 in 2013 while Merten4 had the highest infestation in 2014 (Tables 2.3 and 2.5).

Table 4.2. Infestation (mean ± SE) of winter wheat by *Cephus cinctus* in northeastern Colorado in 2013.

Sites	Mean number of eggs per 50 stems	Total of larvae per 50 stems	Total of pupae per 50 stems	Stems with frass per 50 stems
Merten1.a	0.62 ± 0	14.38 ± 1.02	0.56 ± 0	0.62 ± 0.22
Merten2.a	0.29 ± 0	11.00 ± 0.95	0.47 ± 0	0.11 ± 0.08
Dan1.a	0.31 ± 0	4.69 ± 0.57	0.31 ± 0	0.81 ± 0.21
Dan2.a	0.37 ± 0	5.12 ± 0.54	0.31 ± 0	0.25 ± 0.17
Scott1.a	0	0	0	0
Wickstrom1.a	0.18 ± 0	11.29 ± 0.44	0.59 ± 0	0.17 ± 0.09
Wickstrom2.a	0.11 ± 0	10.35 ± 0.80	0.53 ± 0	1.29 ± 0.11

Table 2.3. Infestation (mean ± SE) of downy brome by *Cephus cinctus* in northeastern Colorado in 2013

Sites	Mean number of eggs per 50 stems	Total of larvae per 50 stems	Total of pupae per 50 stems	Stems with frass per 50 stems
Merten3	0	0.25 ± 0.11	0	0.37 ± 0.12
Merten4	0.12 ± 0	0.29 ± 0.11	0.18 ± 0	0.59 ± 0.19
Dan3	0	0	0	0
Dan4	0	0	0	0
Dan5	0	0	0	0.43 ± 0.13
Scott2	0	0	0	0
Scott3	0	0	0	0
Wickstrom 3	0	0.52 ± 0.15	0.06 ± 0	1.35 ± 0.19

Table 2.4. Infestation (mean ± SE) of winter wheat by *Cephus cinctus* in northeastern Colorado in 2014.

Sites	Mean number of eggs per 50 stems	Total of larvae per 50 stems	Total of pupae per 50 stems	Stems with frass per 50 stems
Merten1.b	0.29 ± 0	15.52 ± 1.01	0.56 ± 0	2.23 ± 0.27
Merten2.b	0.18 ± 0	12.47 ± 0.92	0.71 ± 0	1.12 ± 1.12
Dan1.b	0.13 ± 0	4.00 ± 0.40	0.13 ± 0	0.40 ± 0.27
Dan2.b	0	4.13 ± 0.50	0.27 ± 0	0.26 ± 0.18
Scott1.b	0	0	0	0
Wickstrom1.b	0.35 ± 0	13.24 ± 0.49	0.58 ± 0	0.17 ± 0.09
Wickstrom2.b	0.23 ± 0	13.88 ± 0.71	0.64 ± 0	1.29 ± 0.11

Table 2.5. Infestation (mean ± SE) of downy brome by *Cephus cinctus* in northeastern Colorado in 2014.

Sites	Total of eggs per 50 stems	Total of larvae per 50 stems	Total of pupae per 50 stems	Stems with frass per 50 stems
Merten3	0.05 ± 0	0.24 ± 0.11	0	0.35 ± 0.11
Merten4	0	1.94 ± 0.31	0.17 ± 0	0.58 ± 0.19
Dan3	0	0	0	0
Dan4	0	0	0	0
Dan5	0	0	0	0.40 ± 0.13
Scott2	0	0	0	0
Scott3	0	0	0	0
Wickstrom3	0	0.35 ± 0.14	0.06 ± 0	1.35 ± 0.19

Wheat stem sawfly infestation rates were higher in wheat than in downy brome across all sites over the two years of survey (Figures 2.2 and 2.3).

The host plant was a key factor affecting the wheat stem sawfly infestations at all sites during both years of the survey; $F_{1, 245}$= 272.28, $P < 0.0001$ in 2013 and $F_{1, 242}$= 257.13, $P < 0.0001$ in 2014. Female wheat stem sawflies in northeastern Colorado oviposited more on wheat than downy brome. Wheat fields had 13.5 and 11.8 times more sawflies than downy brome in 2013 and 2014, respectively (Table 2.6). The percentage infestation on wheat and downy brome increased in 2014 (Figures 2.4 and 2.5).

Table 2.6. Wheat stem sawfly infestation (mean number ± SE of sawflies per 50 stems) on wheat and downy brome in northeastern Colorado in 2013 and 2014.

Year	Wheat stem sawfly infestation					
	Wheat crop			Downy brome		
	Mean ± SE	t-test	P-value	Mean ± SE	t-test	P-value
2013	10.03 ± 0.41	24.38	0.0001	0.74 ± 0.38	1.93	0.0543
2014	11.04 ± 0.46	24.01	0.0001	0.94 ± 0.43	2.18	0.0302

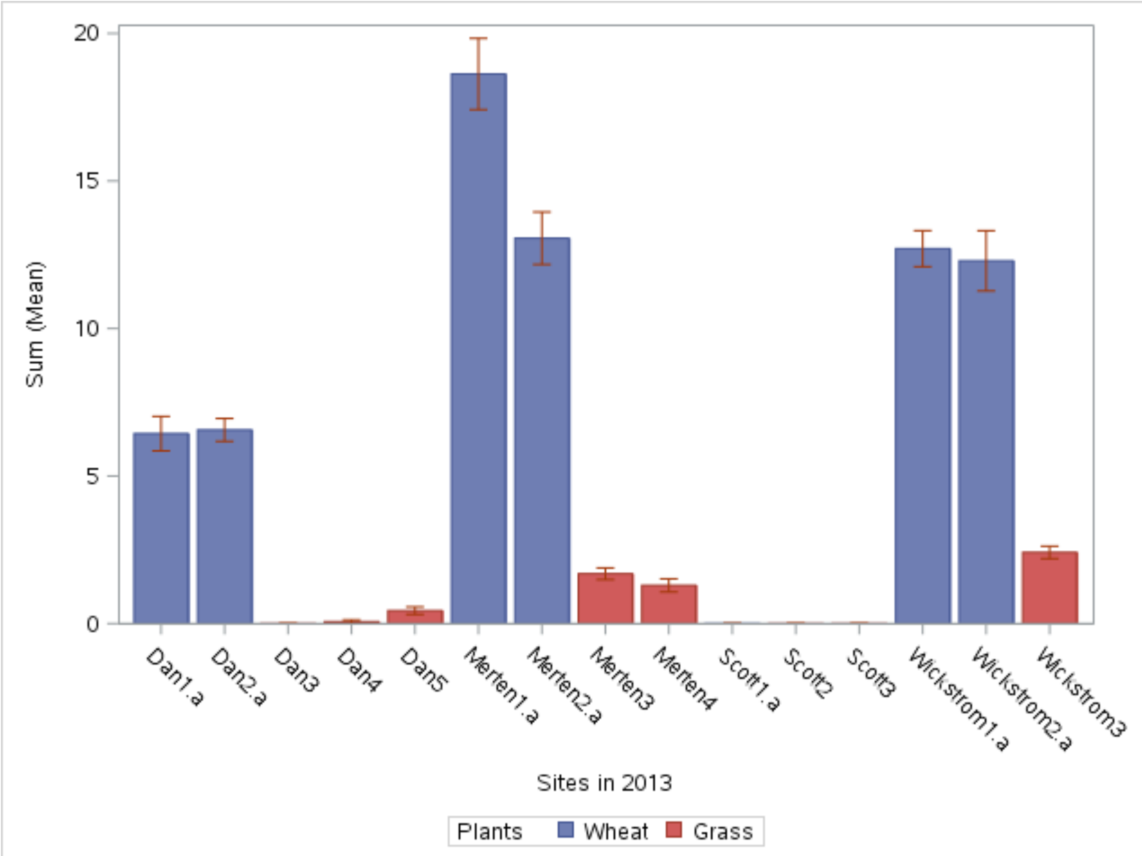

Figure 2.2. Mean of the total wheat stem sawfly infestation in 2013 on winter wheat and downy brome grass in northeastern Colorado.

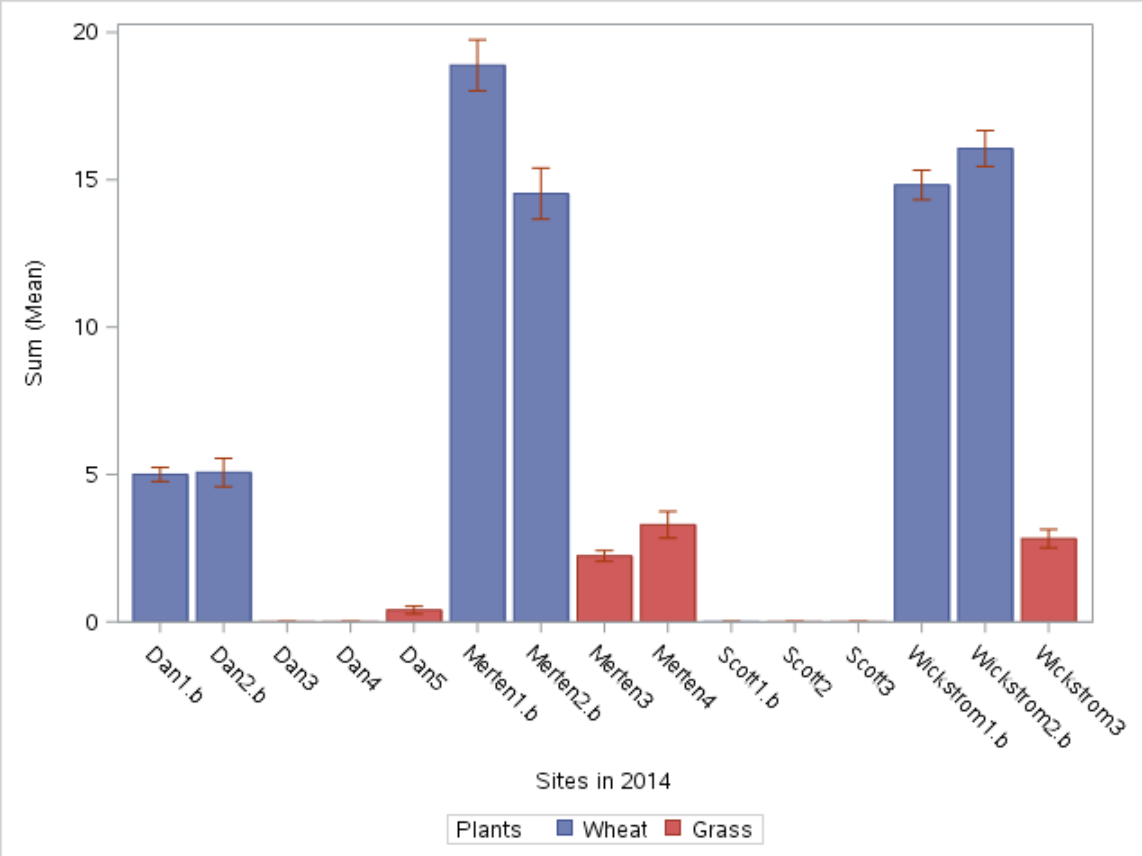

Figure 2.3. Mean of the total wheat stem sawfly infestation in 2014 on winter wheat and downy brome grass in northeastern Colorado.

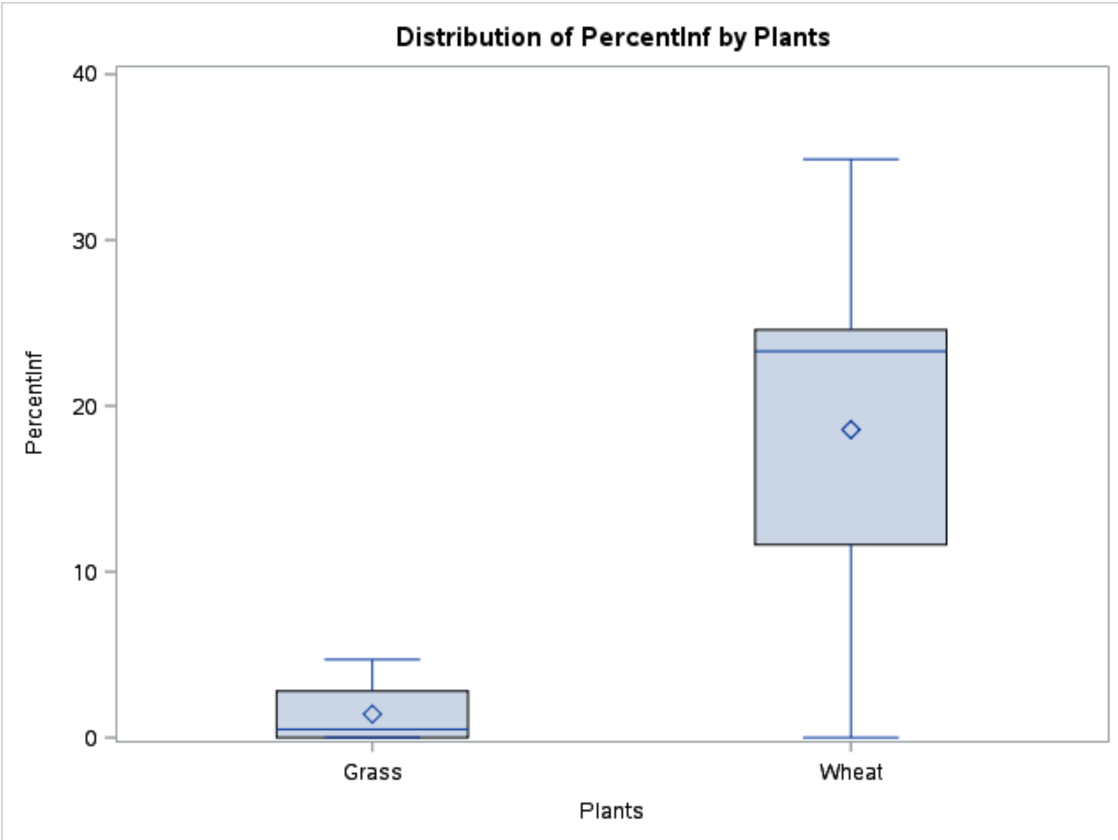

Figure 2.4. Percentage infestation of wheat stem sawfly on winter wheat and downy brome across wheat and downy brome sites in 2013.

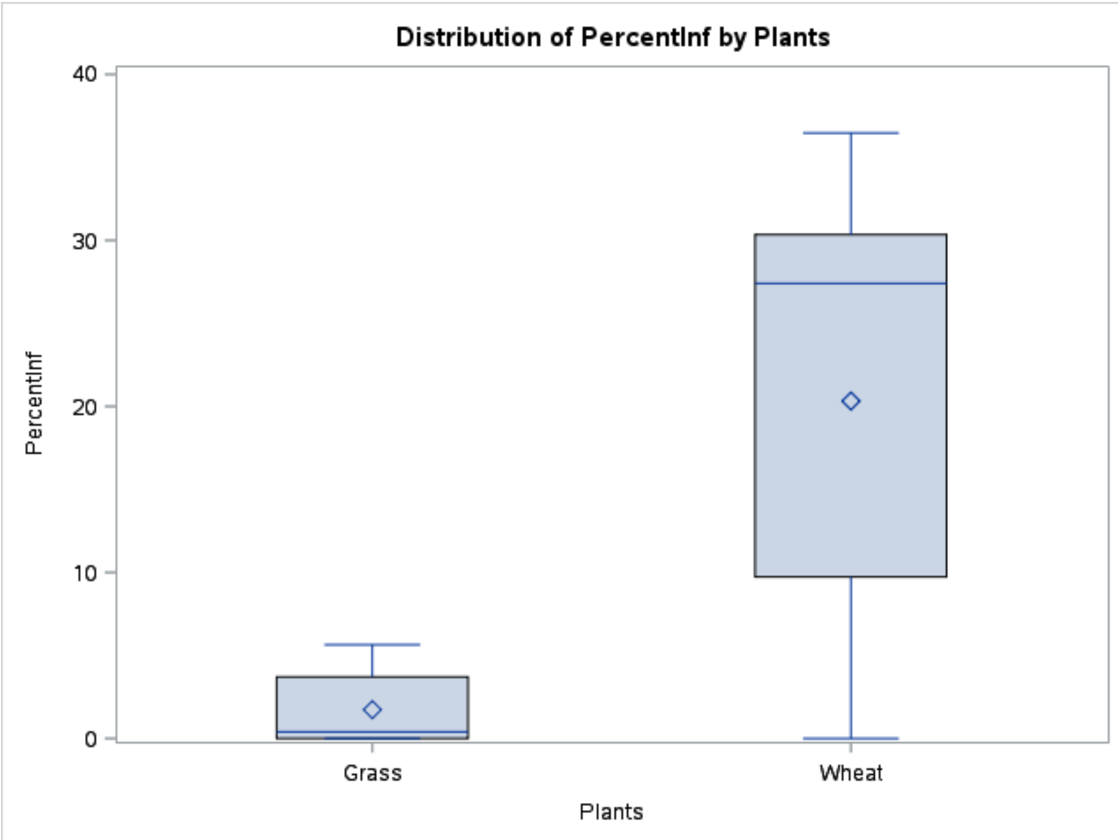

Figure 2.5. Percentage infestation of wheat stem sawfly on winter wheat and downy brome across wheat and downy brome sites in 2014.

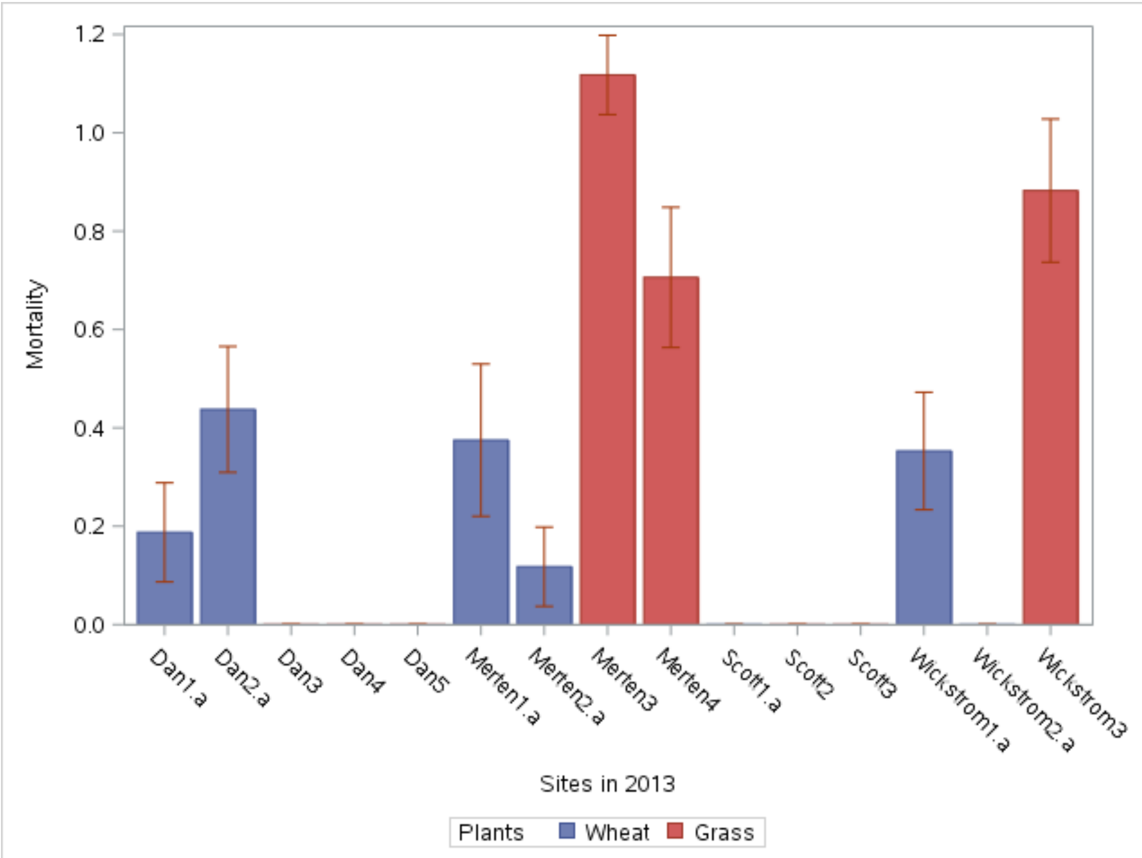

Figure 2.6. Mean of the total wheat stem sawfly larval mortality in winter wheat and downy brome across northeastern Colorado sites in 2013.

Both host plant and site had an effect on larval mortality of the wheat stem sawfly in 2013; $F_{1, 244} = 4.65$, P = 0.03 and $F_{1,244} = 6.33$, P = 0.01, however, in 2014 only host plant was affected larval mortality, $F_{1, 240} = 8.06$, P = 0.005. Mean mortality of overwintering wheat stem sawfly in downy brome was higher than in winter wheat across dates in 2013 and 2014 (Figures 2.6 and 2.7).

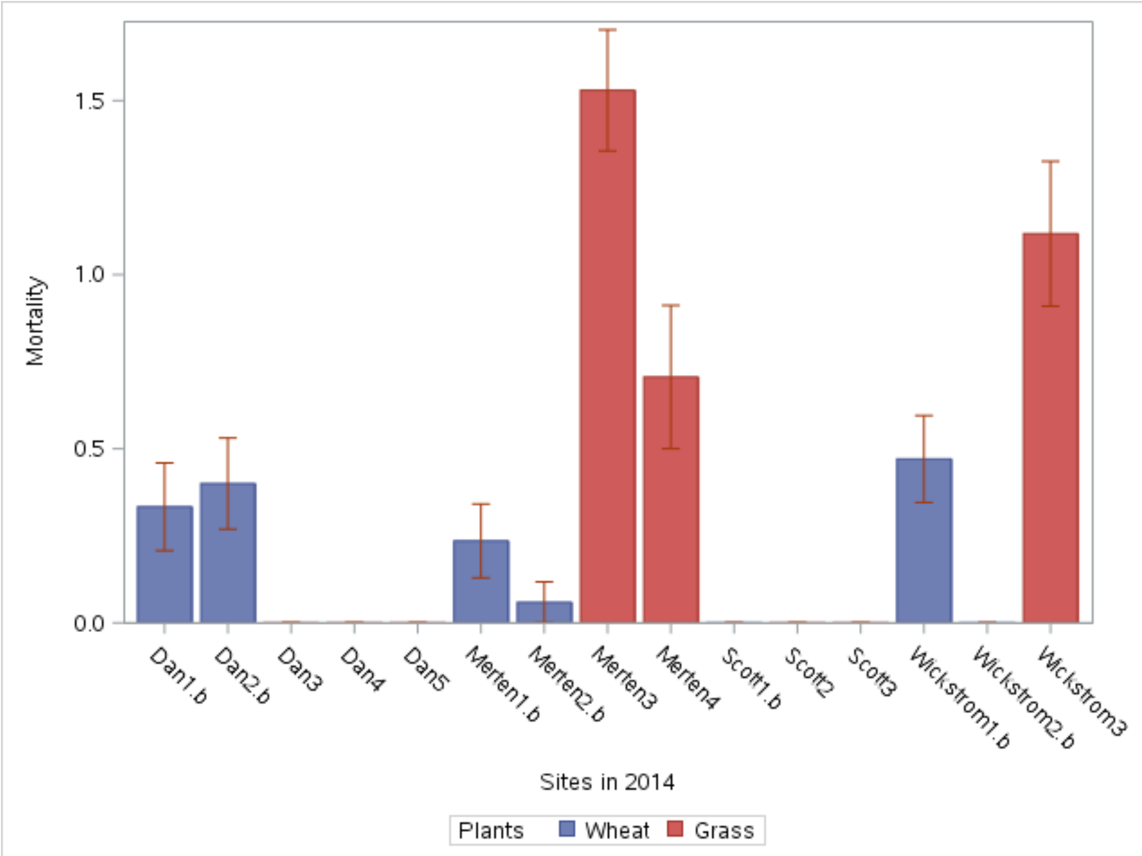

Figure 2.7. Mean of the total wheat stem sawfly larval mortality in winter wheat and downy brome across northeastern Colorado sites in 2014.

Host plant species had a significant effect on the number of adults collected by sweeps during May of 2013: $F_{1,12} = 13.17$, $P = 0.003$, and May 2014: $F_{1,12} = 10.75$, $P = 0.007$. The number of adults collected from wheat fields were 4.9 times to 5.2 times more than adults collected from downy brome locations in 2013 and 2014 (Figures 2.8 and 2.9). The mean number of adults have been collected in the flight period of the two years were not significantly different: $F_{1,26} = 1.39$, $P = 0.25$.

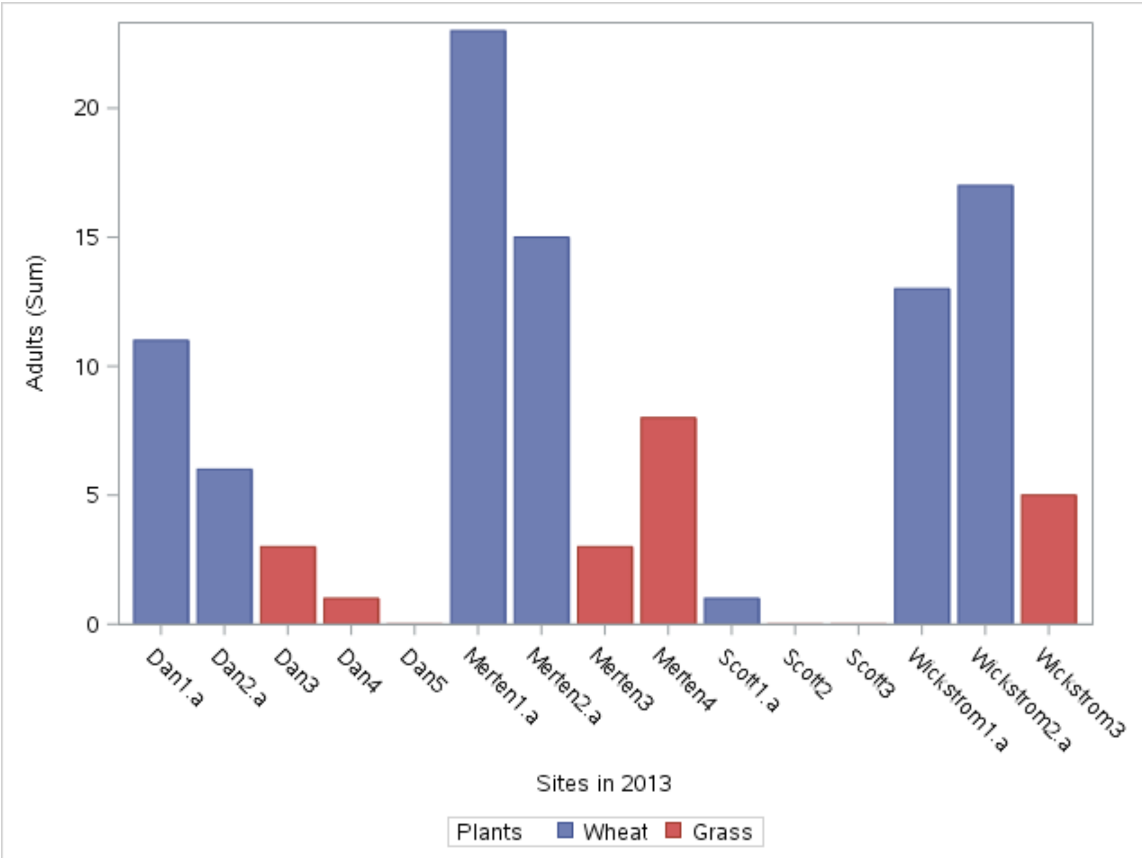

Figure 2.8. Mean number of wheat stem sawfly adults on winter wheat and downy brome across sites in May of 2013.

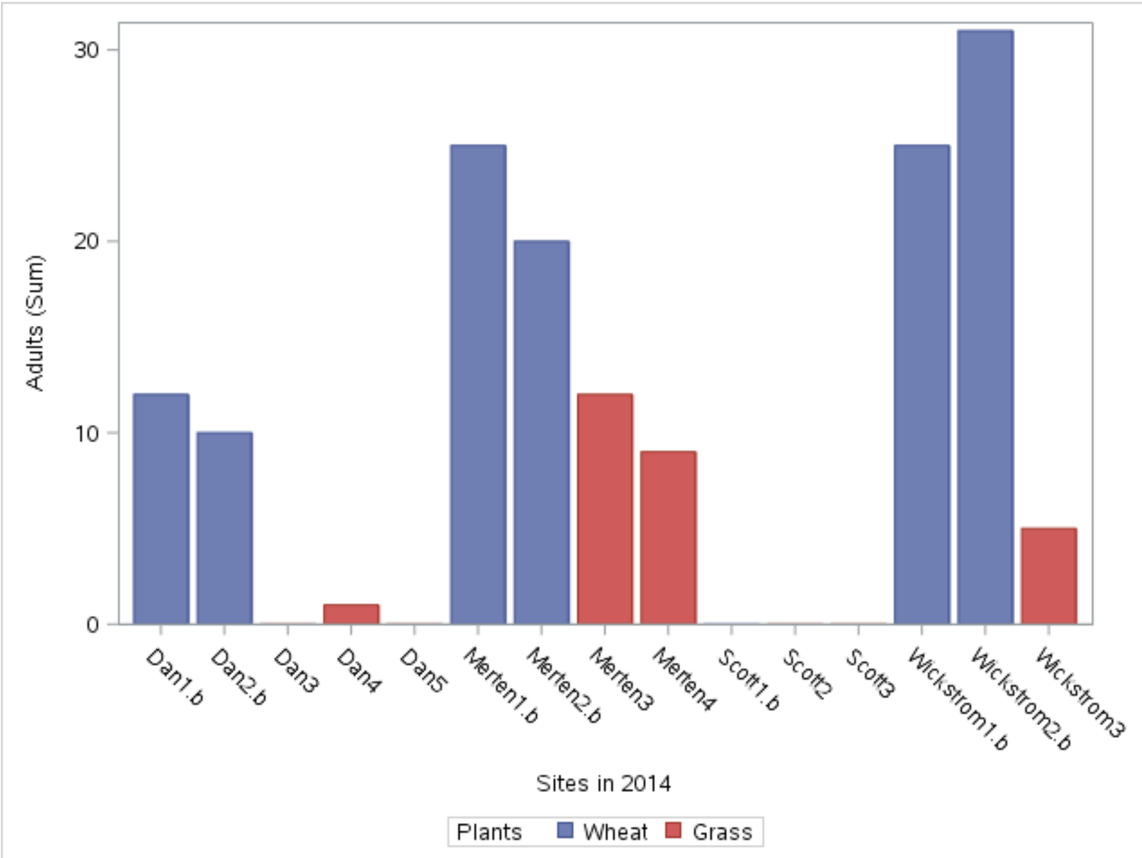

Figure 2.9. Mean number of wheat stem sawfly adults on winter wheat and downy brome across sites in May of 2014.

Discussion

Adult abundance and larval numbers were generally higher on winter wheat than on downy brome. Wheat stem sawfly infestation in winter wheat fields was 11.8 to 13.5 times greater than the mean percent of infestation on downy brome over two years of survey. Also, the adults collected from winter wheat in May during emergence were almost five times more abundant than sawfly adults collected from downy brome. Nevertheless, sawflies will use downy brome as an alternate host.

The quality and availability (proximity to newly emerged adult sawflies) of downy brome were likely important cues in female preference and host selection. Sawflies preferred to lay more eggs on downy brome that were taller and had thicker stems. Furthermore, we found the downy brome was more likely to be attacked when it grew in sites that had higher levels of soil moisture.

While we did not measure soil moisture or the stem height and diameter in this study, we observed these factors during the collecting and processing of samples. Perez-Mendoza *et al*. (2006) reported higher mean infestation by the wheat stem sawfly on downy brome than on wheat in Montana and found significant correlation between stem height and diameter of the downy brome and sawfly oviposition preference. Furthermore, Criddle (1922) reported that the wheat stem sawfly infested smooth brome grass, *Bromus inermis* Leys and Canada wildrye, *Elymus canadensis* (C. L. Hitchcock) Jensen.

Larval mortality of wheat stem sawfly in downy brome was more than 2 times higher than in winter wheat. We observed that the larval mortality on wheat was mostly in the form of moldy, “mushy” dead larvae found in the stems with evidence of fungi or bacteria in the stems. Mortality found in downy brome was more likely due to dry, dead larvae in the stems. Greater mortality was observed in downy brome stems than in winter wheat stems over the two years of the survey.

The results of our study suggests that grass species such downy brome could be used as trap plants attracting the females of wheat stem sawfly but not extremely supportive for larval development, leading to decrease in the sawflies infestation and damage on winter wheat in northeastern Colorado. Similarly, some studies on moths reported that females had high ovipositional preference for most grass species, however, the survivorship of the developed larvae was very low in grasses compared to cultivated maize (Khan *et al*. 1997, Wandera & Mulaa 1997).

We also noticed the larvae found in downy brome stems were smaller in size than larvae that found in wheat stems, however, we did not record larval weight or length in this study. Perez-Mendoza *et al*. (2006) reported that, higher larval survivorship in wheat than in downy brome; furthermore, larvae developed in wheat were heavier than those in downy brome. Our results suggest that larvae of the wheat stem sawfly may survive in downy brome stems but this is a poor host relative to winter wheat. Wheat stem diameters are larger than stem diameters of the most grass species, particularly in drought seasons when grass species may be too small to support larval development (Morrill *et al*. 2000).

Winter wheat seems to be the most suitable host for wheat stem sawfly in northeastern Colorado. However, downy brome is a viable alternate host that may play a significant role in the wheat stem sawfly infestation in northeastern Colorado.

REFERENCES CITED

Ainslie, C.N. (1920). The western grass-stem sawfly. USDA Technical Bulletin No. 841.

Ainslie, C.N. (1929). The western grass-stem sawfly: a pest of small grains. USDA Technical Bulletin No. 157.

Beres, B.L., Dosdall, L.M., Weaver, D.K., Spaner, D.M. & Cárcamo, H.A. (2011). The biology and integrated management of wheat stem sawfly, *Cephus cinctus* (Hymenoptera: Cephidae), and the need for continuing research. The Canadian Entomologist. 143:105–125.

Beres, B. L., McKenzie, R. H., Cárcamo, H. A., Dosdall, L. M., Evenden, M. L., Yang, R. C., & Spaner, D. M. (2012). Influence of seeding rate, nitrogen management, and micronutrient blend applications on pith expression in solid-stemmed spring wheat. Crop Science, 52(3), 1316-1329.

Bird, R. D. (1955). Extension of faunal ranges in the prairie provinces. Entomological Society of Ontario, Annual Report, 86: 33-37.

Callenbach, J. A., & Reinhardt, J. F. (1945). Montana insect pests in 1943-1944. 15th Annual Report of State Entomologist. Mont. Agr. Expt. Sta. Bui. 425, 30 pp.

Criddle, N. (1922). The western wheat stem sawfly in Canada. The report of the Entomological Society, 36, 18-21.

Criddle, N. (1923). The life habits of *Cephus cinctus* Nort. in Manitoba. The Canadian Entomologist, 55: 1–4.

Holmes, N. D., & Peterson, L. K. (1960). The influence of the host on oviposition by the wheat stem sawfly, *Cephus cinctus* Nort. (Hymenoptera: Cephidae). Canadian Journal of Plant Science, 40: 29–40.

Irell, B., & Peairs, F. (2011). Wheat Stem Sawfly: A new pest of Colorado wheat. Colorado State University, U.S. Department of Agriculture.

Khan, Z. R., Ampong-Nyarko, K., Chiliswa, P., Hassanali, A., Kimani, S., Lwande, W., Overholt, W. A., Picketta, J. A., Smart, L. E., & Woodcock, C. M. (1997). Intercropping increases parasitism of pests. *Nature*, *388*(6643), 631-632.

King, K. M. (1929). Insects affecting field crops and gardens in Saskatchewan, 1922-1927. Sci. Agr. 9 : 373-390.

Mack, R. N. (1981). Invasion of *Bromus tectorum* L. into western North America: an ecological chronicle. Agro-ecosystems, 7(2), 145-165.

Montana Agricultural Experiment Station and Montana Extension Service. (1946). The wheat stem sawfly in Montana. 10 pp.

Morrill, W. L., Gabor, J. W., Weaver, D. K., Kushnak, G. D., & Irish, N. J. (2000). Effect of host plant quality on the sex ratio and fitness of female wheat stem sawflies (Hymenoptera: Cephidae). Environmental entomology, 29(2), 195-199.

Morrow, L. A., & Stahlman, P. W. (1984). The history and distribution of downy brome (*Bromus tectorum*) in North America. Weed Science, 2-6.

Perez-Mendoza, J., Weaver, D. K., & Morrill, W. L. (2006). Infestation of wheat and downy brome grass by wheat stem sawfly and subsequent larval performance. Environmental Entomology, 35(5), 1279-1285.

Rashwani, A., 1981. Introduction to the major pests of wheat and barley in the Middle East and North Africa. Tech. Manuel No. 9, 58–63 ICARDA, Aleppo, Syria.

Shanower, T. G., & Hoelmer, K. A. (2004). Biological control of wheat stem sawflies: past and future. Journal of Agricultural and Urban Entomology, 21: 197–221.

Wandera, J., & Mulaa, M. A. (1997). Utilization of wild gramineous plants for management of cereal stem borers in Africa. Insect Sci. Appl, 17, 143-150.

Weiss, M. J., & Morrill, W. L. (1992). Wheat stem sawfly (Hymenoptera: Cephidae) revisited. American Entomologist, 38(4), 241-245.

CHAPTER 3-OVIPOSITIONAL PREFERENCE OF WHEAT STEM SAWFLIES *CEPHUS CINCTUS*

Summary

Insect herbivores generally prefer to oviposit on the most suitable host plants. The wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), infests both cultivated and non-cultivated grasses. Female oviposition behavior may be influenced by different host quality cues such stem height and diameter, volatile production, and growth stage. To expand our understanding of host selection I studied the effect of host quality on female choice. Host selection and oviposition preference by wheat stem sawfly females were studied with a combination of greenhouse choice and no-choice tests using a susceptible (hollow stem) winter wheat variety ('Byrd'), a resistant (solid stem) winter wheat variety ('Bearpaw'), and downy brome raised from field-collected seed. Female sawflies in the no-choice tests laid similar numbers of eggs in Byrd and Bearpaw plants irrespective of growth stage, which were approximately 2.5 times the number of eggs laid on downy brome. Similarly, when given a choice, females laid similar numbers of eggs on Byrd and Bearpaw, but nearly twice as many eggs on either of these two cultivars than on downy brome. Females preferred to oviposit in larger diameter plant stems. However, stem height did not affect female preference for plants at growth stage Zadoks 49, but females did prefer taller stems at growth stage Zadoks 32 and 60. Larval survivorship was lowest in the solid stem Bearpaw and was highest in downy brome and the hollow-stem Byrd. Hollow stem wheat had the highest larval survivorship. These differences suggest that characteristics of the host, such as stem diameter and plant species, determine suitability for oviposition preference and larval survivorship. Our findings support the

importance of the oviposition behavior of the wheat stem sawfly in management tactics, such as trap cropping.

Introduction

Most herbivorous insect species consume one or a few host plants (Futuyma & Moreno 1988). In many insect herbivores, the selection of a suitable site for oviposition is an important life history event. Newly hatched offspring are relatively immobile and are generally not able to search for host plants other than the particular plant chosen by their mother. Therefore, the quality of a host plant for offspring growth and development depends on the choice made by the ovipositing mother. Often, the presumption is that ovipositing mothers choose a host plant that is favorable in terms of offspring survivorship, development, and body size. The positive correlation between oviposition preference and offspring performance is widely known as the 'preference-performance hypothesis' (Mayhew 1998, Gripenberg *et al*. 2010).

Also, optimal foraging would influence host plant choice since insect herbivores may use plant for larval development as well as adult resources (Stephens & Krebs 1986, Gripenberg *et al*. 2010). For example, oviposition preference of *Cephaloleia* beetles is positively linked with host plants that support increased larval survival, female fecundity, and population growth (García-Robledo & Horvitz 2012). While some studies have reported a strong positive correlation between host preference and offspring performance ("mother knows best"), many others report weak correlations, no correlation, or even negative correlations (Rausher 1979, Valladares & Lawton 1991, Mayhew 1997, Scheirs & De Bruyn 2002, Gripenberg *et al*. 2010, García-Robledo & Horvitz 2012). When insect herbivores are not constrained by evolutionary or physiological limitations, preference and performance may not be correlated. For example, as evolution may need several generations to select against females who made a bad decision of choosing low quality hosts, the preference–performance relationship could be negative where insects interact with novel plant species (Chew 1977, Thompson 1988).

Host plant traits may also support the relationship between female preference and offspring performance. Females should be selected to choose high quality host plants when offspring performance differs on different host plants (Craig & Itami 2008). Host plant quality can be measured as the components of the host plant such as nitrogen and carbon content, as well as defensive compounds that affect the insect herbivore performance. Many insect herbivores change the quality of their host plants, affecting both interspecific and intraspecific interactions. Host plant quality influences the fecundity of insect herbivores at both the individual and the population levels (Awmack & Leather 2002).

Use of different host plant species by herbivores can be subject to selection at a variety of spatial scales, leading to changes in oviposition behavior and larval performance on different plant species (Thompson 1988). This is because insect herbivores may encounter many host plant species that differ chemically and physically and, in turn, insect herbivores vary in their responses to plant traits, leading to opportunities for adaptive evolution in response to locally available host plant species (Ladner & Altizer 2005)

The relationship between oviposition preference for a given host plant species and offspring performance (e.g., growth, survival, and reproduction of offspring on those hosts) has been a major issue in the theory of insect-plant interactions (Thompson 1988). The relationship between female preference and offspring performance differs based on the degree of diet specialization. Insects that feed on one type of food or diet would be expected to make a better decision than other insects with different type of diets, since making the decision for females becomes complicated with different choices of host plants (Gripenberg *et al*. 2010).

Host-plant selection by the ovipositing wheat stem sawfly females is affected by several factors such as the growth stage of the plant, the diameter of the stems, and volatile compounds released by plant (Seamans 1945, Morrill *et al*. 2000, Cárcamo *et al*. 2005). Females prefer to oviposit in younger stems and in larger stems (Perez-Mendoza *et al*. 2006). The wheat stem sawfly host selection process involves many host discovery and acceptance cues. Stem diameter, volatiles, and growth stage all influence oviposition preference of wheat stem sawfly (Perez-Mendoza *et al*. 2006, Weaver *et al*. 2009). The objectives of this study were 1) to determine the oviposition preference of wheat stem sawfly for susceptible winter wheat variety (Byrd), resistant winter wheat variety (Bearpaw), and downy brome, and 2) to determine the influence of the host quality for these plants on wheat stem sawfly female's oviposition behavior.

Materials and Methods

Insects

Wheat stem sawflies were obtained as larvae from field-collected grass and wheat stubble samples. The stubble samples were held in plastic zippered storage bags (30 cm wide by 37.5 cm long) at 2-3 ˚C for at least 100 days so that wheat stem sawfly larvae could complete diapause. After the diapause period, the stubble samples were placed in pots with soil. Pots were placed in cages (75 × 75 × 115 cm) with clear plastic front and back panels permitting observation of insect activity and polyester netting (160 µm mesh) side panels for ventilation. There were three zippered openings in the front panel of cage to allow movement of potted plants into and out of the cage. Two smaller sleeve openings (18 cm diameter) on the zippered opening permit addition or removal of insects while preventing escape. The stubble was lightly moistened twice weekly with water to prevent desiccation of the developing larvae. These cages were held at room temperature (22-27°C) under a LD 14.5:9.5h photoperiod until adult sawflies emerged 4-5 weeks later. The cages were checked daily to collect the newly emerged sawfly adults. The adults were removed and placed in glass 2-liter Mason jars containing a 50% sucrose-water solution and moistened filter paper to provide them with nutrition and moisture until they could be used for experiments. Adults used in the experiments had emerged within the previous 24 hours.

Plants

Two winter wheat cultivars (the wheat stem sawfly susceptible 'Byrd' and the resistant 'Bearpaw') and the invasive grass downy brome were used in the choice and no-choice experiments described below. Two vernalized seedlings of one of the winter wheat varieties or downy brome were planted in each round standard pot (13cm x 13cm). The pots were held in a greenhouse with supplemental light (400 watt high pressure sodium lights) at a 14.5: 9.5 h LD photoperiod. Daytime temperature was 23-25 ˚C and the overnight temperature was 20-22 ˚C. Plants were grown in soil mix (7 parts Fafard 2SV custom potting mix, 2 parts Planters Mix, 1 part perlite, and 5g Scotts Osmacote 14-14-14). The plants were watered three to four times weekly. Plants used in the experiments were between GS Zadoks 32 (two nodes visible), GS Zadoks 49 (awns first visible) and GS Zadoks 60 (heading stage). GS Zadoks 32 and GS Zadoks 49 stages represent the range of plant growth stages infested under field conditions. However, in our preliminary experiment, the wheat stem sawfly also laid eggs on GS Zadoks 60 (heading stage) plants, so we included the heading stage in the experiment. Plants were fertilized with

Peters General Purpose Fertilizer 20-20-20 (J. R. Peters, Allentown, PA) at 100 ppm in aqueous solution twice each week starting at Zadoks 13 (three unfolded leaves).

Choice Tests

In order to determine whether ovipositing wheat stem sawflies exhibited a preference between the wheat cultivars and downy brome, we conducted choice tests in screened cages (75cm x 75cm x 115cm) using the following comparisons: Byrd vs. Bearpaw, Byrd vs. downy brome, and Bearpaw vs. downy brome. For each choice trial, we placed eight pots, four of each variety, inside the cage. Ten females and five males were released within the cage and allowed to mate and oviposit for one day (Weaver et al. 2009). Choice tests were repeated using plants at one of the following developmental stages: GS Zadoks 32, GS Zadoks 49, and GS Zadoks 60. Fifteen replicates were run for each choice combination.

After oviposition, adults were removed from the cages. Then plants were left for three days to allow the eggs to swell so that they could be more visible and easily counted. After three days, one plant from each pot was removed and dissected under a stereomicroscope to count the number of eggs laid. In order to assess any mortality effects of the different plants (sawfly performance), the remaining plant in each pot was left for three weeks to allow the sawfly larvae to complete development. Stems where no wasp emerged were dissected and numbers of live and dead larvae were recorded. Host plant quality was measured in terms of stem height (measured from the soil surface to the highest extended leaf in the stem in cm) and diameter (measured near the soil surface in mm). Plant height and diameter were used as measures of host quality influencing the preference of the wheat stem sawfly females.

No-Choice Tests

To examine female sawfly oviposition behavior in the absence of choice, we conducted the following no-choice tests: Byrd alone, Bearpaw alone, or downy brome alone. Each trial was conducted in a screened cage with eight pots as described above. As with the choice tests, 10 females and five males were introduced to each cage for 24 hours. The number of eggs laid were counted for one of the two plants; the other plant was left for three weeks after which time the stems were dissected to count the number of larvae that were dead or alive. These no-choice trials were repeated using plants at Zadoks 32, Zadoks 49, and Zadoks 60. Fifteen replicates were used for each treatment of the no-choice tests.

Data Analysis

Choice Tests

The effects of plant growth stage (GS Zadoks 32, GS Zadoks 49, and GS Zadoks 60) and different plant types (Byrd, Bearpaw, and downy brome) on the preference of the wheat stem sawflies for the group of choice tests were analyzed using two-way analysis of variance (ANOVA). Differences in the number of eggs laid on different host plants for each plant growth stage: GS Zadoks 32, GS Zadoks 49, and GS Zadoks 60 in the choice tests were analyzed using one-way ANOVA.

To compare the number of stems that contained wheat stem sawfly eggs across the different plants and choices, infestation was modeled as a binary response variable (1 = infested stem, 0 = not infested stem). Data were analyzed using a logistic regression in SAS (Proc Glimmix) treating plant type as a fixed effect, plant height and plant diameter as covariates, and replicate as a random factor.

To compare the number of stems of the second plant containing dead or live larvae, larval mortality was modeled as a binary response variable (1 = dead larvae, 0 = live larvae). Data were analyzed using logistic regression in SAS (Proc Glimmix) treating plant type as a fixed effect, plant height and diameter as covariates, and replicate as a random factor.

No-Choice Tests

The effects of the two factors, development stages (GS Zadoks 32, GS Zadoks 49, and GS Zadoks 60) and host plants (Byrd, Bearpaw, and downy brome) on the number of eggs and larvae of the wheat stem sawflies in no-choice tests were analyzed using two-way ANOVA. For each plant growth stage of the no choice tests, the infestation differences on plants were analyzed using three separate one-way ANOVAs.

Results

Choice Tests

In choice test, the development stage influenced number of eggs and larvae; $F_{2,261} = 13.18$, $P < 0.0001$. The growth stages of Zadoks 32 and 60 had ten percent higher infestation rate than Zadoks 49. Also plant hosts affected the females preference and infestation; $F_{2,261} = 631.12$, $P < 0.0001$. Wheat cultivars “Byrd” and “Bearpaw” had greater number of eggs and larvae, 2.2 times more than downy brome.

The interaction between development stages and host plants was also significant; $F_{4,261} = 4.22$, $P < 0.0025$.

For group of choice tests, there was no significant difference in the mean of wheat stem sawfly infestation between Zadoks 32 growth stage and the heading stage Zadoks 60; $t = -1.36$, df = 261, P = 0.3649. However, the infestation mean was significantly different between Zadoks 32 and Zadoks 49 growth stages; t = 3.61, df = 261, P = 0.0011also between Zadoks 49 and Zadoks 60 growth stages; t = -4.97, df = 261, P <0.0001.

No-Choice Tests

Development stage affected the number of eggs and larvae in no-choice tests; $F_{2,126}$ = 12.31, P < 0.0001 growth stages of Zadoks 32 and 60 had higher infestation, 1.06 and 1.10 times more than GS Zadoks 49. The host plant played a significant role in the female preference; $F_{2,126}$ = 425.58, P < 0.0001), wheat cultivars had higher infestation, 1.8 times more than downy brome. The interaction between development stages and host plants was significant; $F_{4,126}$ = 52.73, P < 0.0001).

The early stage Zadoks 32 and the heading stage Zadoks 60 were not different in the number of eggs and larvae for no-choice tests; t = -1.82, df = 126, P = 0.1662. However, the infestation mean was significantly different between Zadoks 32 and Zadoks 49 growth stages; t = 3.09, df = 126, P = 0.0070 also between Zadoks 49 and Zadoks 60 growth stages; t = -4.91, df = 126, P <0.0001.

Choice Tests in Cages at GS Zadoks 32

In choice test between Byrd and Bearpaw the number of eggs and larvae was not different at stage Zadoks 32; F(1,14) = 0.85, P = 0.3712. The mean infestation for Byrd was 19.20 and for Bearpaw mean infestation was 19.87. However, the number of eggs and larvae was greater in Byrd than downy brome at stage Zadoks 32; F(1,14) = 403.72, P < 0.0001. The wheat stem sawfly laid 2.4 times more eggs in Byrd than downy brome grass. In choice test of Bearpaw and downy brome we observed higher infestation in Bearpaw than downy brome grass: F(1,28) = 408.59, P < 0.0001. The females laid 2.6 times more in Bearpaw than downy brome grass (Figure 3.1).

No-Choice Tests in Cages at GS Zadoks 32

In no choice tests, wheat stem sawfly females strongly preferred to lay eggs on the two wheat cultivars compared to downy brome; females laid 2.5 times more eggs on Bearpaw than they did on downy brome and 2.4 times more eggs on Byrd than on downy brome ($F_{2,42}$ = 531.83, P < 0.001) (Figure 3.2).

Choice Tests in Cages at GS Zadoks 49

The number of eggs and larvae of wheat stem sawfly in the choice tests were different at growth stage of Zadoks 49. In the choice test between Byrd and Bearpaw, the females laid more eggs on Bearpaw; F(1,14) = 18.20, P = 0.0002. However, the females preferred Byrd over downy brome; F(1,14) = 668.50, P < 0.0001 as well as Bearpaw over downy brome: F(1,14) = 155.62, P < 0.0001 (Figure 3.3).

No-Choice Tests in Cages at GS Zadoks 49

In no choice tests, the number of eggs and larvae was different between plants; $F_{2,42}$ = 13.45, P < 0.0001. The females laid slightly more eggs on Byrd and Bearpaw 1.23 and 1.16 times over downy brome grass at growth stage Zadoks 49 in no choice tests (Figure 3.4).

Choice Tests in Cages at GS Zadoks 60

In choice tests, the wheat varieties at Zadoks 60 did not differ in terms of the number of eggs and larvae laid: $F_{1,14} = 0.10$, $P = 0.75$. The hollow stem wheat "Byrd" had higher infestation over downy brome in choice test at stage Zadoks 60; $F_{1,28} = 103.42$, $P < 0.0001$ also the solid stem wheat cultivar "Bearpaw" was preferred by the wheat stem sawfly over downy brome: $F_{1,14} = 104.75$, $P < 0.0001$ (Figure 3.5).

The wheat stem sawfly females likely preferred to lay eggs on Byrd and Bearpaw over downy brome so, the overall infestation percentages in the choice tests were higher on both wheat cultivars, 60.62% for Byrd and 59.13% for Bearpaw, and 39.19% for downy brome grass.

No-Choice Tests in Cages at GS Zadoks 60

In no-choice tests, the number of eggs and larvae differed among Byrd, Bearpaw, and downy brome grass; $F_{2,42} = 178.09$, $P < 0.0001$ at growth stage Zadoks 60. The wheat varieties Byrd and Bearpaw had higher infestation than downy brome grass (Figure 3.6)

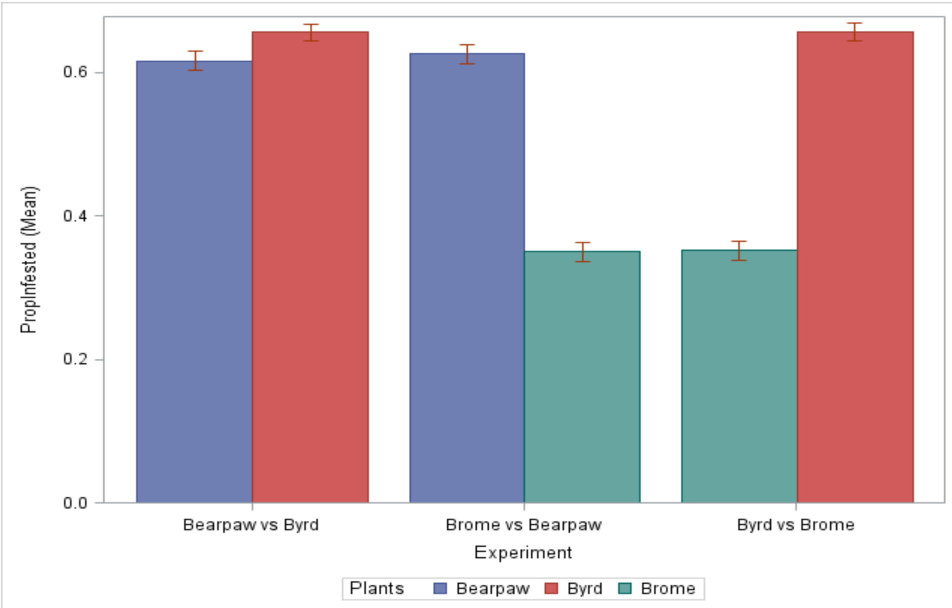

Figure 3.1. Infestation in Bird, Bearpaw and downy brome, expressed as proportion of infested stems in choice tests, growth stage Zadoks 32.

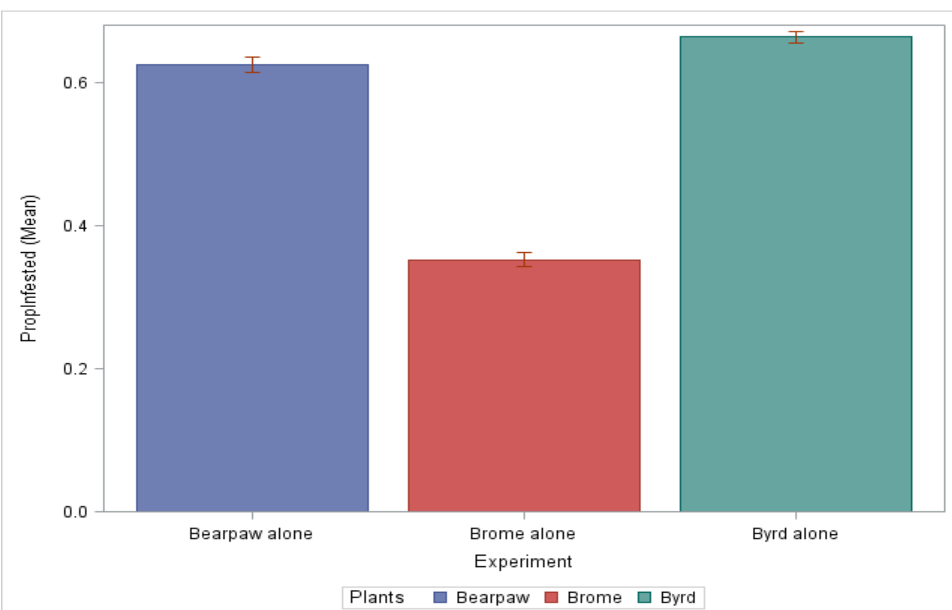

Figure 3.2. Infestation in Bird, Bearpaw and downy brome, expressed as proportion of infested stems in no-choice tests, growth stage Zadoks 32.

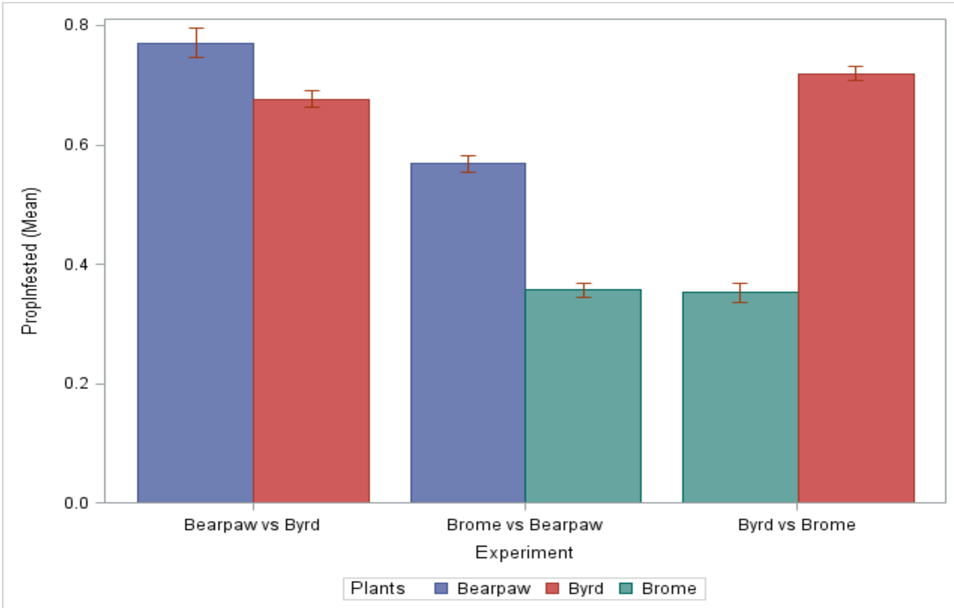

Figure 3.3. Infestation in Bird, Bearpaw and downy brome, expressed as proportion of infested stems in choice and no-choice tests, growth stage Zadoks 49.

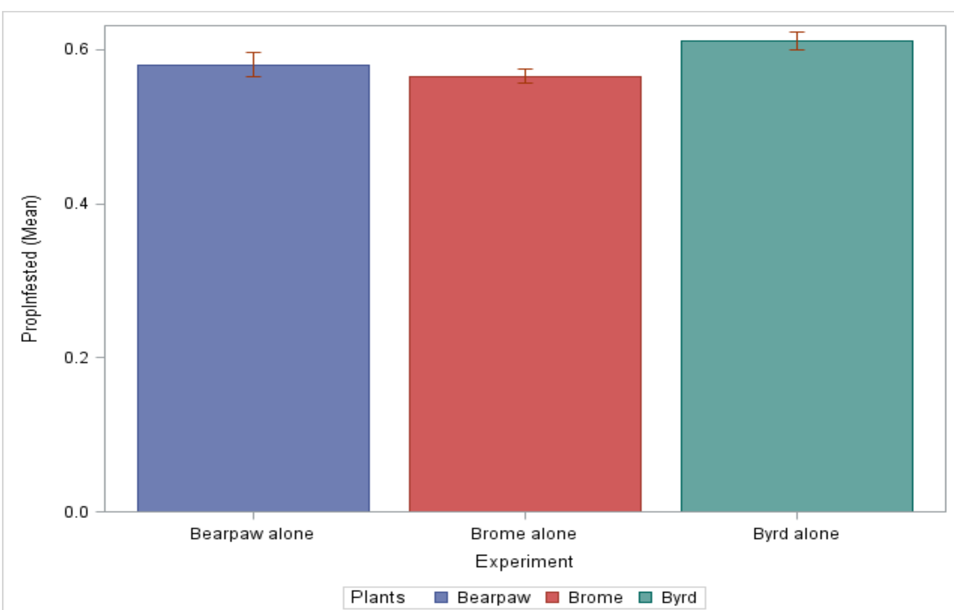

Figure 3.4. Infestation in Bird, Bearpaw and downy brome, expressed as proportion of infested stems in no-choice tests, growth stage Zadoks 49.

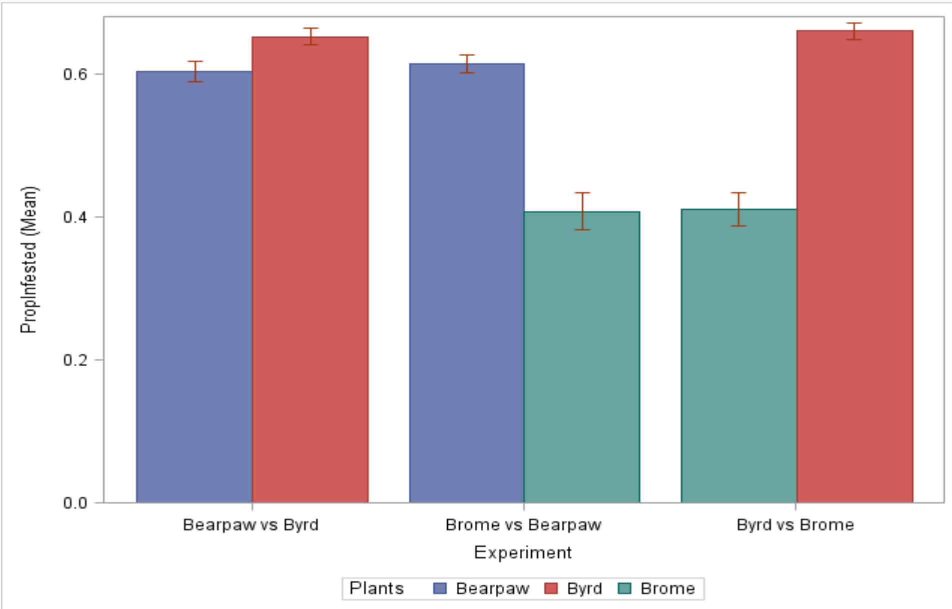

Figure 3.5. Infestation in Bird, Bearpaw and downy brome, expressed as proportion of infested stems in choice tests, growth stage Zadoks 60.

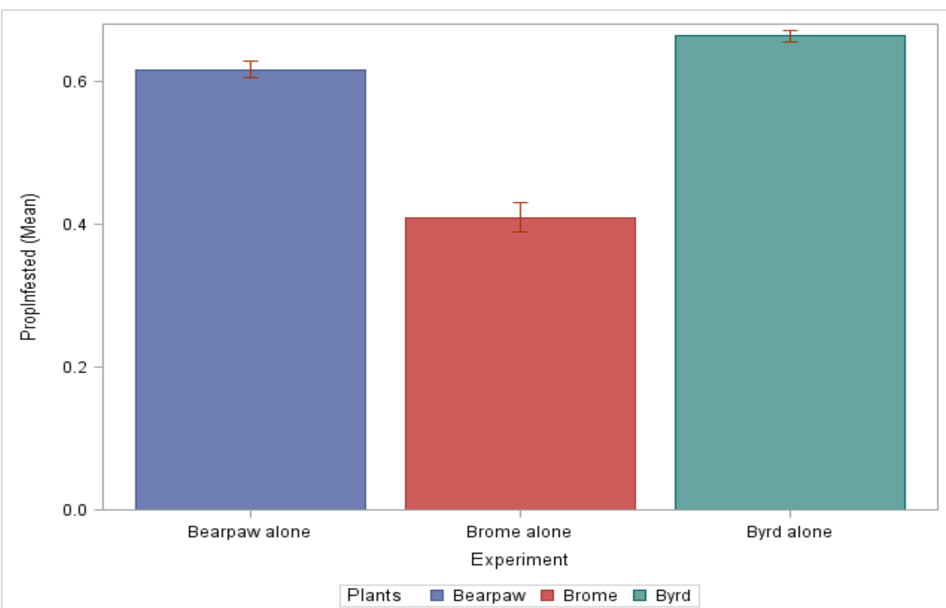

Figure 3.6. Infestation in Bird, Bearpaw and downy brome, expressed as proportion of infested stems in no-choice tests, growth stage Zadoks 60.

Wheat Stem Sawfly Females Preference and Host Quality at GS Zadoks 32

There was a significant difference in number of eggs in different plants (Byrd, Bearpaw and downy brome); $F_{2,231.6} = 13.84$, $P < 0.0001$). Plant height and plant diameter affecting sawfly preference; $F_{1,2165} = 309.24$, $P < 0.0001$) and $F_{1,2165} = 221.90$, $P < 0.0001$. Female wheat stem sawfly laid more eggs in Bearpaw and Byrd as 62.87% and 61.62% than in downy brome 33.19%.

Plant height and plant diameter had significant relationship with the number of eggs (Figures 4 and 5). For every one unit change in plant height and plant diameter, the log odds of eggs increases by 0.4877 and 7.1274, respectively. The plant height was greater in Byrd than Bearpaw and downy brome at stage Zadoks 32. Plant diameters were similar in different plants at growth stage Zadoks 32 (Figures 3.7 and 3.8).

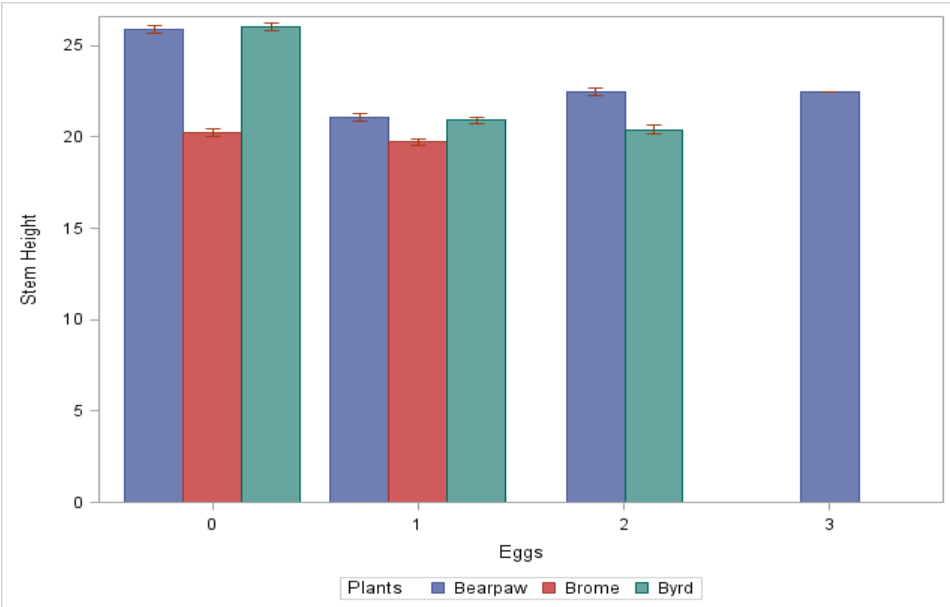

Figure 3.7. Plant height and number of wheat stem sawfly eggs in Bird and Bearpaw winter wheats and downy brome at growth stage Zadoks 32.

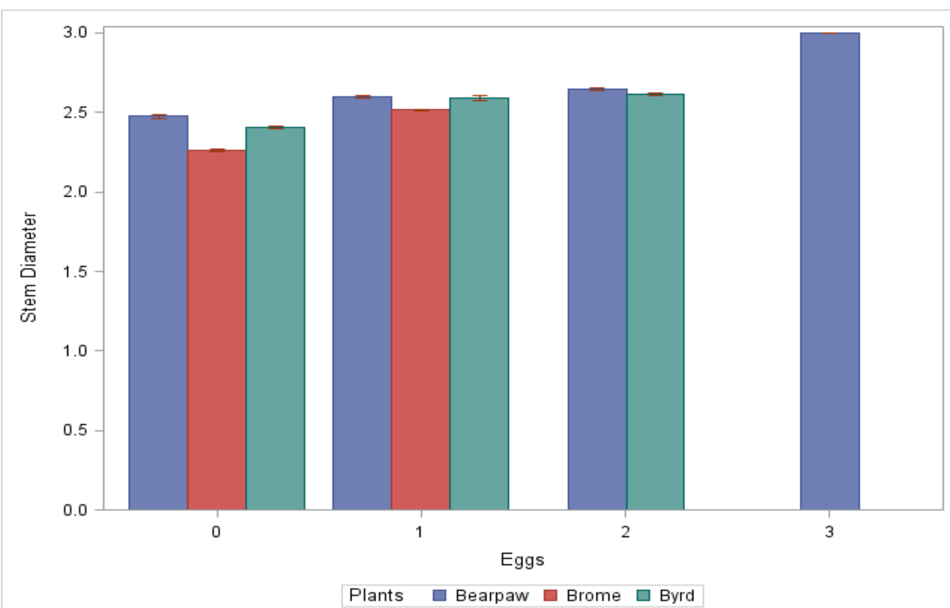

Figure 3.8. Plant diameter and number of wheat stem sawfly eggs in in Bird and Bearpaw winter wheats and downy brome at growth stage Zadoks 32.

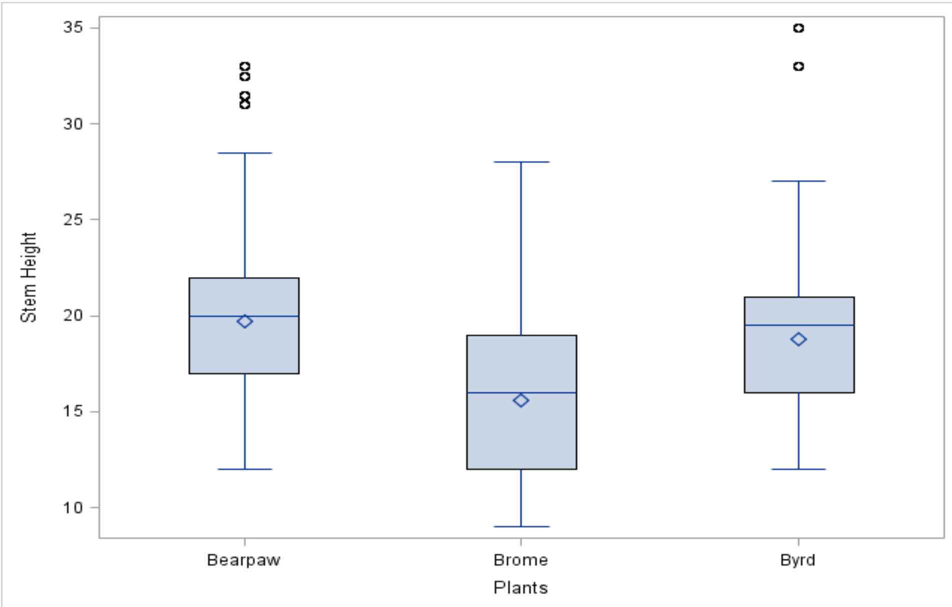

Figure 3.9. Plant height in choice and no-choice tests of Bird and Bearpaw winter wheats and downy brome at growth stage Zadoks 32.

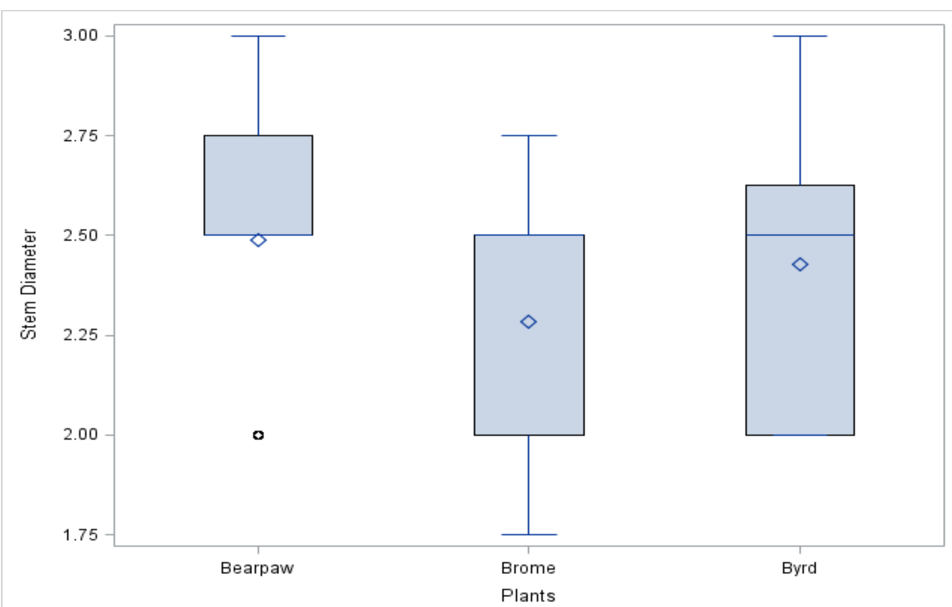

Figure 3.10. Plant diameter in choice and no-choice tests of Bird and Bearpaw winter wheats and downy brome at growth stage Zadoks 32.

Wheat Stem Sawfly Females Preference and Host Quality at GS Zadoks 49

There was a significant difference in number of eggs in different plants; $F_{1,754.5} = 11.20$, $P < 0.0001$. Plant diameter affected the number of eggs; $F_{1,1980} = 455.39$, $P < 0.0001$. However plant height did not affect the number of eggs laid by female sawflies; $F_{1, 1980} = 3.18$, $P = 0.07$ (Figures 3.11 and 3.12). The percentage of eggs was higher in Byrd as 59.86% than in Bearpaw as 53.79% and 61.62% and downy brome 44.34%.

For every one unit change in plant diameter, the log odds of eggs increases by 5.3374. The plant height was greater in Byrd than Bearpaw and downy brome at GS Zadoks 49. Plant diameter was greater in Bearpaw than Byrd and downy brome at GS Zadoks 49 (Figures 3.13 and 3.14).

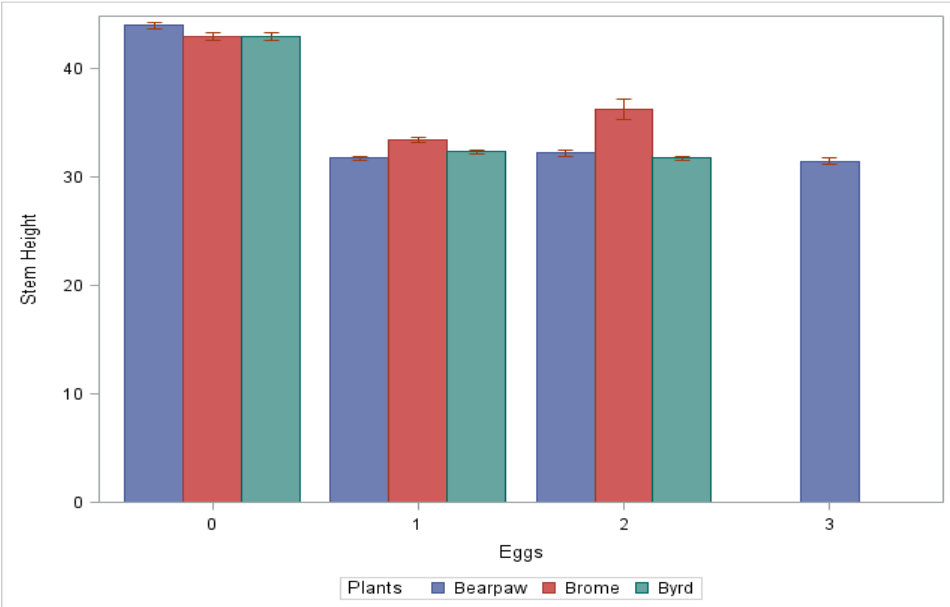

Figure 3.11. Plant height and number of wheat stem sawfly eggs in Bird and Bearpaw winter wheats and downy brome at growth stage Zadoks 49.

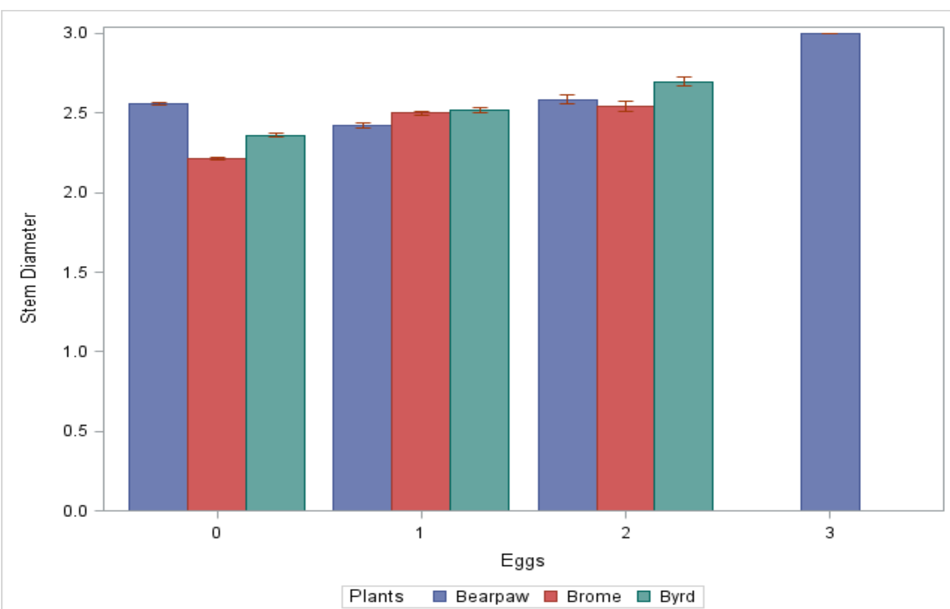

Figure 3.12. Plant diameter and number of wheat stem sawfly eggs in Bird and Bearpaw winter wheats and downy brome at growth stage Zadoks 49.

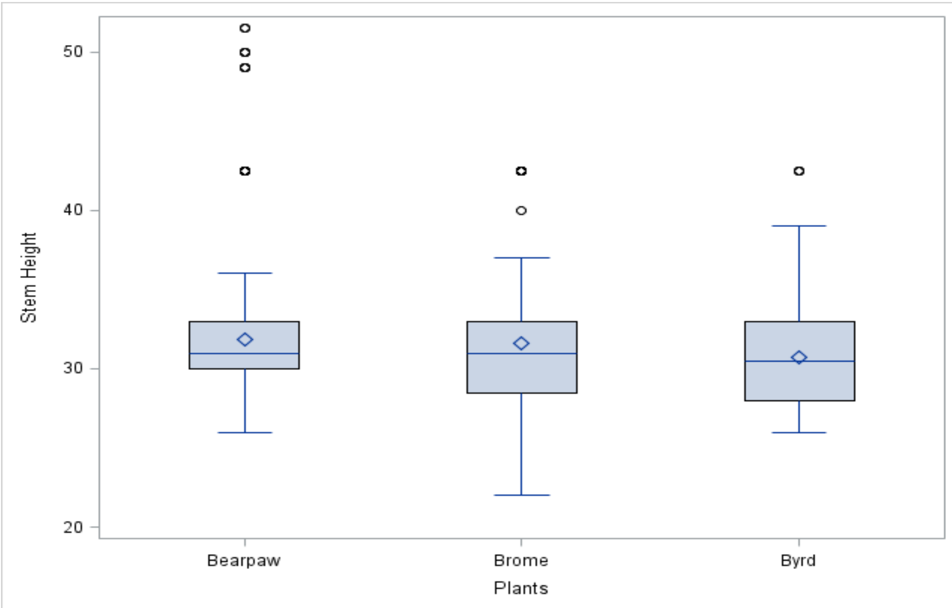

Figure 3.13. Plant height in choice and no-choice tests of Bird and Bearpaw winter wheats and downy brome at growth stage Zadoks 49.

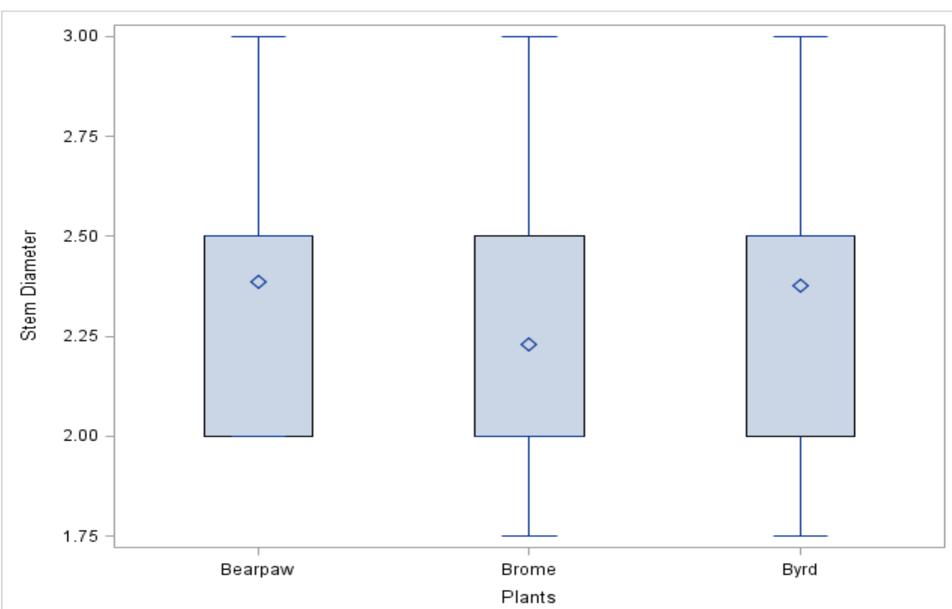

Figure 3.14. Plant diameter in choice and no-choice tests of Bird and Bearpaw winter wheats and downy brome at growth stage Zadoks 49.

Wheat Stem Sawfly Females Preference and Host Quality at GS Zadoks 60

There was a significant relationship between number of eggs and host plants; $F_{1,680}$ = 36.99, P < 0.0001, plant height; $F_{1, 1960}$ = 415.74, P < 0.0001 and plant diameter; $F_{1,1960}$ = 206.03, P < 0.0001. The percentage of eggs in Byrd and Bearpaw are similar as 61.19% and 60.73 while the percentage of eggs in downy brome was 40.06%.

Number of eggs in all plants was positively associated with both plant diameter and height (Figures 3.15 and 3.16). For every one unit change in plant height and plant diameter, the log odds of eggs increases by 0.1044 and 4.6417, respectively. The plant height was similar in Byrd and Bearpaw at GS Zadoks 60. Plant diameter was greater in Bearpaw and Byrd at GS Zadoks 60 than downy brome (Figures 3.17 and 3.18).

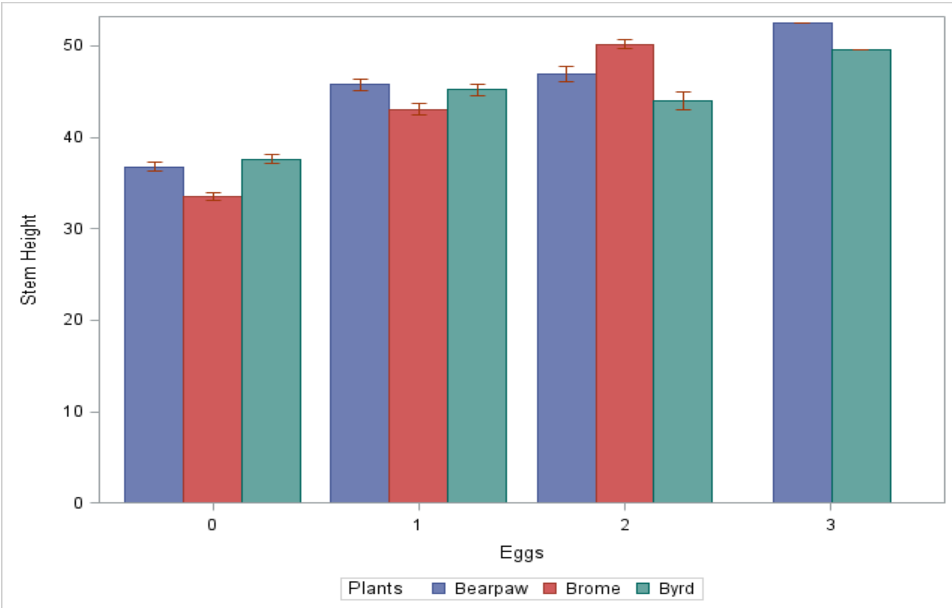

Figure 3.15. Plant height and number of wheat stem sawfly eggs in Bird and Bearpaw winter wheats and downy brome at growth stage Zadoks 60.

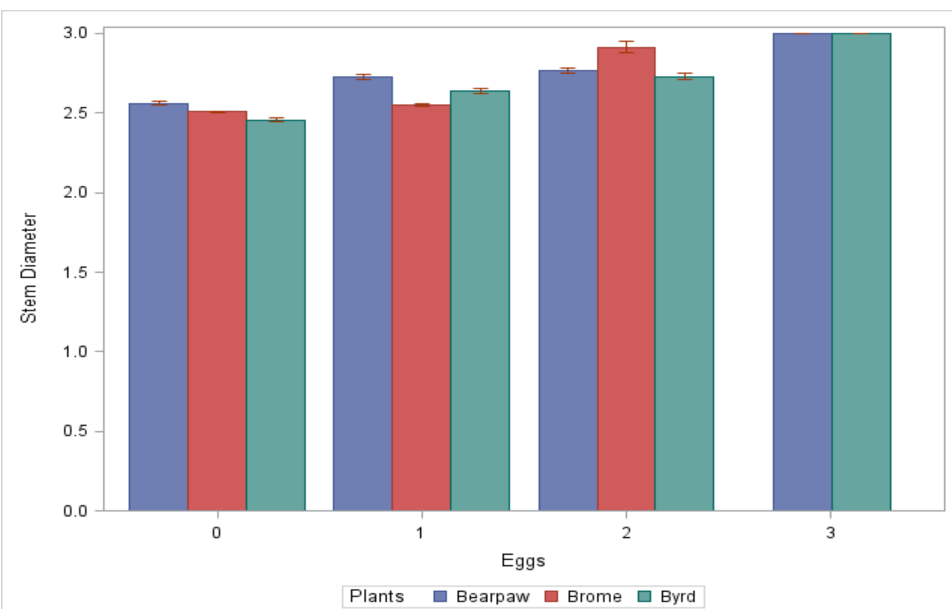

Figure 3.16. Plant diameter and number of wheat stem sawfly eggs in Bird and Bearpaw winter wheats and downy brome at growth stage Zadoks 60.

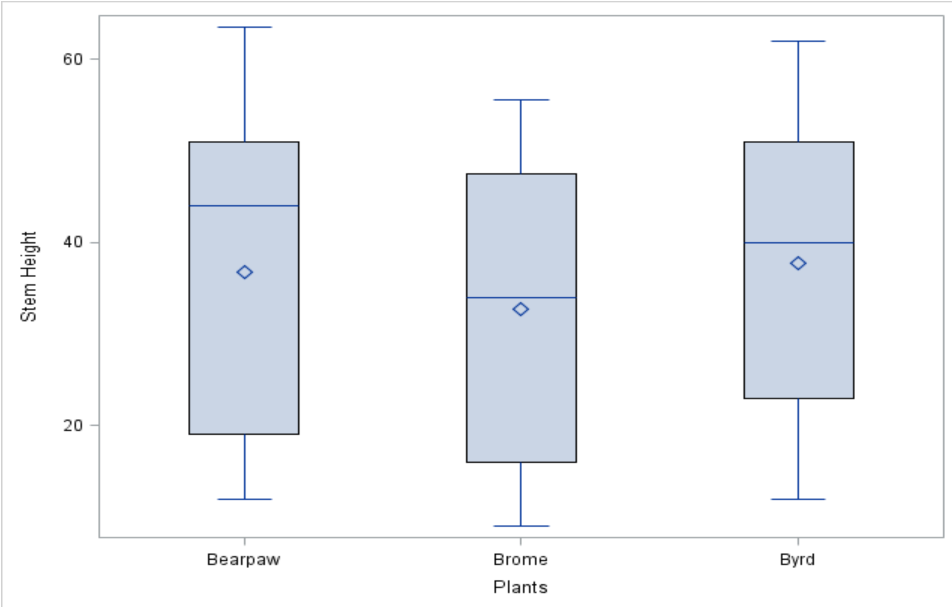

Figure 3.17. Plant height in choice and no-choice tests of Bird and Bearpaw winter wheats and downy brome at growth stage Zadoks 60.

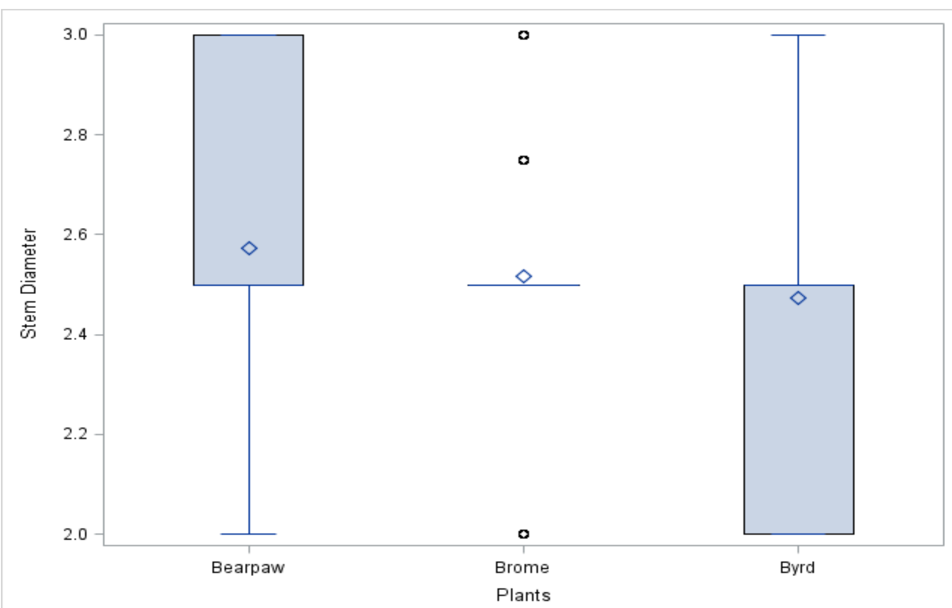

Figure 3.18. Plant diameter in choice and no-choice tests of Bird and Bearpaw winter wheats and downy brome at growth stage Zadoks 60.

Wheat Stem Sawfly Larvae Survivorship at GS Zadoks 32

The survivorship of the wheat stem sawfly larvae was higher in Byrd than Bearpaw and downy brome. The mortality rate was higher in Bearpaw (38.24% mortality) than in downy brome (33.18% mortality) and no dead larvae were observed on Byrd (Figure 3.19). Plant height was greater in Bearpaw and Byrd and plant diameters were similar in different plants at stage Zadoks 32 (Figures 3.20 and 3.21).

Larval survivorship was significantly correlated with plants height at growth stage Zadoks 32: $F_{1,1254} = 316.89$, $P = 0.036$ and plant diameter: $F_{1,1254} = 281.11$, $P = 0.038$. However, different plant hosts did not affect the number of live larvae at GS Zadoks 32.

Wheat Stem Sawfly Larvae Survivorship at GS Zadoks 49

The survivorship of the wheat stem sawfly larvae was higher in Byrd than Bearpaw and downy brome as 99.28% in Byrd, 95.47in downy brome and 77.93 in Bearpaw. The mortality rate was higher in Bearpaw and downy brome than in Byrd (Figure 3.19). Plant height was similar in Bearpaw, Byrd and downy brome and plant diameter was greater in Bearpaw than Byrd and downy brome at GS Zadoks 49 (Figures 3.22 and 3.23).

Numbers of live larvae were significantly correlated with different plants at growth stage 49 Zadoks: $F_{1,740.5} = 57.47$, $P < 0.0001$ and plant diameter: $F_{1,1249} = 49.99$, $P < 0.0001$. However, plant height did not have significant effect on number of live larvae: $F_{1,1249} = 0.20$, $P = 0.66$.

Wheat Stem Sawfly Larval Survivorship at GS Zadoks 60

The survivorship of the wheat stem sawfly larvae was higher in Byrd, 100%, than downy brome, 94.99%, and Bearpaw, 67.51%. The survival rate was lower in Bearpaw than downy brome (Figure 3.19). Plant diameter was greater in Bearpaw and Byrd than downy brome at GS Zadoks 60. Plant height was similar in Bearpaw and Byrd at (Figures 3.24 and 3.25).

The survivorship of wheat stem sawfly larvae was correlated with plant diameter: $F_{1,2231} = 266.26$, $P < 0.0001$, plant height: $F_{1,\ 2231} = 211.29$, $P < 0.0001$ and different plants: $F_{1,305.1} = 117.93$, $P < 0.0001$) at GS 60 Zadoks.

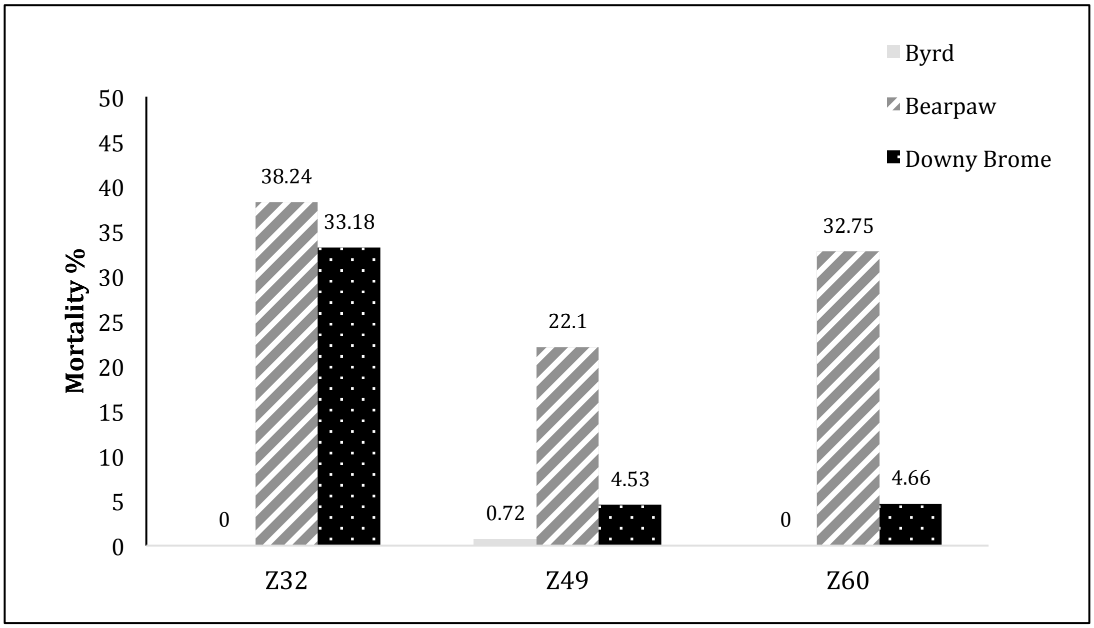

Figure 3.19. Mortality of wheat stem sawfly larvae at Zadoks 32, Zadoks 49, and Zadoks 60.

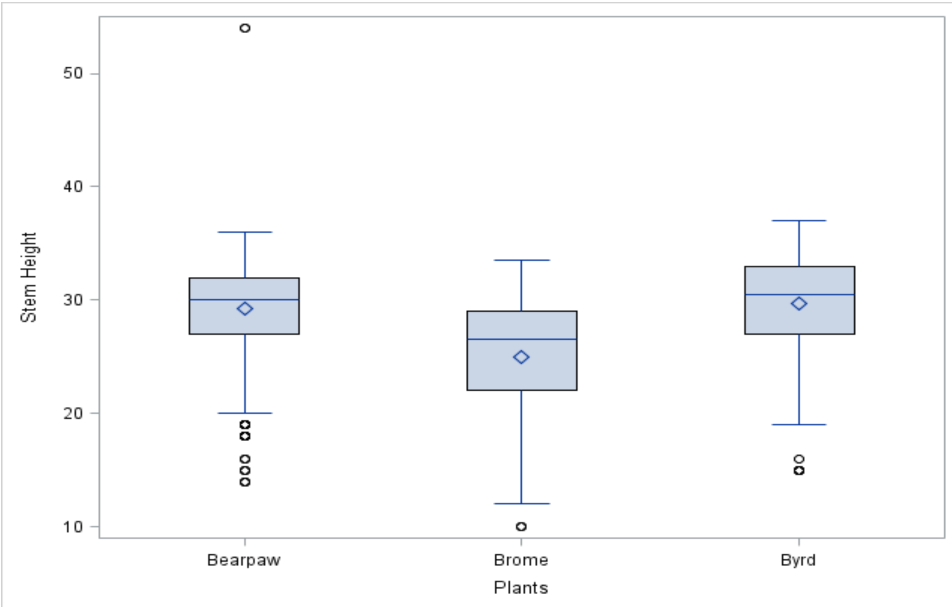

Figure 3.20. Plant height in choice and no-choice tests of the second seedling for Byrd and Bearpaw winter wheats and downy brome at growth stage Zadoks 32.

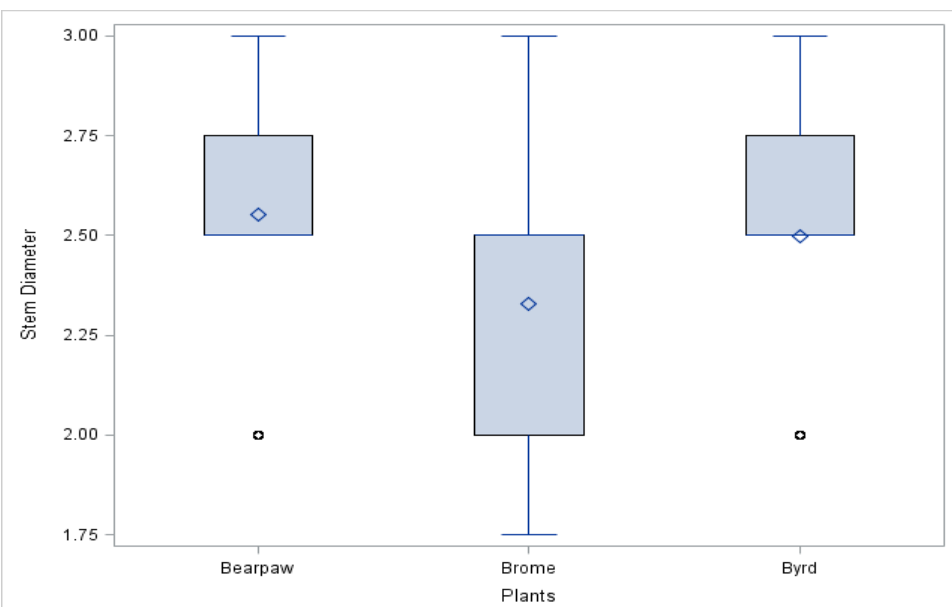

Figure 3.21. Plant diameter in choice and no-choice tests of the second seedling for Byrd and Bearpaw winter wheats and downy brome at growth stage Zadoks 32.

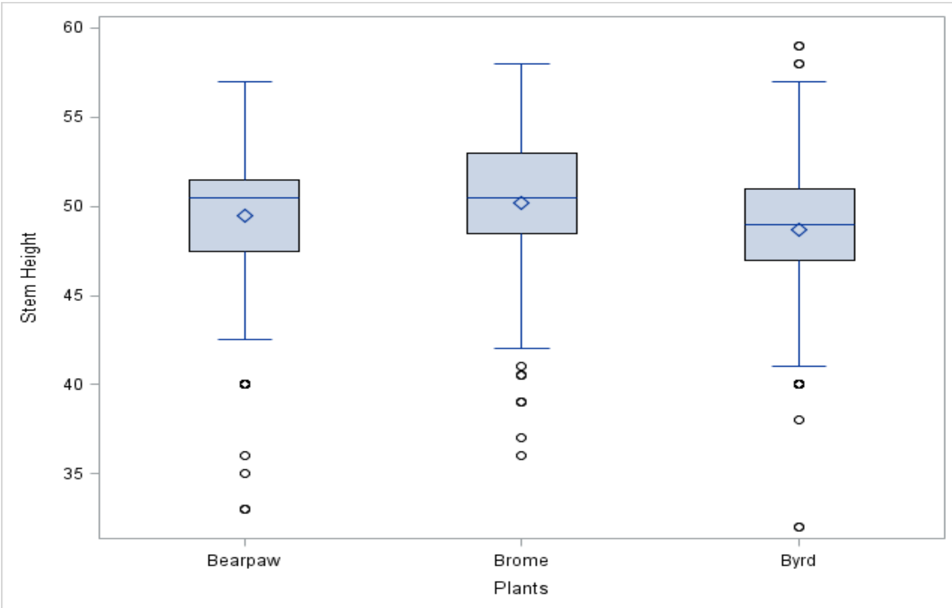

Figure 3.22. Plant height in choice and no-choice tests of the second seedling for Byrd and Bearpaw winter wheats and downy brome at growth stage Zadoks 49.

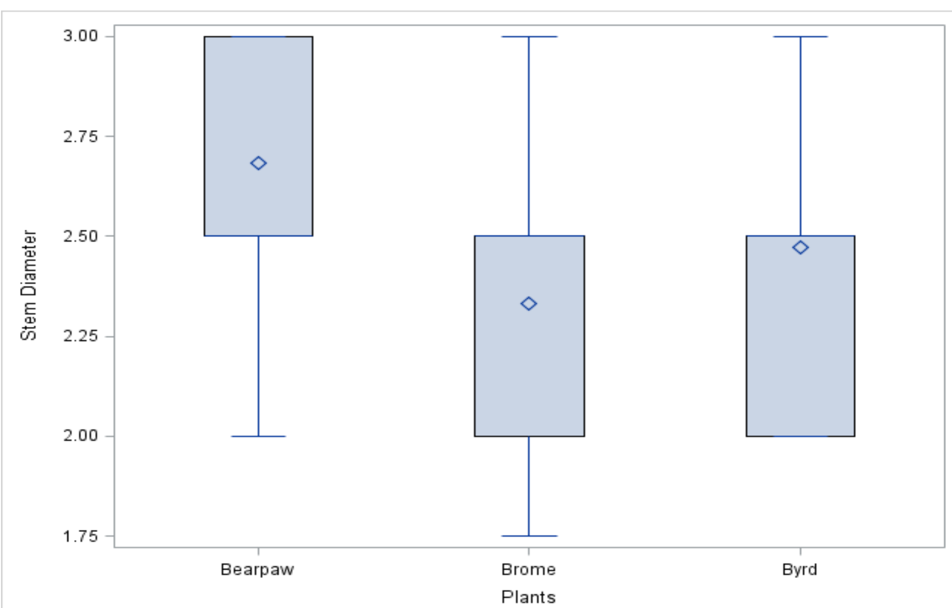

Figure 3.23. Plant diameter in choice and no-choice tests of the second seedling for Byrd and Bearpaw winter wheats and downy brome at growth stage Zadoks 49.

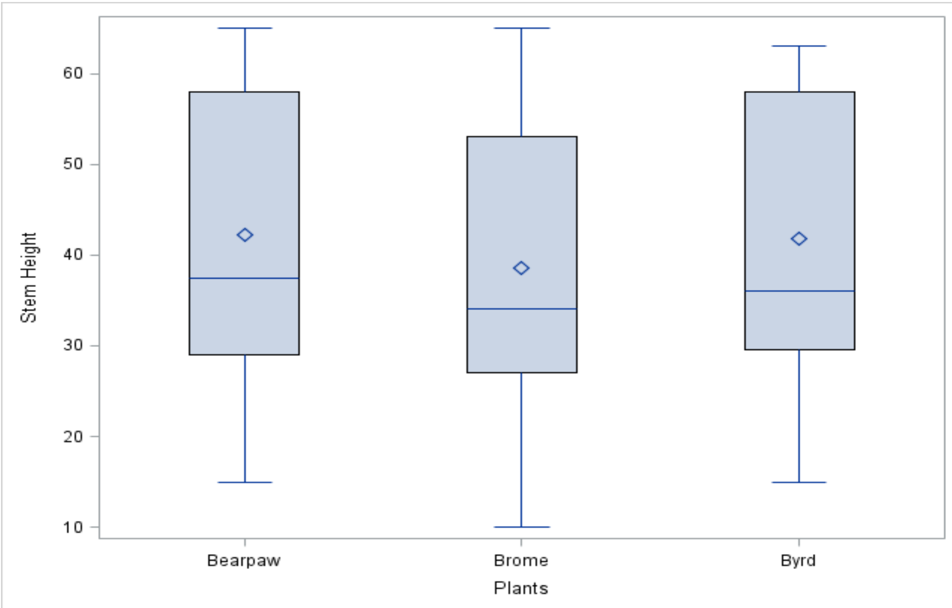

Figure 3.24. Plant height in choice and no-choice tests of the second seedling for Byrd and Bearpaw winter wheats and downy brome at growth stage Zadoks 60.

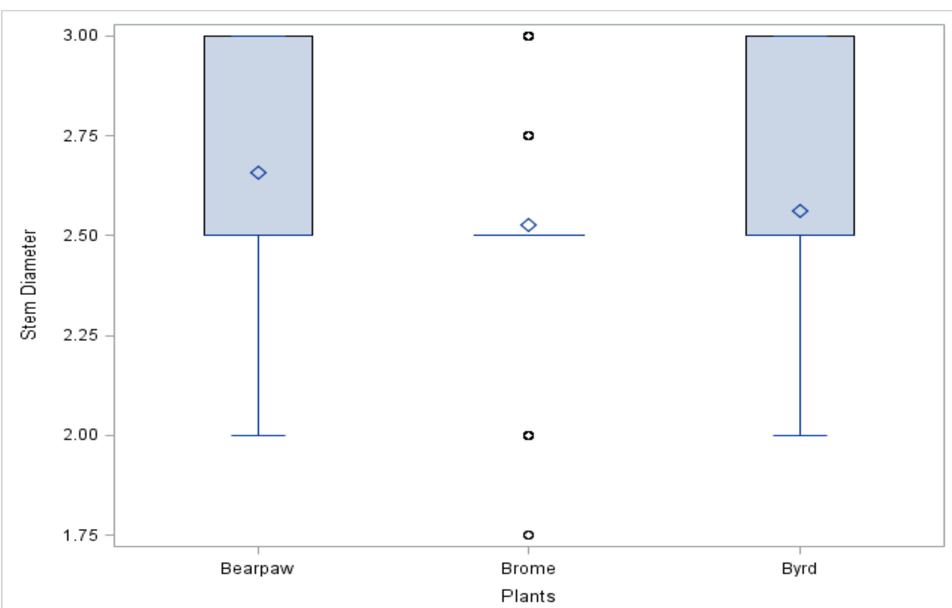

Figure 3.25. Plant diameter in choice and no-choice tests of the second seedling for Byrd and Bearpaw winter wheats and downy brome at growth stage Zadoks 60.

Discussion

In both choice and no-choice tests, wheat stem sawflies preferred both wheat cultivars, the hollow-stemmed Byrd and solid-stemmed Bearpaw, over downy brome. The female preferred both wheat cultivars over downy brome irrespective of plant development stages. There was no significant difference in infestation between Byrd and Bearpaw. Semiochemicals are known to be important cues for host acceptance and oviposition behavior of the wheat stem sawflies (Piesik *et al*. 2008, Weaver *et al*. 2009). Although we did not compare the volatile profiles of these two cultivars in our study, one possibility is that sawflies could not discriminate between both wheat cultivars because the plant volatiles released from Byrd and Bearpaw are similar. However, Weaver *et al*. (2009) reported that two spring wheat cultivars, 'Reeder' and 'Conan', released different amounts of some volatiles compounds. Reeder plants release higher amounts of (Z)-3-hexenyl acetate and wheat stem sawflies could discriminate between wheat cultivars. Some studies have showed that female wheat stem sawflies use the released volatile compounds from host plants as cues to select the suitable host plant for oviposition (Piesik *et al*. 2008).

Larval mortality was observed on Bearpaw, presumably due to the solid-stem trait of this cultivar and on downy brome due to small stem diameter not supporting larval development. Larval mortality was lower in Byrd and downy brome compared to Bearpaw.

Plant height and diameter are known to influence host plant selection by wheat stem sawfly females (Holmes & Peterson 1960). We found the stem diameter of both wheat cultivars and downy brome of the same growth stage influenced positively the number of eggs laid by wheat stem sawfly. Wheat stem sawflies may use some cues such as plant volatiles, host quality, and contact when selecting a suitable host plants (Bruce *et al*. 2005). Female wheat stem sawflies choose host plants with larger stems as oviposition sites in the same host species (Holmes & Peterson 1960, Morrill *et al*. 1992, Perez-Mendoza *et al.* 2006). Perez-Mendoza *et al*. (2006) reported that oviposition preference of female wheat stem sawflies seems to be influenced by stem diameter. Furthermore stem diameter and height are correlated.

Our data showed that stem diameter of downy brome grass was smaller than either of the wheat varieties and that female wheat stem sawflies infested mostly the larger stems of downy brome grass. Similarly, Perez-Mendoza *et al*. (2006) reported that female sawflies laid eggs in larger diameter downy brome grass stems and that 75% of the uninfested stems had smaller diameters than infested stems. These finding support our hypothesis that wheat stem sawfly females should choose stems with larger diameters as oviposition sites regardless the host species. Morrill *et al*. (2000) reported that grass species have smaller stems in diameter than wheat crop when drought stress occurred in fields had economic damage of wheat sawflies. Holmes and Peterson (1960) also reported that female wheat stem sawflies oviposit in larger diameter wheat stems. Our study showed that both winter wheat cultivars “Byrd” and “Bearpaw” had similar stem diameter at a given development stage and that they were larger than downy brome. Cárcamo *et al*. (2016) reported that plant volatiles had an effect on the oviposition of wheat stem sawfly on the hollow-stemmed durum spring wheat “AC Avonlea” which had less infestation by wheat stem sawfly eggs than solid-stemmed spring wheat “AC Lillian” although “AC Avonlea” had larger plant diameter than “AC Lillian”.

The wheat stem sawfly larval survivorship was higher in the hollow-stemmed cultivar “Byrd” than in the solid-stemmed cultivar “Bearpaw” or downy brome grass. Our findings support this in two ways. First, the solidness trait likely affects larval feeding inside the stem, so larvae tend to girdle the upper portion of the stem mostly between the second and third internode rather than girdling the lower part of the stem where overwintering occurs. Second, we have observed multiple larvae (up to three) within the same stem of Bearpaw while we have not recorded more than one larva per stem in Byrd. Similar results were reported by Morrill *et al*. (1992). Also, the high consistency of the pith tissue in the solid-stemmed cultivar “Bearpaw” had an effect on the larval movement and increased the larval mortality within the stem while hollow-stemmed “Byrd” permits larval development and movement. Farstad (1940) reported that there is a relationship between the pith tissue of the plant and larval development of the wheat stem sawfly and generally, larvae hatched from eggs surrounded by tissue are not able to survive and move. Roberts (1954) found there was correlation between solidness of the stem and larval mortality. O'Keeffe *et al*. (1960) reported significant differences in sawfly survival and stem solidness. The wheat stem sawfly larvae still could survive in the solid-stemmed Bearpaw in our study because we observed variation in solidness trait of the pith in this cultivar. Females sometimes deposit their eggs within the solid stem at a spot with reduced pith tissue so both eggs and larvae could complete development. O'Keeffe *et al*. (1960) reported same observation.

In conclusion, our study showed that both wheat cultivars, hollow-stemmed “Byrd” and solid-stemmed “Bearpaw”, were more preferred over downy brome by wheat stem sawfly. The plant stages had a role in the infestation and oviposition preference. Stem solidness was positively associated with larval mortality. The solid-stemmed cultivar “Bearpaw” can affect the oviposition and survivorship of sawfly larvae. Downy brome grass had lower host quality (stem height and stem diameter) that lead to lower eggs deposit and larvae survivorship compared to hollow-stemmed cultivar.

REFERENCES CITED

Awmack, C. S., & Leather, S. R. (2002). Host plant quality and fecundity in herbivorous insects. Annual Review of Entomology, 47(1), 817-844.

Bruce, T. J., Wadhams, L. J., & Woodcock, C. M. (2005). Insect host location: a volatile situation. Trends in plant science, 10(6), 269-274.

Cárcamo, H.A., Beres, B.L., Clarke, F., Byers, R.J., Mundel, H.H., May, K., and DePauw, R. (2005). Influence of plant host quality on fitness and sex ratio of the wheat stem sawfly (Hymenoptera: Cephidae). Environmental Entomology, 34: 1579–1592.

Cárcamo, H. A., Beres, B. L., Larson, T. R., Klima, C. L., & Wu, X. H. (2016). Effect of Wheat cultivars and blends on the oviposition and larval mortality of *Cephus cinctus* (Hymenoptera: Cephidae) and parasitism by *Bracon cephi* (Hymenoptera: Braconidae). *Environmental Entomology*, nvv231.

Chew, F. S. (1977). Coevolution of pierid butterflies and their cruciferous foodplants, II. Distribution of eggs on potential foodplants. Evolution 31:568–579.

Craig, T. P., & Itami, J. K. (2008). Evolution of preference and performance relationships. Pp. 20–28 in K. J. Tilmon, ed. Specialization, speciation and radiation. University of California Press, Berkeley and Los Angeles, California.

Futuyma, D. J., & Moreno, G. (1988). The evolution of ecological specialization. Annual Review of Ecology and Systematics, 207-233.

García-Robledo, C. & Horvitz, C. C. (2012). Parent–offspring conflicts, "optimal bad motherhood" and the "mother knows best" principles in insect herbivores colonizing novel host plants. Ecology and Evolution, 2: 1446–1457.

Farstad, C. W. (1940). The development of Western wheat stem sawfly (*Cephus cinctus* Nort) in various host plants as an index of resistance.

Gripenberg, S., Mayhew, P. J., Parnell, M., & Roslin, T. (2010). A meta-analysis of preference–performance relationships in phytophagous insects. Ecology Letters, 13: 383–393.

Holmes, N. D., & Peterson, L. K. (1960). The influence of the host on oviposition by the wheat stem sawfly, *Cephus cinctus* Nort. (Hymenoptera: Cephidae). Canadian Journal of Plant Science, 40: 29–40.

Ladner, D. T. & Altizer, S. (2005). Oviposition preference and larval performance of North American monarch butterflies on four *Asclepias* species. Entomologia Experimentalis et Applicata, 116: 9–20.

Mayhew, P. J. (1997). Adaptive patterns of host plant selection by phytophagous insects. Oikos, 79, 417–428.

Mayhew, P. J. (1998). Testing the preference–performance hypothesis in phytophagous insects: lessons from chrysanthemum leafminer (Diptera: Agromyzidae). Environmental Entomology, *27*(1), 45-52.

Morrill, W.L., Gabor, J.W., Hockett, E.A., and Kushnak, G.D. (1992). Wheat stem sawfly (Hymenoptera: Cephidae) resistance in winter wheat. Journal of Economic Entomology, 85: 2008–2011.

Morrill, W.L., Gabor, J.W., Weaver, D.K., Kushnak, G.D., and Irish, N.J. (2000). Effect of host plant quality on the sex ratio and fitness of female wheat stem sawflies (Hymenoptera: Cephidae). Environmental Entomology, 29: 195–199.

O'Keeffe, L.E., Callenbach, J.A., and Lebsock, K.L. (1960). Effect of culm solidness on the survival of the wheat stem sawfly. Journal of Economic Entomology, 53: 244–246.

Perez-Mendoza, J., Weaver, D. K., & Morrill, W. L. (2006). Infestation of wheat and downy brome grass by wheat stem sawfly and subsequent larval performance. Environmental Entomology, 35(5), 1279-1285.

Piesik, D., Weaver, D. K., Runyon, J. B., Buteler, M., Peck, G. E., & Morrill, W. L. (2008). Behavioural responses of wheat stem sawflies to wheat volatiles. Agricultural and Forest Entomology, 10: 245–253.

Rausher, M. D. (1979). Larval habitat suitability and oviposition preference in 3 related butterflies. Ecology, 60, 503–511.

Roberts, D. W. A. (1954). Sawfly resistance in wheat. I. Types of resistance. Canada. Jour. Agr. Sci. 34: 582-597.

Scheirs, J. & De Bruyn, L. (2002). Integrating optimal foraging and optimal oviposition theory in plant–insect research. Oikos, *96*(1), 187-191.

Seamans, H.L. (1945). A preliminary report on the climatology of the wheat stem sawfly (*Cephus cinctus* Nort.) on the Canadian prairies. Scientific Agriculture, 25: 432–457.

Stephens, D. W. & Krebs, J. R. (1986) Foraging Theory, Princeton University Press.

Thompson, J. N. (1988). Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. Entomologia Experimentalis et Applicata, 47(1), 3-14.

Valladares, G. & Lawton, J.H. (1991). Host plant selection in the holly leaf-miner: does mother know best? The Journal of Animal Ecology, 60, 227– 240.

Weaver, D.K., Buteler, M., Hofland, M.L., Runyon, J.B., Nansen, C., Talbert, L.E., Lamb, P., and Carlson, G.R. (2009). Cultivar preferences of ovipositing wheat stem sawflies as influenced by the amount of volatile attractant. Journal of Economic Entomology, 102: 1009–1017.

CHAPTER 4-BEHAVIORAL RESPONSES OF WHEAT STEM SAWFLY *CEPHUS CINCTUS* TO WINTER WHEAT CULTIVARS AND DOWNY BROME

Summary

The wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), infests both cultivated and uncultivated host plants. Females use visual and chemical cues from plants to identify a suitable host for oviposition. In this study, a Y-tube bioassay was developed to evaluate female wheat stem sawfly behavior in response to an airflow that passed over winter wheat *Triticum aestivum* L. and downy brome *Bromus tectorum* L. Choice and no-choice tests were conducted with three host plants: a susceptible wheat cultivar ’Byrd’, a resistant wheat cultivar ‘Bearpaw’, and downy brome. Separate tests were conducted using plants at three different growth stages: Zadoks 32, 49, and 60. Adult females were attracted to wheat cultivars Byrd and Bearpaw over downy brome when given choices of Bearpaw vs. Byrd, Bearpaw vs. downy brome and Byrd vs. downy brome at all growth stages. The female’s response speed did not differ when given a choice between the two wheat cultivars Byrd and Bearpaw. However, females were faster in making a choice (100% ≈ 86% females made fast choice) toward each of wheat cultivars over downy brome in the choice tests of Bearpaw vs. downy brome and Byrd vs. downy brome at all growth stages. Downy brome was least preferred (31.82%) by female wheat stem sawfly in choice test of Bearpaw vs. downy brome at Zadoks 32 while it was equally preferred (49.21%) in the choice test of ‘Byrd vs. downy brome’ at growth stage of Zadoks 60. Our olfactometer experiments only provide a limited picture of the oviposition and host selection behavior of female wheat stem sawflies. Collection and analysis of volatile compounds from

these host plants is needed to identify volatile attractants in which may help in sawfly management.

Introduction

The wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), is a serious pest of wheat, *Triticum aestivum* L. (Cyperales: Poaceae), in the northern Great Plains of North America. Infestation by sawflies causes damage that reduces the harvested yield (Ainslie 1920, Holmes 1977, Morrill *et al.* 1992). Management approaches include breeding for host plant resistance and agronomic practices such tillage and using insecticides to control the adults during the flight (Weaver *et al.* 2004). The larvae feed and develop within the stems and overwinter as larvae in the lower portion of the stems near the soil surface. Sawfly adults emerge in the spring when wheat initiates growth. Males emerge before females, so the mating period is short; the first females to emerge are more likely to mate. Females spend their adult life searching for suitable hosts and oviposition sites (Ainslie 1920, Weiss *et al.* 1992). Sawfly adults have limited time to locate and select suitable host plants due to their short life span of about one week after emergence (Morrill *et al.* 2000, Perez-Mendoza *et al*. 2006). Also, sawflies need to oviposit eggs in young and moist green stems. In the field, sawflies were observed ovipositing eggs in green stems of wheat between growth stages of Zadoks 32 and 49 (Zadoks *et al.* 1974, Holmes & Peterson 1960). Female wheat stem sawflies select taller, larger, and more developed stems in the young and advanced plants as oviposition sites (Holmes and Peterson 1960). Thus, wheat cultivars that develop slowly can be more attractive for wheat stem sawflies as suitable oviposition sites and serve as a trap crop during their flight. The wheat stem sawfly life cycle more closely matches the development of winter wheat stems rather than the stem development of spring wheat, which makes winter wheat an efficient trap crop to be planted around spring wheat crop (Morrill and Kushnak 1999; Morrill *et al*. 2001). Limited information is available about the plant characteristics and plant volatiles emission of the suitable plant development stages for wheat stem sawfly oviposition (Buteler *et al*. 2010).

Plants emit chemical cues that are dependent on species, plant tissues, and environmental conditions. Many plant volatiles are correlated and elicit many insect behavioral responses. Insect herbivores use chemical cues to locate suitable habitats and host plants, evaluation of host quality, locating mates, and locating herbivorous prey in the case of predators and parasitoids (Reinecke & Hilker 2014). Wheat stem sawflies may use the volatile compounds of host plants as cues that help to identify the suitability of stems for oviposition (Piesik *et al*. 2008, Weaver *et al*. 2009). Some volatile compounds that influence the wheat stem sawfly behavior have been identified including the green-leaf volatiles (Z)-3-hexenyl acetate and (Z)-3-hexenol, 6-methyl-5-hepten-2-one, and the terpene bocimene (Piesik *et al*. 2008, Buteler *et al*. 2010). Spring wheat cultivars release different amounts of (Z)-3-hexenyl acetate and cultivars such "Reeder" that release high amounts of (Z)-3-hexenyl acetate are preferred by wheat stem sawflies (Weaver *et al*. 2009). Plant semiochemicals that elicit insect behaviors could be used as management tools to control pest infestation of crops (Agelopoulos *et al.* 1999, Reddy *et al.* 2004, Martel *et al.* 2005). Plant semiochemicals can be applied in pest management in a variety of approaches. It could be used in trap crops (Katsoyannos & Guerin 1984, Martel *et al.* 2005, Ruther & Mayer 2005) or as pheromones in trap catches in mass trapping, monitoring, and attracticides (Nakamuta *et al.* 1997, Reddy *et al.* 2004, Leskey *et al.* 2005).

Plant semiochemicals can be applied in a 'push–pull' strategy to manipulate the insect behavior, abundance, and distribution without using toxins or pesticides (Khan *et al.* 2006, Cook *et al.* 2007). Volatiles compounds that have a repellent effect to pests can be used as a pest management approach (Pettersson *et al.* 1994, Borden *et al.* 1997). Other volatiles act as defense compounds in plants can be used as application treatment to protect the plant from pest infestation (Pettersson *et al.* 1994).

Understanding the interactions of insect behavior and host plant and chemical ecology is important in order to develop appropriate management practices that take advantage of plant semiochemicals (Cook *et al.* 2007). Understanding the relationship between host plant chemicals and wheat stem sawfly behavior could help in developing safe non-chemical based management approaches to reduce sawfly damage (Cossé *et al.* 2002). It may also help to identify unattractive, resistant wheat cultivars that could be planted along with trap crops (Piesik *et al*. 2008). Olfactometer bioassays have been used in chemical ecology studies to understand how insect herbivores locate their hosts. Detailed studies have been conducted on the host locating behavior of some parasitoid wasps that lay eggs inside insect herbivores. Findings of these studies have reported that parasitoid wasps use volatile compounds released by plants under attack by insect herbivores to find the ovipositing hosts (Ballhorn & Kautz 2013). Piesik *et al.* (2008) studied wheat stem sawfly behavioral responses to volatiles of wheat plants and seven synthetic volatiles compounds in a Y-tube olfactometer. Females significantly responded to wheat plants while males did not. The synthetic volatiles compounds tests showed that females preferred (*Z*)-3-hexenyl acetate, β-ocimene, and (*Z*)-3-hexen-1-ol, while males did not respond to these volatiles.

The present study aimed 1) to evaluate the female wheat stem sawfly behavior in response to an airflow that passed over wheat cultivars: susceptible winter wheat cultivar ‘Byrd’, the resistant winter wheat cultivar ‘Bearpaw’, and downy brome, 2) to understand the behavioral responses of females for different growth stages of plants, and 3) to measure the response speed of females in the Y-tube olfactometer to different hosts in choice tests.

Results of this study will increase our understanding about host selection and oviposition behavior of female wheat stem sawfly. Also findings from this study will give further insight into the interactions between sawfly oviposition behavior and host plant chemicals.

Materials and Methods

Plants

All plants were grown in a greenhouse located at Colorado State University, Fort Collins, Colorado, USA. Two winter wheat cultivars, the wheat stem sawfly susceptible cultivar 'Byrd' and the sawfly resistant 'Bearpaw', and the invasive grass downy brome were used as plant material in the Y-tube olfactometer experiments (see below). Two vernalized seedlings of one of the winter wheat cultivars or downy brome were planted in a standard round pot (13 cm x 13 cm) filled with soil mix (7 parts Fafard 2SV custom potting mix, 2 parts Planters Mix, 1 part perlite, and 5g Scotts Osmacote 14-14-14). The pots were held in a greenhouse with supplemental light (400 watt high pressure sodium lights) at a 14.5: 9.5 h LD photoperiod. Daytime temperature was 23-25 ˚C and the overnight temperature was 20-22 ˚C. The plants were watered three to four times weekly. Plants used in the experiments were at three different growth stages: GS Zadoks 32 (two nodes visible), GS Zadoks 49 (awns first visible), and GS Zadoks 60 (heading stage) (Zadoks *et al.*, 1974). GS Zadoks 32 and GS Zadoks 49 stages represent the range of plant growth stages infested under field conditions. However, in a preliminary experiment, we observed that wheat stem sawfly females laid eggs on wheat plants at GS Zadoks 60 (heading stage); therefore, I included the heading stage in the experiment. Plants were fertilized with

Peters General Purpose Fertilizer 20-20-20 (J. R. Peters, Allentown, PA) at 100 ppm in aqueous solution twice each week. Fertilizing started when plants reached a developmental stage of Zadoks 13 (three unfolded leaves).

Insects

Wheat stem sawfly larvae were collected in wheat stubble. Random stubble samples were collected from a wheat field infested with sawflies in northeastern Colorado. The stubble samples were held in plastic zippered storage bags at 2-3 ˚C in a dark walk-in cooler for at least 100 days to allow the wheat stem sawfly larvae to complete its obligatory diapause. After the diapause period, the stubble samples were placed in pots with soil, which were placed in cages (75 × 75 × 115 cm) with clear plastic front and back panels permitting observation of insect activity and polyester netting (160-µm mesh) side panels for ventilation. There are three zippered openings in the front panel of cage to allow movement of potted plants into and out of the cage. The two smaller sleeve openings (18 cm diameter) on the zippered opening permit addition or removal of insects while preventing escape. The stubble was lightly moistened twice weekly with water to prevent desiccation of the developing larvae. These cages were held at room temperature (22-27˚C) under a LD 14.5:9.5h photoperiod until adult sawflies emerged 4-5 weeks later. The cages were checked daily to collect the newly emerged sawfly adults. Only females were collected and transferred from the cages with 15 mL plastic centrifuge tubes to prevent touching the insects. Females were held at room temperature until trials were started. All bioassays were conducted with females that had emerged within 24 hours.

Y-tube Olfactometer

Y-tube bioassays were conducted to determine whether wheat stem sawfly females could discriminate between different host plants based on the emitted volatiles from plants. Three plants were tested with the Y-tube olfactometer: susceptible winter wheat (Byrd), resistant winter wheat (Bearpaw), and downy brome. All plants were grown in the greenhouse as previously described.

The olfactometer consists of a Y-shaped glass tube (arm dimensions: 0.5 cm inside diameter, 5.75 cm length) connected to two vertical cylindrical glasses (61 cm long and 18 cm in diameter), in which the plant sources were placed (one in each chamber) (Figure 4.1). The air flow source was provided with a diaphragm air pump at a rate of 1.0 L/min. Air was charcoal-filtered using pelleted activated charcoal and delivered by 1.27cm diameter PTFE tubing to the bottom of the glass cylinder-enclosed chamber containing the potted plant. Air entered each chamber via a 1.27cm diameter PTFE tube in the bottom of the chamber and exited via a 1.27cm PTFE tube at the top where the two arms of the Y-tube were connected. The common arm of the Y-tube was placed and covered by a black box (20 x 20 cm) made from heavy black leather in order to avoid visual stimuli. Fiber optic dual gooseneck halogen lamps were used as a light source and were focused onto the Y-junction of the olfactometer to stimulate female movement and response (de Kogel *et al*. 1999). I placed a wire inside the Y-tube glass, extending from the main arm into the Y-junction of the olfactometer to assist the female as she approached the test junction (Piesik *et al*. 2008).

Figure 4.1. Y-tube olfactometer for recording responses of *Cephus cinctus* females to plant volatiles. (see Material & Methods for further explanation).

Bioassay

I used three choice tests: Byrd vs. Bearpaw, Byrd vs. downy brome, and Bearpaw vs. downy brome, and three no-choice tests: Byrd vs. Byrd, Bearpaw vs. Bearpaw, and downy brome vs. downy brome. Each choice and no-choice test was repeated using plants at one of three different plant stages: GS Zadoks 32 (two nodes visible), GS Zadoks 49 (awns first visible), and GS Zadoks 60 (heading stage). A tube containing one female was attached to the main arm of the Y-tube olfactometer. For each replicate, one female wheat stem sawfly was released at the entrance of the base tube of the apparatus. Each female was observed for 10 minutes. Females that did not make a choice by the end of the observation time and remained in the common arm of the Y-tube were scored as having made 'no-choice' (Bertschy *et al*. 1997). A female was scored as making a choice if she entered either arm and crossed more than half of the distance from the intersection of the Y-tube. Females that made a choice within the first five minutes were scored as a 'fast response or choice' while female that made a choice within last five minutes scored as a 'slow response'. Fifteen replicates were conducted for each choice or no-choice test. The position of the plants in the chambers, as well as the position of the two arms of the olfactometer, were shifted after each replicate in order to avoid positional preference. Five females for each replicate were released, one female at a time, and a total of 75 females were used for each choice and no-choice test. All tests were conducted in a room at temperature of 22° C and 51% humidity.

Data Analysis

Choice and no-choice test results were analyzed with a two-sided binomial test to assess the proportion of females made choice toward a particular plant volatile in each experiment. For statistical analyses of the response speed of females, a chi-square test was applied, using the total number of females that made fast or slow choice for a particular plant ($\alpha = 0.05$). The number of females that made a choice toward a particular plant in a particular test was compared to the other females made choice when the same plant was paired or given with different partners, using the chi-square test ($\alpha = 0.05$).

Results

Wheat stem sawfly females exhibited a stronger preference for both winter wheat cultivars over downy brome over all plant growth stages. The odors emitted by Bearpaw and Byrd did not appear to differ as females did not differentiate between the resistant wheat and suitable wheat cultivars. Females showed a greater preference for Byrd over downy brome in both plant growth stages Zadoks 32 and 49 (Figures 4.2 and 4.3). Sawflies preferred Bearpaw over downy brome while sawflies equally preferred Byrd and downy brome at growth stage Zadoks 60 (Figure 4.4). Females choice of Bearpaw (68.2%) over downy brome was strongest at the youngest wheat at growth stage Zadoks 32 (Table 4.1).

Female wheat stem sawflies showed the highest preference (49.2%) for downy brome over wheat Byrd at growth stage Zadoks 60 among all plant growth stages (Tables 4.1, 4.2 and 4.3). The greatest number of females making a choice in the Y-olfactometer bioassays was 69 females out of 75 females in the test between Byrd and Bearpaw cultivars at growth stage

Zadoks 60, while the lowest number of females that made choice was 26 females out of 75 in the choice of Bearpaw vs. Byrd at growth stage Zadoks 32 (Table 4.1).

Table 4.1. Proportion of wheat stem sawfly females responding to a particular plant in the choice and control tests in a Y-tube olfactometer at growth stages Zadoks 32.

Bioassays	Binomial Proportion*	Proportion	Z	Two-sided *P*-value	n**	95% Confidence Limits	
						Lower	Upper
Choice Tests							
Bearpaw vs. Downy Brome	Bearpaw	0.6818	2.9542	0.0031	66	0.5556	0.7911
Bearpaw vs. Byrd	Byrd	0.4677	-0.5080	0.6115	62	0.3398	0.5988
Byrd vs. Downy Brome	Brome	0.3548	-4.0931	0.0001	65	0.1477	0.3687
Control Tests							
Bearpaw vs. Bearpaw	Bearpaw	0.5500	0.4472	0.6547	20	0.3153	0.7694
Byrd vs. Byrd	Byrd	0.5232	0.2182	0.8273	21	0.2978	0.7429
Downy Brome vs. Downy Brome	Brome	0.6111	0.9428	0.3458	18	0.3575	0.8270

*test the null hypothesis that the population proportion equals 50%.
**n = number of females that made a choice out of 75 females in choice tests.
**n = number of females that made a choice out of 25 females in no-choice control tests.

Table 4.2. Proportion of wheat stem sawfly females responding to a particular plant in the choice and control tests in a Y-tube olfactometer at growth stage Zadoks 49.

Bioassays	Binomial Proportion*	Proportion	Z	Two-sided *P*-value	n**	95% Confidence Limits	
						Lower	Upper
Choice Tests							
Bearpaw vs. downy brome	Bearpaw	0.5625	1.0000	0.3173	64	0.4328	0.6863
Bearpaw vs. Byrd	Byrd	0.4545	-0.7385	0.4602	66	0.3314	0.5819
Byrd vs. Downy Brome	Brome	0.3968	-1.6378	0.1015	63	0.2757	0.5280
Control Tests							
Bearpaw vs. Bearpaw	Bearpaw	0.5455	0.4264	0.6698	22	0.3221	0.7561
Byrd vs. Byrd	Byrd	0.5238	0.2182	0.8273	21	0.2978	0.7429
Downy Brome vs. Downy Brome	Brome	0.5500	0.4472	0.6547	20	0.3153	0.7694

*test the null hypothesis that the population proportion equals 50%.
**n = number of females that made a choice out of 75 females in choice tests.
**n = number of females that made a choice out of 25 females in no-choice control tests.

Table 4.3. Proportion of wheat stem sawfly females responding to a particular plant in the choice and control tests in a Y-tube olfactometer at growth stage Zadoks 60.

Bioassays	Binomial Proportion*	Proportion	Z	Two-sided *P*-value	n**	95% Confidence Limits	
						Lower	Upper
			Choice Tests				
Bearpaw vs. Downy Brome	Bearpaw	0.5909	1.4771	0.1396	66	0.4629	0.7105
Bearpaw vs. Byrd	Byrd	0.5072	0.1204	0.9042	69	0.3841	0.6298
Byrd vs. Downy Brome	Brome	0.4921	-0.1260	0.8997	63	0.3638	0.6211
			Control Tests				
Bearpaw vs. Bearpaw	Bearpaw	0.5455	0.4264	0.6698	22	0.3221	0.7561
Byrd vs. Byrd	Byrd	0.5238	0.2182	0.8273	21	0.2978	0.7429
Downy Brome vs. Downy Brome	Brome	0.5263	0.2294	0.8185	19	0.2886	0.7555

*test the null hypothesis that the population proportion equals 50%.
**n = number of females that made a choice out of 75 females in choice tests.
**n = number of females that made a choice out of 25 females in no-choice control tests.

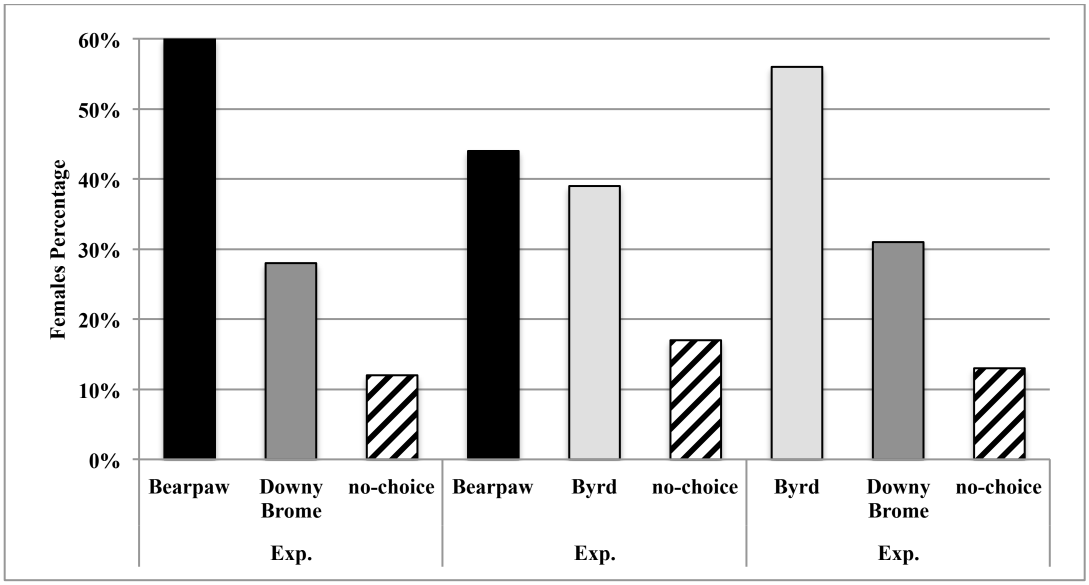

Figure 4.2. The percentage of wheat stem sawfly females making a choice in the choice test in the Y-tube olfactometer at Zadoks 32.

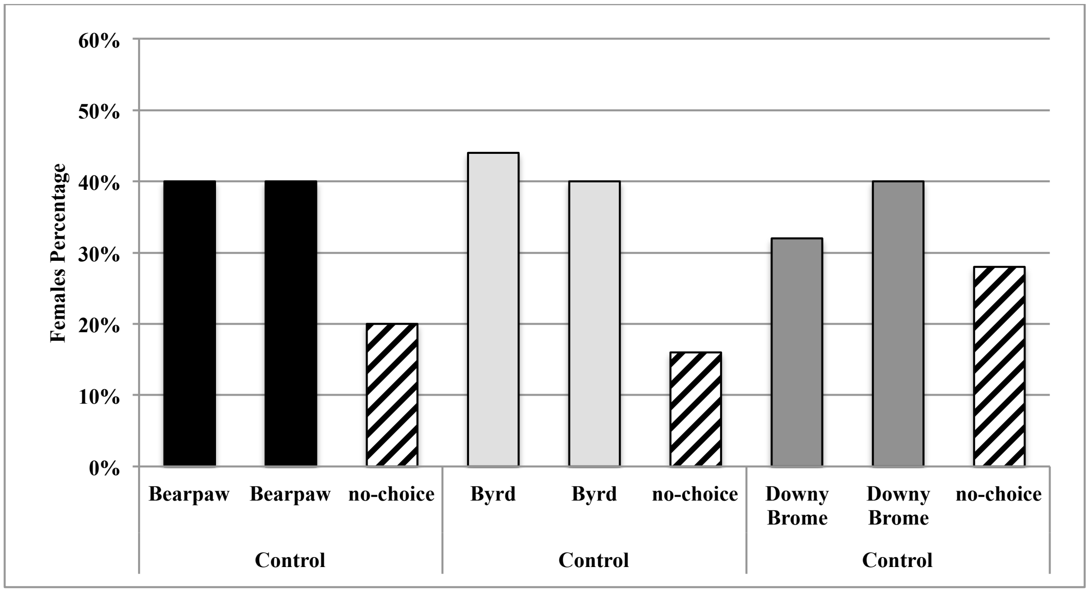

Figure 4.3. The percentage of wheat stem sawfly females making a choice in the control test in the Y-tube olfactometer at Zadoks 32.

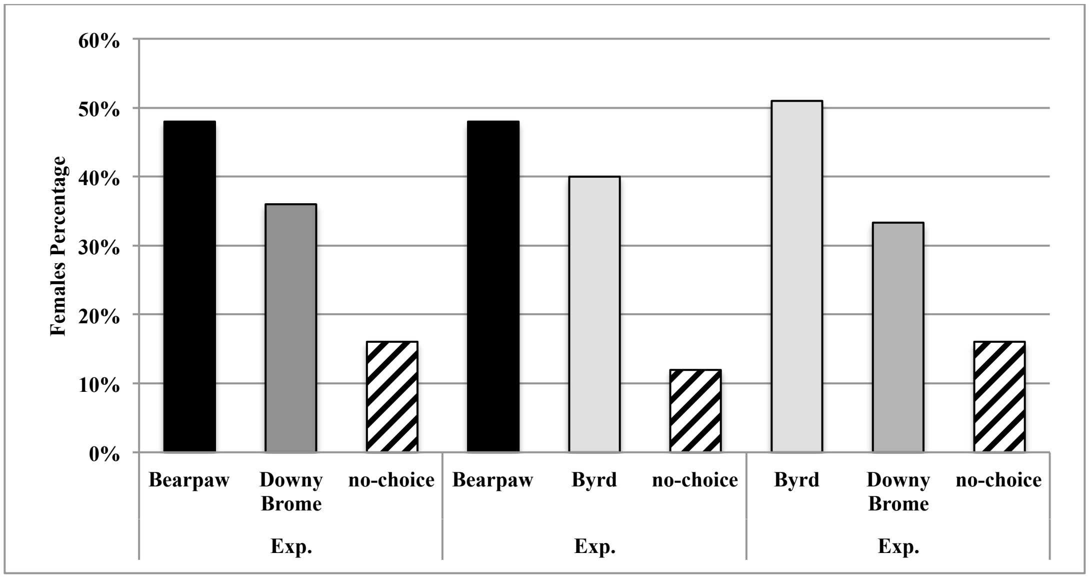

Figure 4.4. The percentage of wheat stem sawfly females making a choice in the choice test in the Y-tube olfactometer at Zadoks 49.

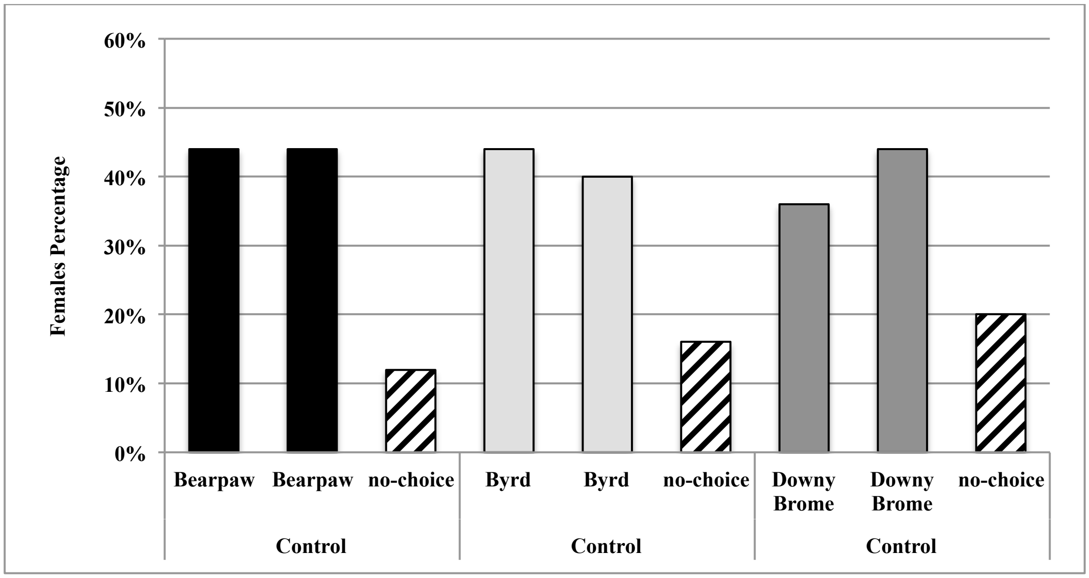

Figure 4.5. The percentage of wheat stem sawfly females making a choice in the control test in the Y-tube olfactometer at Zadoks 49.

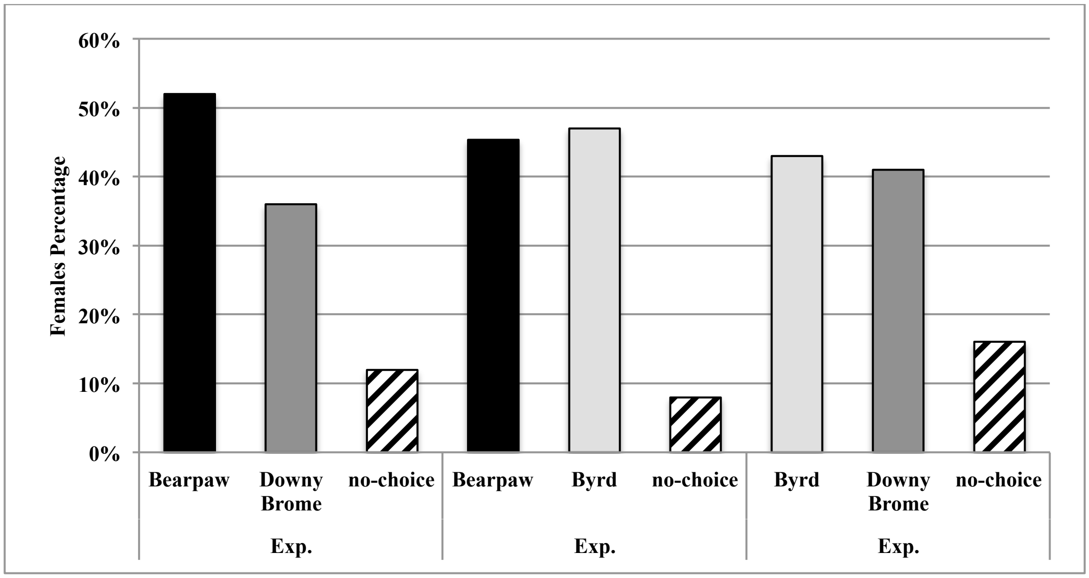

Figure 4.6. The percentage of wheat stem sawfly females making a choice in the choice test in the Y-tube olfactometer at Zadoks 60.

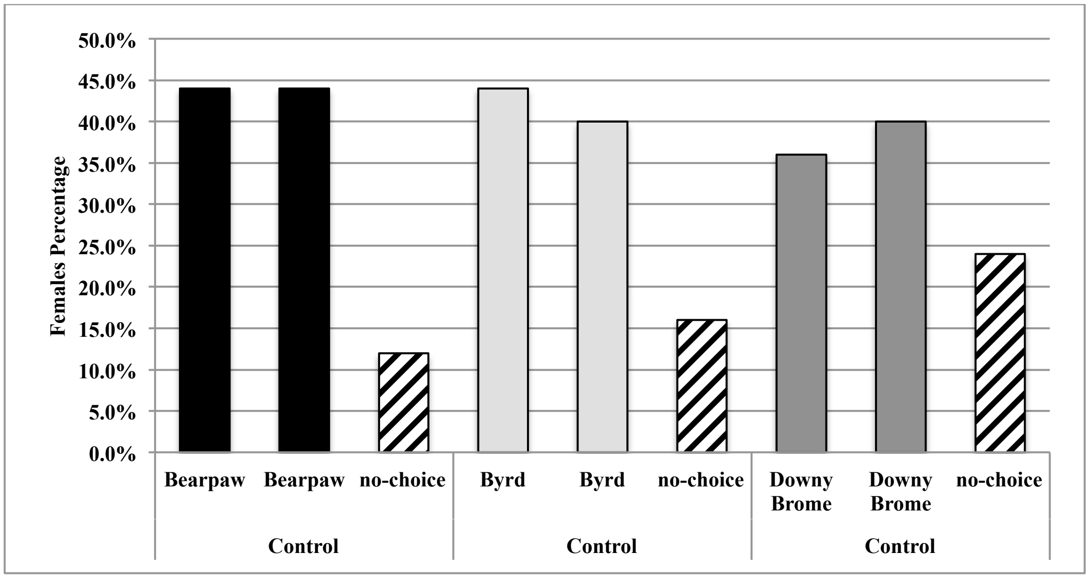

Figure 4.7. The percentage of wheat stem sawfly females making a choice in the control test in the Y-tube olfactometer at Zadoks 60.

Of the females that responded within the first five minutes in the Y-tube olfactometer, 88.57% made a fast choice toward the wheat cultivar Byrd (X^2 = 7.11, P = 0.0077) versus downy brome and 100% of the females made fast choice toward Bearpaw (X^2 = 12.45, P = 0.0004) over downy brome at Zadoks 32 (Figure 4.5).

In the choice test of Bearpaw versus downy brome, 77.78% of the females made a fast choice toward Bearpaw (X^2 = 8.80, P = 0.003) over downy brome, while 92.86% of the females made a fast choice toward Byrd (X^2 = 22.30, P < 0.0001) over downy brome at Zadoks 49 (Figure 4.6).

In the choice bioassay of Byrd versus downy brome at Zadoks 60, the females that made a fast choice toward downy brome were increased to (25%) compared to the female percent that made a fast response toward downy brome at Zadoks 49 and 32. In the choice bioassay between Bearpaw and downy brome, 86.21% of the females made fast choice toward wheat cultivar Bearpaw (X^2 = 15.74, P < 0.0001) over downy brome at GS Zadoks 60 (Figure 4.7).

The female's response speed in the choice test of wheat cultivars Byrd vs. Bearpaw was not significantly different at Zadoks 32 Byrd (X^2= 0.40, P = 0.53), Zadoks 49 (X^2 = 1.93, P = 0.17), or Zadoks 60 (X^2 = 0.28, P = 0.60).

The wheat stem sawfly females showed stronger preference for odor of Byrd (64.5%, X^2 = 3.95, P = 0.05) in the choice test of Byrd vs. downy brome than the female preference for odor of Byrd (46.7%) in the choice test of Bearpaw vs. Byrd at Zadoks 32. However, the female preference for odor of Byrd in the choice tests of Byrd vs. downy brome and Bearpaw vs. Byrd was not significantly different at either the plant growth stage of Zadoks 49 (X^2 = 2.86, P = 0.09) or Zadoks 60 (X^2 = 0.0001, P = 0.99). However, the female preference for the odor released by Bearpaw in the choice tests of Bearpaw vs. Byrd and Bearpaw vs. downy brome did not differ at

Zadoks 32 ($X^2 = 3.00$, $P = 0.08$), Zadoks 49 ($X^2 = 0.04$, $P = 0.84$) and Zadoks 60 ($X^2 = 1.31$, $P = 0.25$). Also, females exhibited similar preference for odor of downy brome when it was paired with each of winter wheat cultivars Bearpaw or Byrd in all plant growth stages Zadoks 32, 49 and 60 except in Bearpaw vs. downy brome at growth stage of Zadoks 32.

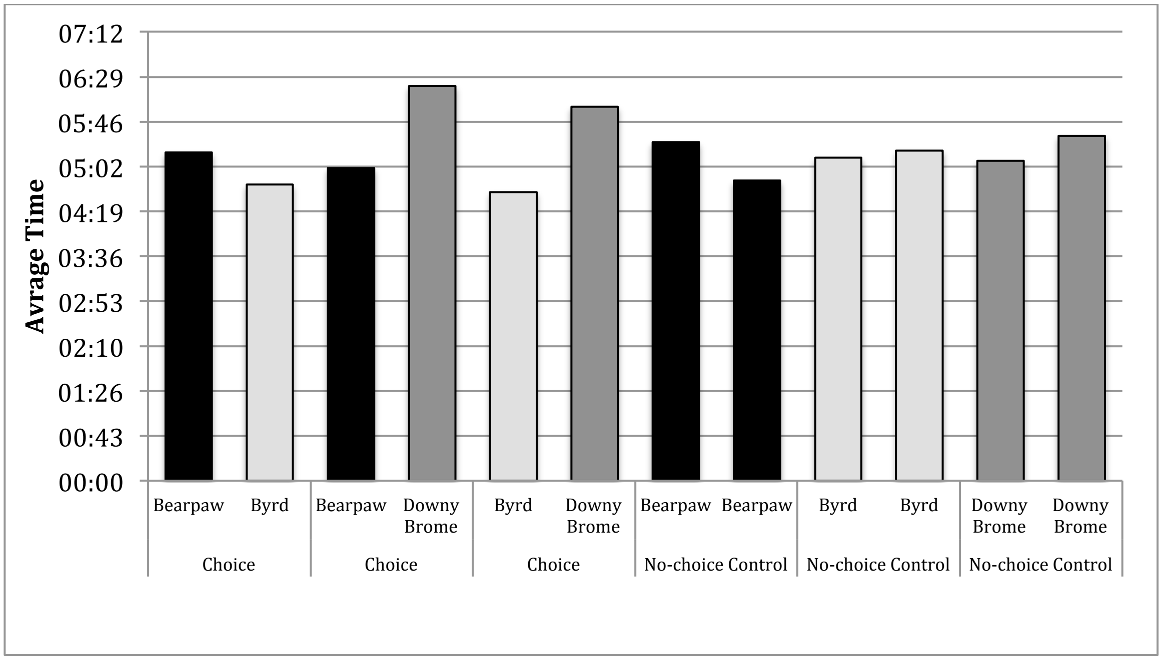

Figure 4.8. Average choice time by wheat stem sawfly at Zadoks 32 in Y-tube olfactometer.

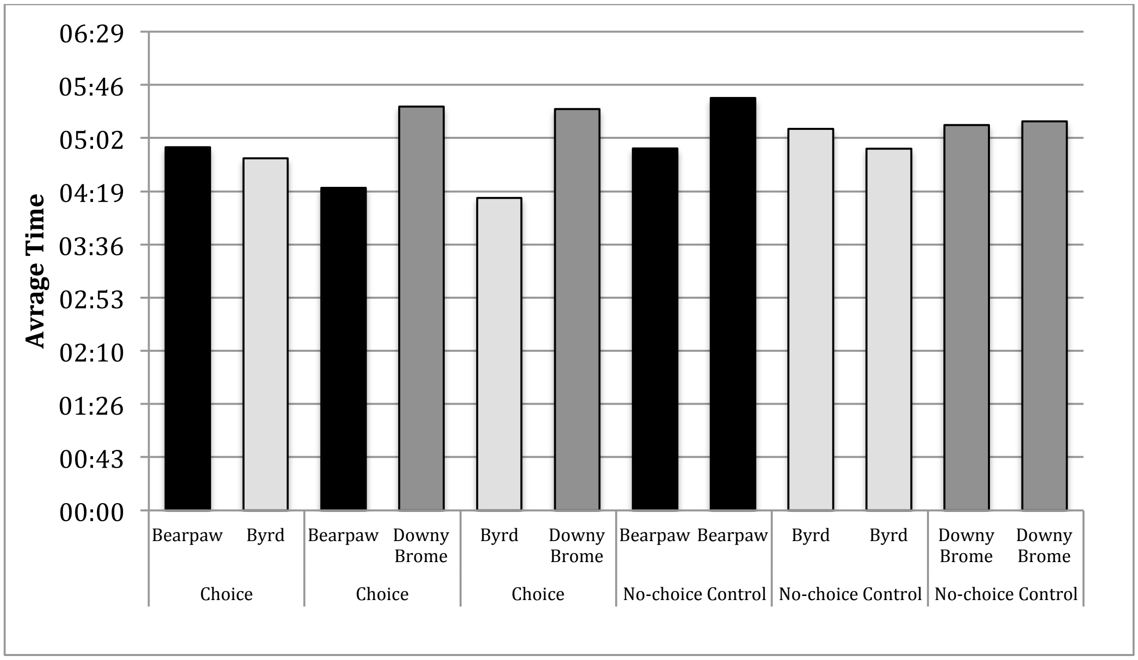

Figure 4.9. Average choice time by wheat stem sawfly at Zadoks 49 in Y-tube olfactometer.

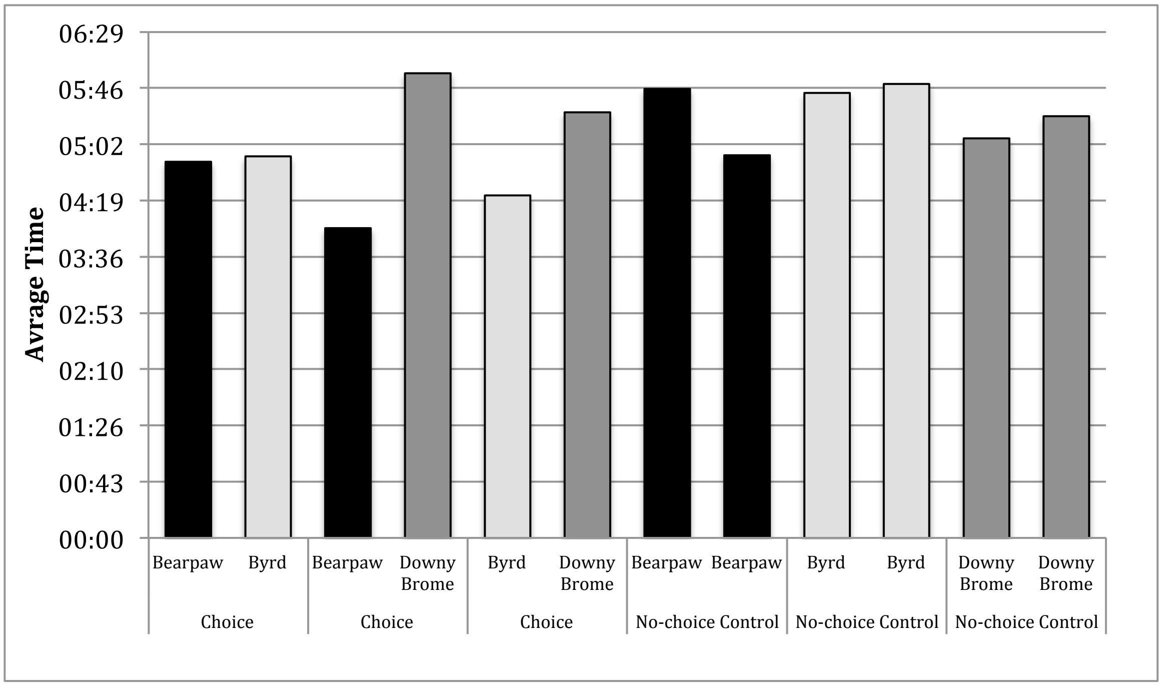

Figure 4.10. Average choice time by wheat stem sawfly at Zadoks 60 in Y-tube olfactometer.

Discussion

The results of the Y-tube olfactometer bioassays demonstrated a clear attraction of wheat stem sawfly females toward winter wheat cultivars compared to downy brome. However, sawflies appeared to be unable to distinguish the odor of Byrd plants from those of Bearpaw and as they responded similarly to both wheat cultivars. This might be because the volatile compounds emitted from both winter wheat cultivars Byrd and Bearpaw are not significantly different. However, Weaver *et al*. (2009) reported significant differences between spring wheat cultivars in the amount of the volatile compound (Z)-3-hexenyl acetate; 'Reeder' released the highest amount of this volatile and wheat stem sawflies preferred this cultivar over another in the choice tests. In my study, I was unable to collect, successfully extract, and identify the volatile compounds from my plant materials.

Plant growth stage had an impact on the female preference for plant odor. Fewer females made a choice toward downy brome in the choice test of Bearpaw vs. downy brome and Byrd vs. downy brome when these plants were at Zadoks 32 while downy brome at growth stage of Zadoks 60 was preferred by wheat stem sawflies. Likely, the younger plants of winter wheat produced the most attractive volatiles while downy brome produced the lower amount of these volatiles, and as the plants grew older these volatiles were decreased in winter wheat, which made wheat cultivars and downy brome similarly preferred by the females.

A higher proportion of females (69 out of 75) made a choice between Byrd and Bearpaw when plants were at Zadoks 60. Also, we have found that the average time for females to make a choice toward plant odor when plants are at Zadoks 32 was longer than the average time was needed for the female to make the choice toward plant odors at growth stages of Zadoks 49 and 60. These results suggest that the volatiles released by wheat cultivars were similar, potentially confusing the females and increasing the response time. Also, we assumed that females could distinguish between odors of Byrd and Bearpaw at Zadoks 49 and 60 but not at Zadoks 32. The emission of plant volatiles can be variable depending on the tissue type and plant age (Smagghe & Diaz 2012).

The female response speed to plants odor varied between different plants and females were more likely to make faster response toward wheat cultivars Byrd and Bearpaw and a slower response to downy brome odor. We speculate that the odor released by winter wheat has higher amount of the attractive volatiles than these released by downy brome and sawflies could differentiate between wheat odors and downy brome in the choice tests in Y-tube olfactometer.

Bertschy *et al*. (1997) found that females of the biological control agents of the cassava mealybug *Acerophagus coccois* Smith responded faster than the other tested species in the Y-tube olfactometer in the choice test between infested and healthy plant odors and they did not differentiate between the odors of infested cassava plants by the cassava mealybug and healthy leaves.

In our study, host plants and plant growth stages played a role in the behavioral response in Y-tube olfactometer. As discussed above, host plants of winter and downy brome and the growth stage of plants were detected as factors that significantly affected behavioral choice and the response speed of the females.

Our study shows that the use of olfactometer experiments to analyze wheat stem sawfly behavior is a useful approach to understand the behavior and decision making of this pest using the odor of plant as a cue to define the host quality. This can lead to the development of trap crop strategies to reduce sawflies damage and infestation. Future study involving the collection and analysis of volatile compounds from these wheat cultivars and downy brome is needed to better understand the interaction between sawflies behavior and their hosts semiochemicals.

REFERENCES CITED

Agelopoulos, N., Birkett, M.A., Hick, A.J, Hooper, A.M., Pickett, J.A., Pow, E.M., Smart, L.E., Smiley, D.W.M., Wadhams, L.J. & Woodcock, C.M. (1999) Exploiting semiochemicals in insect control. Pesticide Science, 55, 225–235.

Ainslie, C.N. (1920). The western grass-stem sawfly. United States Department of Agriculture. Bulletin 841.

Ballhorn, D. J., & Kautz, S. (2013). How useful are olfactometer experiments in chemical ecology research? *Communicative & Integrative Biology*, *6*(4), e55602.

Bertschy, C., Turlings, T. C., Bellotti, A. C., & Dorn, S. (1997). Chemically-mediated attraction of three parasitoid species to mealybug-infested cassava leaves. Florida Entomologist, 383-395.

Borden, J.H., Chong, L.J. Savoie, A. & Wilson, I.M. (1997) Responses to green leaf volatiles in two biogeographic zones by striped ambrosia beetle, Trypodendron lineatum. Journal of Chemical Ecology, 23, 2471–2479.

Buteler, M., Weaver, D. K., Bruckner, P. L., Carlson, G. R., Berg, J. E., & Lamb, P. F. (2010). Using agronomic traits and semiochemical production in winter wheat cultivars to identify suitable trap crops for the wheat stem sawfly. The Canadian Entomologist, 142(03), 222-233.

Cook, S.M., Khan, Z.R. & Pickett, J.A. (2007) The use of push- pull strategies in integrated pest management. Annual Review of Entomology, 52, 375–400.

Cossé, A. A., Bartelt, R. J., Weaver, D. K., & Zilkowski, B. W. (2002). Pheromone components of the wheat stem sawfly: identification, electrophysiology, and field bioassay. Journal of chemical ecology, 28(2), 407-423.

de Kogel, W. J., Koschier, E. H., & Visser, J. H. (1999). Y-tube olfactometer to determine the attractiveness of plant volatiles to western flower thrips. Proceedings of the Section Experimental and Applied Entomology of the Netherlands Entomological Society (Vol. 10, pp. 131-135).

Holmes, N.D. (1977) The effect of the wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), on the yield and quality of wheat. Canadian Entomologist, 109, 1591–1598.

Holmes, N.D. & Peterson, L.K. (1960) The influence of the host on oviposition by the wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae). Canadian Journal of Plant Science, 40, 29–46.

Katsoyannos, B.I. & Guerin, P.M. (1984) Hexanol: a potent attractant for the black fig fly, *Silba adipata*. Entomologia Experimentalis et Applicata, 35, 71–74.

Khan, Z.R., Midega, C.A.O., Hassanali, A., Pickett, J.A., Wadhams L.J. & Wanjoya, A. (2006) Management of witchweed, *Striga hermonthica*, and stemborers in sorghum, *Sorghum bicolor*, through intercropping with greenleaf desmodium, *Desmodium intortum*. International Journal of Pest Management, 52, 297–302.

Leskey, T.C., Zhang, A. & Herzog, M. (2005) Nonfruiting host tree volatile blends: novel attractants for the plum curculio (Coleoptera: Curculionidae). Environmental Entomology, 34, 785–793.

Martel, J. W., Alford, A. R., & Dickens, J. C. (2005). Laboratory and greenhouse evaluation of a synthetic host volatile attractant for Colorado potato beetle, *Leptinotarsa decemlineata* (Say). Agricultural and Forest Entomology, *7*(1), 71-78.

Morrill, W.L., Gabor, J.W. & Kushnak, G.D. (1992) Wheat stem sawfly (Hymenoptera: Cephidae) damage and detection. Journal of Economic Entomology, 85, 2413–2417.

Morrill, W.M., Gabor, J.W., Weaver, D.K., Kushnak, G.D. & Irish, N.J. (2000) Effect of host plant quality on the sex ratio and fitness of female wheat stem sawflies (Hymenoptera: Cephidae). Environmental Entomology, 29, 195–199.

Morrill, W.L. & Kushnak, G.D. (1999) Planting date influence on the wheat stem sawfly (Hymenoptera: Cephidae) in spring wheat. Journal of Agricultural and Urban Entomology, 16, 123–128.

Morrill, W.L., Weaver, D.K., and Johnson, G.D. (2001). Trap strip and field border modification for management of the wheat stem sawfly *Cephus cinctus* Norton (Hymenoptera: Cephidae). Journal of Entomological Science, 36: 34–35.

Nakamuta, K., Leal, W.S., Nakashima, T., Tokoro, M., Ono, M. & Nakanishi, M. (1997) Increase of trap catches by a combination of male sex pheromones and a floral attractant in longhorn beetle, *Anaglyptus subfasciatus*. Journal of Chemical Ecology, 23, 1635–1639.

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

Pettersson, J., Pickett, J.A., Pye, B.J., Quiroz, A., Smart, L.E., Wadhams, L.J. & Woodcock, C.M. (1994) Winter host component reduces colonization by bird-cherry-oat aphid, *Rhopalosiphum padi* (l.) (Homoptera, Aphididae), and other aphids in cereal fields. Journal of Chemical Ecology, 20, 2565–2574.

Piesik, D., Weaver, D. K., Runyon, J. B., Buteler, M., Peck, G. E., & Morrill, W. L. (2008). Behavioural responses of wheat stem sawflies to wheat volatiles. Agricultural and Forest Entomology, 10(3), 245-253.

Reddy, A., Gadi, V.P. & Guerrero, A. (2004) Interactions of insect pheromones and plant semiochemicals. Trends Plant Science, 9, 253–261.

Reinecke, A., & Hilker, M. (2014). Plant Semiochemicals–Perception and Behavioural Responses by Insects. Annual Plant Reviews volume 47: Insect-Plant Interactions, 115-153.

Ruther, J. & Mayer, C.J. (2005) Response of garden chafer, *Phyllopertha horticola*, to plant volatiles: from screening to application. Entomologia Experimentalis et Applicata, 115, 51–59.

Smagghe, G., & Diaz, I. (2012). Arthropod-plant interactions: novel insights and approaches for IPM (Vol. 14). Springer Science & Business Media.

Weaver, D.K., Buteler, M., Hofland, M.L., Runyon, J.B., Nansen, C., Talbert, L.E., and Carlson, G.R. (2009). Cultivar preferences of ovipositing wheat stem sawflies as influenced by the amount of volatile attractant. Journal of Economic Entomology, 102: 1009–1017.

Weaver, D.K., Sing, S.E., Runyon, J.B. & Morrill, W.L. (2004) Potential impact of practices on wheat stem sawfly (Hymenoptera: Cephidae) and associated parasitoids. Journal of Agricultural and Urban Entomology, 21, 271–287.

Weiss, M.J., Morrill, L.W. & Reitz, L.L. (1992) Wheat stem sawfly (Hymenoptera: Cephidae) revisited. American Entomologist, **38**, 241–245.

Zadoks, J.C., Chang, T.T., and Konsak, C.F. (1974). A decimal code for growth stages of cereals. Weed Research, 14: 15–21.