

GENETIC BASIS OF WHEAT RESISTANCE TO THE WHEAT STEM SAWFLY

by

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## DEDICATION

To my parents, Maria Helena and Euclydes, whose encouragement and constant love sustained me throughout my life.

To my siblings, Marco Antônio and Ana Lúcia, whose friendship over the years is immeasurable.

To my husband, João Luiz, who has shared many uncertainties, challenges, and sacrifices for completing this dissertation.

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## ABSTRACT

The wheat stem sawfly, *Cephus cinctus* Norton (WSS), has been a serious pest of wheat in the Northern Great Plains of North America for decades. Host plant resistance has been most consistently effective and is the foundation for integrated pest management of the WSS; therefore, the main objective of this research was to enhance the repertoire of genetic resistance to WSS available for exploitation by wheat breeders through marker-assisted selection (MAS). We used two strategies to achieve our goal. First, we better characterized resistance provided by previously identified quantitative trait loci (QTL). For that, we developed populations of near-isogenic lines (NILs) polymorphic for resistance QTLs on chromosomes 2D (*Qwss.msub-2D*), 3B (*Qss.msub-3BL*) and 4A (*Qwss.msub-4A.1*). Near-isogenic lines were tested greenhouse cage trials and in the field at both WSS-infested and uninfested locations. The *Qwss.msub-4A.1* was shown to affect host plant attractiveness to foraging females, but did not change oviposition preference after females landed on the wheat stem. The *Qwss.msub-2D* and *Qss.msub-3BL* were shown to be associated with decreased preference for oviposition. The *Qss.msub-3BL* was also shown to be associated with temporal patterns of stem solidness expression, with the allele derived from the spring wheat cultivar Conan providing decreased infestation, high neonatal mortality, high levels of early stem solidness, and a rapid rate of pith retraction during stem maturation. Together, these studies characterized the effect of each one of these QTLs on WSS resistance. This information will help wheat breeders and entomologists to better integrate management strategies to reduce damage caused by the WSS. Our second strategy, involved the identification of new marker-trait associations for WSS resistance. For that, we used a panel of 244 elite spring wheat lines from North America. By conducting an association mapping study we were able to identify several QTLs for WSS resistance, including QTLs for early and late stem solidness, heading date, stem cutting, and larval mortality. This study showed that improvements in WSS management may be obtained using alleles that already exist in elite germplasm. Collectively, our research provided wheat breeders with a better tool kit for improving resistance to the WSS.

## CHAPTER ONE

## LITERATURE REVIEW

The Wheat Stem Sawfly as a Pest of Wheat

The wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae) (WSS) is historically a pest of major economic importance in wheat, *Triticum aestivum* L., in the Northern Great Plains of North America. Early records indicate that the WSS originally developed in native grass species of the genera *Agropyron* and *Elymus* (Ainslie, 1929), but as European settlers began to plow large areas of native grasses to cultivate wheat, WSS populations adapted to this new and widely distributed host plant. As a result, in 1895, the WSS was reported as a pest of wheat in Canada for the first time (Fletcher, 1896). Through the years, WSS infestations followed the westward movement of wheat production in both Canada and the United States, causing economic losses in Alberta, Saskatchewan, Manitoba, North Dakota, South Dakota, and Montana (Ainslie, 1929; Painter, 1953; Wallace and McNeal, 1966; Weiss et al., 1992). More recently, damaging WSS infestations have expanded southward into parts of Wyoming, Nebraska and Colorado.

Historically, spring wheat has been more severely attacked by the WSS because winter wheat crops mature earlier and escape attack. But in 1996, a study showed that WSS populations have adapted to winter wheat early maturation phenology by synchronizing emergence patterns (Morrill and Kushnak, 1996). In addition to spring and winter wheat, the WSS has been reported infesting other crop species, such as durum

wheat (*Triticum durum* Desf.), barley (*Hordeum vulgare* L.), spelt (*Triticum spelta* L.), and triticale (Beres et al., 2011a).

Other closely related species of the WSS are also important pests of wheat in other parts the world. *Cephus pygmaeus* (L.) (Hymenoptera: Cephidae) is the main sawfly species attacking cereal crops in Western Europe, North Africa and the Middle East (Joukhadar et al., 2013), while in China, *Cephus fumipennis* Eversmann (Hymenoptera: Cephidae) is the dominant species. Together, the WSS and these closely related species infest vast areas of wheat crops, causing severe economic losses throughout the world.

### The Wheat Stem Sawfly

#### Life Cycle

Adults emerge from May to July (Criddle, 1922) and copula usually occurs immediately after emergence. Females are ready to lay eggs a few hours after copulation. Each female produces 33 to 50 eggs (Holmes, 1978) and usually a single egg per female is deposited inside a wheat stem, although several females can oviposit in the same stem. Adults survive 5 to 8 days, depending on temperature and other environmental conditions, and during this time they are not known to feed (Wallace and McNeal, 1966).

Larvae hatch approximately 7 days after oviposition and immediately start feeding on the parenchyma tissue and vascular bundles of the plant. Because larvae are cannibalistic, only one larva will grow inside each stem (Wallace and McNeal, 1966). By the time plants are nearly mature, the larva moves downward to the base of the plant, and

girdles the inside of the stem near the soil surface, weakening the stem that, consequently, lodges when exposed to rain or wind. The larva overwinters in the stub inside a sealed hibernaculum and in the following spring, it goes through pre-pupal and pupal periods, before adult emergence (Ainslie, 1929).

#### Host Selection and Oviposition Preference

Host selection by the WSS, as by any other herbivorous insect, is a process that includes habitat location, host location, and host acceptance and use. Throughout this process, insects use visual, olfactory, gustatory, and tactile stimuli from plants as cues to discriminate suitable hosts from non-suitable ones (Bernays and Chapman, 1994). These multiple sensorial inputs generated from plants are processed and integrated by insects' central nervous system to generate a behavioral response that can be either the acceptance or the rejection of the host.

Plant traits used as cues by herbivorous insects during the host selection process vary among and within plant species due to genetic and environmental factors. Female insects react to plant variability by showing a host preference hierarchy that often reflects a fine-tuned adaptation for optimal host selection and utilization (Thompson, 1983; Mayhew, 1997; Gripenberg et al., 2010). In the case of the WSS, the cryptic larva is constrained within the stem in which the egg was laid, so maternal choices during host plant selection are important determinants of progeny fitness and survival. Thus, female choices for oviposition are expected to be under strong selective forces to optimize host selection and utilization (Jaenike, 1978). Not surprisingly, host selection by the WSS is a multi-step process that involves multiple plant cues. Briefly, WSS host selection starts

with external host evaluation, characterized by walking on leaves and stems, followed by internal host evaluation through ovipositor insertions into the stem, and a final decision of host acceptance or rejection when choosing to oviposit (Buteler et al., 2009).

Plant chemical cues usually mediate host location and landing, though visual stimuli might also be involved (Renwick, 1989). Wheat plants have a characteristic 'green' odor due to eight volatile compounds of 6-carbon aldehydes and alcohols (Hatanaka, 1993). Two of these eight volatile compounds, (Z)-3-hexenyl acetate and (Z)-3-hexen-1-ol, are attractive to WSS females (Piesik et al., 2008). In fact, foraging WSS have been shown to distinguish the two spring wheat varieties, 'Reeder' and 'Conan' (WestBred, LLC), possibly due to differences in emission of the behaviorally active volatile, (Z)-3-hexenyl acetate (Weaver et al., 2009). Besides 'green' odor, a terpenoid compound,  $\beta$ -ocimene, and a carotenoid derivative, 6-methyl-5-hepten-2-one, also released by wheat plants have been shown to elicit behavioral activity from WSS females (Piesik et al., 2008).

While in contact with the plant, both physical factors and chemical cues are involved in host acceptance or rejection by herbivore insects (Renwick, 1989). For instance, the length and diameter of the stems are important physical factors to be assessed by the WSS (Holmes and Peterson, 1960). Females can use as oviposition-sites stems within a limited range of diameter, since they need to be able to encircle the stem with their legs during oviposition (Farstad, 1940). Generally, larger stems are preferred possibly due to higher food availability (Holmes and Peterson, 1960), which in turn affects progeny physiological parameters, such as egg loads of females, weight, and

longevity (Morril et al., 2000). Another important feature assessed by WSS ovipositing females is the stage of plant growth. Wheat plants can only be used as a host during stem elongation period and before the emergence of the inflorescence (Holmes and Peterson, 1960). That is why early in the flight period main stems are preferred for oviposition, but later in the season less mature tillers are used as oviposition sites.

Non-volatile contact stimuli from wheat plants might also play a role in WSS host acceptance, though it is less well understood. Insects' ovipositor harbor gustatory sensilla that can detect non-volatile compounds, which either stimulate or deter oviposition (Hilker and Meiners, 2002). An evidence of the importance of such compounds is given by the WSS behavior while surveying the stem: females repeatedly insert and withdraw their saw-like ovipositor inside the stem before egg laying (Buteler et al., 2009). Similar behavior was also reported for *C. pygmaeus* (Ries, 1926) and for the gall-inducing sawfly, *Euura lasiolepis* (Hymenoptera: Tenthredinidae) (Roininen et al., 1999). The mechanism by which these non-volatile compounds play a role in oviposition is better understood for *E. lasiolepis* and according to Roininen et al. (1999) a willow plant phenolic glucoside, tremulacin, elicits probes with the ovipositor and stimulates oviposition.

Insect oviposition behavior can also be affected by the presence of conspecific eggs and larvae on the host plant, and both visual and chemical cues can help females assess the host infestation status (Nufio and Papaj, 2001). For instance, damage caused by *Trichoplusia ni* (Hubner) (Lepidoptera: Noctuidae) larva when feeding on cotton leaves changes plant odor chemistry reducing its attractiveness as oviposition sites to *T. ni*

females (Landolt, 1993). Wheat stem sawfly infestation induces quantitative changes in volatile production by wheat plants (Peck, 2004). Nevertheless, WSS females neither prefer nor avoid previously infested hosts (Buteler et al., 2009).

After deciding on laying an egg in the stem, the WSS should also decide whether a male or a female egg will be laid. Sawflies have a haplo-diploid sex determination system by which females develop from fertilized eggs and have a full complement of 18 chromosomes (Mackay, 1955), whereas males develop from unfertilized eggs (arrhenotoky). Mated females store sperms in the spermatheca, and during oviposition, females control the release of the sperm and fertilization. Females adjust their offspring sex ratio to host factors, such as diameter of the stem (Cárcamo et al., 2005). Larger diameter stems produce more females, probably because females are larger than males and, therefore, require more resources (Charnov, 1982).

The information detailed above highlights the level of complexity of WSS host location and selection. Further understanding of WSS plant preference and oviposition behavior could lead to improvements in pest management.

### Plant Damage

The damage in wheat plants is caused by the stem-mining larvae. They feed on parenchyma tissue and vascular bundles, affecting plants photosynthetic capacity (Macedo et al., 2005), that consequently cause reductions on kernel weight (Morrill et al., 1994), grain quality, and yield (Morril et al., 1992, 1994). The amount of grain that can be harvested is also reduced by mature larvae that cut the base of the stems, causing them

to lodge when exposed to rain or wind (Criddle, 1922). Lodged stems are time-consuming and expensive to recover (Luginbill Jr. and Mc Neal, 1954).

### Pest Management

The cryptic life cycle of the WSS coupled with its prolonged adult emergence interval greatly reduces the range of control measures and makes WSS management challenging (Beres et al., 2011a).

Cultural Control. Cultural methods of insect control were the first to be recommended against this pest. Early recommendations included destruction of stubs through fire, deep plowing, early harvest, destruction of WSS reservoirs by mowing grassy borders, and the use of trap crops (Beres et al., 2011a). Some of these practices were shown to be inefficient or harmful for the soil and were discouraged. Promising results were obtained using crop traps (Seamans 1928), but inconsistent control and impractical seedling operations prevented its adoption by wheat producers. Currently, cultural methods that help manage WSS populations include crop rotation using immune crops such as oats (*Avena sativa* L.), soybeans (*Glycine max* L.) and sunflower (*Helianthus annuus* L.), and delayed planting in fields prone to WSS attack (Beres et al., 2011a). However, these methods are limited in scope due to other economic agronomic considerations by growers.

Chemical Control. Numerous insecticides have been tested against the WSS but limited success has been achieved, because immature stages are protected from insecticides inside the stem and adults that emerge after spraying have reduced exposure

to the chemical product (Gall and Dogger 1967, Holmes 1978, Anonymous 1997). In the 1960s, satisfactory results were obtained when granular insecticides were applied in the furrow with the wheat seed, but mainly when infestation was low and the oviposition occurred in the lower internodes of the stem where insecticide was more concentrated (Holmes and Peterson, 1963). Recently, the Montana Department of Agriculture issued a special local needs pesticide registration authorizing the use of the systemic organophosphate insecticide, Thimet 20-G®, for WSS control in spring and winter wheat. However, due to its toxicity to mammals, fish, and birds, Thimet 20-G® must be incorporated into the soil and application cannot be done later than 85 days before harvest. Also, the use of this insecticide can negatively impact the population of WSS natural enemies.

Biological Control. According to Beres et al. (2011), nine species of Hymenoptera can parasitize the WSS in native and exotic grasses but only two species, *Bracon cephi* Gahan and *Bracon lissogaster* Muesebeck (Hymenoptera: Braconidae), have been recorded parasitizing WSS in wheat fields. Parasitism rates in wheat are inferior to rates observed in grasses (Runyon, 2001). Nevertheless, parasitoid species have been shown to have a positive effect on wheat plant fitness (Buteler et al., 2008). Thus, the use of agronomic practices that reduces disturbance on parasitoids' habitat can help conserve parasitoids in wheat fields and increase natural biological control of the WSS. Biological control of the WSS was also tried using introduced biocontrol agents. Two parasitoid species of *C. pygmaeus*, *Collyria calcitrator* (Gravenhorst) and *Collyria coxator* (Villers)

(Hymenoptera: Ichneumonidae), were introduced in North America, but introductions failed to produce positive results (Davis, 1955).

Despite decades of research on chemical, biological, and cultural methods of control, limited success has been achieved in mitigating losses due to this pest. Currently, host plant resistance is the most consistently effective method of WSS control and is the foundation for integrated pest management of WSS. Due to its importance, wheat resistance to the WSS is reviewed independently under the next topic.

### Plant Genetic Resistance

Plant-insect interactions are commonly observed in nature. Some of these relationships are beneficial, but most of them involve plant predation (Fürstenberg-Hägg et al., 2013). In fact, scientists estimate that more than a million insect species feed on plant tissues (Howe and Jander, 2008) and that virtually all plant species are used as food by at least one insect species (Ehrlich and Raven, 1964). Although apparently passive, plants respond to herbivory with a varying array of defense strategies that aim to escape, combat, or compensate for herbivory. Such diversity of defense mechanisms is the result of a millions of years-arm race between plants and herbivores caused by reciprocal selection (Ehrlich and Raven, 1964).

Plant defense mechanisms are broadly categorized as tolerance and resistance (Stout, 2013). Tolerance relates to the ability of a plant to regrow and/or reproduce after herbivore attack. It includes plant traits that mitigate herbivore damages, such as the increasing net photosynthetic rate after damage or the ability to reallocate carbon stores

from roots to shoots after damage (Strauss and Agrawal, 1999). Plant resistance, on the other hand, comprises all plant traits that reduce the extent of abnormal changes in a plant physiological process caused by a herbivore (Stout, 2013). Resistance can be further subdivided according to the trophic level of organisms involved in the defense (direct or indirect) or the manner in which plant defense mechanisms are regulated (constitutive or induced).

Direct plant resistance refers to plant traits that directly affect herbivores biology or behavior, while indirect resistance refers to plant traits that promote the effectiveness of higher trophic levels (insect's natural enemies) and consequently increase herbivore mortality (Quintero et al., 2013). Resistance is said to be constitutive when it is expressed regardless of the presence of the herbivore. On the contrary, inducible resistance is only expressed or expressed in a greater extent after injury by a herbivore (Stout, 2013).

#### Wheat Resistance to the Wheat Stem Sawfly

The main type of resistance used against the WSS over the past five decades is the stem solidness. Solid stems limit larval growth and movement, reducing insects' survival (Wallace and McNeal, 1966). The solid stem trait was first observed in a landrace from Portugal, S-615, which was used as the source of resistance in the first North American solid-stemmed cultivar in the 1940s, 'Rescue' (Platt et al., 1948). Following 'Rescue', several solid or semi-solid stemmed varieties were developed.

Some of the reasons that led to the wide-spread use of solid-stemmed varieties are the efficiency in WSS control and ease of selection in large populations. Selection for a constitutive type of resistance, such as stem solidness, can be accomplished in the

absence of insect's pressure, allowing efficient screening of segregating materials at research sites that are free from pest infestation. The problem with the ability to rapidly select for solidness in the absence of the pest is that it limited the potential to make new entomological observations during the selection for the most solid stems. As a consequence, other types of resistance mechanisms have long been neglected.

Neglecting other resistance mechanism is problematic because stem solidness-based resistance is not always reliable. As early as 1948, the solid-stemmed variety 'Rescue' showed stem cutting in some sites as high as 20% (Platt et al., 1948). One explanation for such higher-than-expected level of stem cutting is that the degree of solidness is influenced, among other factors, by photoperiod and light intensity during stem elongation (Beres et al., 2011b). Hence additional resistance mechanisms could potentially improve WSS control in wheat fields.

A second type of resistance mechanism that has been more recently investigated is the host plant attractiveness to ovipositing females. Lack of attractiveness could reduce the chances of infestation, therefore providing a level of resistance to WSS. Weaver et al. (2009) showed for the first time that wheat varieties differed in the emission of an attractive compound and that ovipositing females overwhelmingly preferred the more attractive wheat variety as a site for egg laying. This suggested that WSS uses plant volatiles while selecting its host.

Some of the WSS parasitoid species might also use plant volatiles to find their host. Peck (2004) showed that WSS infestation can induce changes in volatile production in wheat plants. Synthetic volatiles matching those produced by WSS-infested plants

generated a positive electrophysiological response from parasitoids antennae. Some of these synthetic volatiles were also shown to generate a significant attractive response from *B. cephi* and *B. lissogaster* (Perez, 2009). These results suggest that indirect plant defense mechanism might also play a role in wheat defense against the WSS.

#### Genetic Basis of Wheat Resistance to the Wheat Stem Sawfly

Traits that show continuous phenotypic variation are designated quantitative traits, while the genomic regions containing one or multiple genes that affect variation in quantitative traits are called quantitative trait loci (QTL). The detection and localization of QTLs is possible through their linkage to polymorphic marker loci. During meiosis, genes and markers segregate via chromosome recombination (crossing-over). Those genes and markers that are tightly-linked will be inherited together more frequently than those located further apart, because the probability of recombination between markers and genes increases with physical distance (Collard et al., 2005). If a QTL is linked to a polymorphic marker locus, then individuals with different marker alleles will have different mean values of the quantitative trait (Mackay et al., 2009).

Two methods are commonly used to genetically map quantitative traits in crop species, bi-parental QTL mapping and association mapping (Mackay et al., 2009). In bi-parental mapping, two parental lines that differ for traits of interest are crossed and a segregating population is derived. The population is phenotyped and genotyped using polymorphic markers and then analytical techniques are employed to determine linkages between markers and genomic regions controlling traits of interest. In an association

mapping approach, a diverse panel of lines is sampled and all the historical recombination events are exploited. Because multiple generations separate parental haplotypes and panel lines, crossing-over has uncoupled all but the most tightly-linked markers from the genes of interest (Rafalski, 2010). Thus, an association mapping approach allows for a higher resolution in QTL localization and for the ability to sample a large amount of allelic variation. It is important to notice though, that in an association mapping approach, false marker-trait associations may arise from the non-random distribution of genotypes among panel lines (population structure) (Mackay and Powell, 2007). In such cases, statistical tests that correct for false positive rates should be employed (Kang et al., 2008).

The first QTL mapping study that aimed to identify genomic regions associated with WSS resistance was done by Cook et al. (2004). A mapping population was derived from a solid- by hollow-stemmed winter wheat cross to allow the investigation of the genetic basis of stem solidness, the primary mechanism of resistance used against the WSS. Most of the solid stem variation in winter wheat was shown to be controlled by a major QTL on chromosome 3B, named *Qss.msub-3BL* (Cook et al., 2004). Later, Kalous et al. (2011) showed that the same QTL controls most of the variation for stem solidness in spring wheat. This genomic region was also shown to be associated with stem solidness in durum wheat (Houshmand et al., 2007). Other minor genes, including *Qss.msub-3DL*, may modify the degree of stem solidness provided by *Qss.msub-3BL* (Lanning et al., 2006).

As quantitative trait, stems range from hollow to solid. Lines with intermediate solidness (semi-solid) have been observed to have more resistance than is explained by the level of solidness. For instance, the semi-solid spring wheat lines Conan and 'Scholar' differ for WSS resistance, despite the similarity in the degree of stem solidness. Indeed, new evidences suggest that the semi-solid line Conan has a different solid stem allele that is probably involved with an additional mechanism of resistance to the WSS (Talbert et al., 2014). Further studies are necessary to elucidate the resistance mechanism provided by this allele.

Solid stem cultivars have historically produced lower yields than hollow-stemmed cultivars (McNeal et al., 1965; Weiss and Morrill, 1992). Since most of variation in stem solidness is caused by the *Qss.msub-3BL* locus, it has been hypothesized that this locus is also responsible for reduction in yield. To test this hypothesis, a recent study compared the performance of near-isogenic lines (NILs) for alleles at *Qss.msub-3BL* in six genetic backgrounds and under different environments. In general, the allele for stem solidness at the 3B QTL does not cause reduction in yield (Sherman et al., 2015). Low yielding performance of solid stem lines previously observed may be attributed to poor genetic backgrounds.

In addition to the solid stem QTL, other QTLs for resistance to WSS have been identified on multiples chromosomes in wheat (Sherman et al., 2010; Joukhadar et al., 2013). Sherman et al. (2010) identified QTLs associated with WSS resistance on chromosomes 1B, 2D, 4A and 5B using a recombinant inbred line (RIL) population derived from a cross between the spring wheat lines Conan and Reeder. Quantitative trait

loci on chromosomes 1B and 5B were both associated with heading date. Lines that head late in the season escape infestation, because their stems are not suitable for egg-laying during the WSS oviposition period. The QTLs on chromosomes 2D and 4A explained 19% and 29% of the variation for WSS infestation and stem cutting, respectively. In both cases, Conan alleles provided resistance against the WSS. Interestingly, these two QTLs did not cosegregate with morphological traits (e.g. heading date, plant height, and solid stems) known to affect WSS infestation and cutting. Thus, they provide resistance through new but unknown mechanisms.

Although the resistance mechanisms provided by the 4A and 2D QTLs are unknown, evidences suggest that at least one of them might be associated with host plant attractiveness to ovipositing females. The wheat varieties Conan and Reeder used by Sherman et al. (2010) in the bi-parental mapping study were also used by Weaver et al. (2009) to study WSS preference for oviposition. Reeder has shown to be significantly preferred by ovipositing females and to release a greater amount of an attractive compound that influences females' preference for oviposition (Weaver et al., 2009). Thus, it is possible that either the 4A or the 2D QTLs identified by Sherman et al. (2010) is involved with volatile production and host preference.

Joukhadar et al. (2013) identified three novel QTLs associated with resistance to the WSS using an association mapping panel comprised of wheat landraces, synthetic hexaploid wheat and elite lines from West Asia. The novel QTLs are located on chromosomes 1D, 6B, and 7A. Analysis of gene ontology for the molecular marker associated with the 1D QTL showed that this locus is linked to genes associated with cell

defense response and apoptosis. Resistance mechanisms for QTLs on chromosomes 6B and 7A have not been determined. Previously identified QTLs on chromosomes 3B and 5B were also detected by Joukhadar et al. (2013).

Other alleles known to influence plant susceptibility to the WSS are found at the Rht-B1 and Rht-D1 loci controlling plant height on chromosomes 4B and 4D, respectively. Taller plants are preferred by sawfly females for oviposition (Buteler et al., 2009).

Numerous studies have identified QTLs for resistance to the WSS. Though important, these discoveries are a preliminary step in developing new varieties of wheat resistant to the WSS. Further studies are needed to validate QTLs in additional backgrounds and environments and to determine the mechanism of resistance provided by novel QTLs.

### Research Objectives

Host plant resistance is the foundation for integrated pest management of the WSS; therefore, the main objective of this research was to enhance the repertoire of genetic resistance to WSS available for exploitation by wheat breeders through marker assisted selection. To achieve this goal, we divided this research into two parts. In the first part, we performed a set of experiments to first, validate previously identified QTLs for WSS resistance and second, to further investigated the resistance mechanism provided by some of these QTLs. In the second part of this research, we conduct an association mapping study using an elite panel of spring wheat lines from North America to

characterize the genetic variation for stem solidness and WSS resistance and to identify new marker-trait associations for traits related to WSS resistance.

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## CHAPTER TWO

### HOST PLANT QUANTITATIVE TRAIT LOCI AFFECT SPECIFIC BEHAVIORAL SEQUENCES IN OVIPOSITION BY A STEM-MINING INSECT

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## HOST PLANT QUANTITATIVE TRAIT LOCI AFFECT SPECIFIC BEHAVIORAL SEQUENCES IN OVIPOSITION BY A STEM-MINING INSECT

The following chapter has been submitted to the journal BMC Plant Biology.

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### Abstract

#### Background

Host plant selection for oviposition is an important determinant of progeny performance and survival for phytophagous insects. Specific cues from the plant influence insect oviposition behavior; but, to date, no set of host plant quantitative trait loci (QTLs) have been shown to have an effect on behavioral sequences leading to oviposition. Three QTLs in wheat (*Triticum aestivum* L.) have been identified as influencing resistance to the wheat stem sawfly (WSS) (*Cephus cinctus* Norton). Wheat near-isogenic lines (NILs) for each of the three QTLs were used to test whether foraging WSS were able to discriminate variation in plant cues resulting from allelic changes.

## Results

A QTL on chromosome 3B (*Qss-msub-3BL*) previously associated with stem solidness and larval antibiosis was shown to affect WSS oviposition behavior, host preference, and field infestation. Decreased preference for oviposition was also related to a QTL allele on chromosome 2D (*Qwss.msub-2D*). A QTL on chromosome 4A (*Qwss.msub-4A.1*) affected host plant attractiveness to foraging females, but did not change oviposition preference after females landed on the stem.

## Conclusions

These findings show that oviposition decisions regarding potential plant hosts require that phytophagous WSS have the capability to discriminate plant cues associated with allelic variation and combinations of alleles across host plant quantitative loci. Allele types in a host plant QTL associated with differential survival of immature progeny can affect maternal choices for oviposition. The multidisciplinary approach used here may lead to the identification of plant genes with important community consequences, and may complement the use of antibiosis due to solid stems to control the wheat stem sawfly in agroecosystems.

## Background

Plant traits that influence host selection for oviposition in phytophagous insects vary among and within plant species due to genetic and environmental factors. Female insects react to variability among host plants by showing a host preference hierarchy that often reflects adaptation for optimal host selection and utilization [1], [2], [3]. Genetic

variation in host species controlling insect oviposition influences ecology (e.g. host range, population dynamics) and evolution of the host and the insect. It also may allow for new opportunities to use behavioral manipulation methods to improve insect management in agroecosystems.

Several studies have identified host plant quantitative trait loci (QTLs) associated with insect oviposition preference [4]-[8] and immature performance and survival [5], [9]-[12]. Oviposition preference may include long-distance detection of suitable plants, with subsequent physical and sensory evaluation of the host after the insect has alighted on the plant. Ideally, females should select plants for oviposition which will optimize larval performance and survival. However, no study has linked host plant QTLs to behavioral sequences leading to insect oviposition or that maternal oviposition choices for specific allele types at a plant locus are associated with differential survival of immature offspring.

The wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae) (WSS) was originally identified as a pest of many large-stemmed grass species in western United States [13]. As European settlers began to farm the shortgrass prairie, large areas of native grasses were displaced to cultivate wheat, *Triticum aestivum* L. Shortly after wheat crops were planted, *C. cinctus* populations adapted to this new and widely distributed plant host [14]. This resulted in the common name for the insect to change from ‘western grass stem sawfly’ to ‘wheat stem sawfly’ [15]. The WSS is now a major pest of wheat in the Northern Great Plains of North America [16]. Adults emerge from wheat stubs from May to July and copulation occurs soon after emergence [17]. Females lay eggs inside

wheat stems and the larvae feed on parenchymal tissues and bore through vascular bundles of the plant [18]-[20]. When the plant is nearly mature, the larva cuts the base of the stem in preparation for overwintering diapause [15]. Infested stems show reduced grain quality and yield; cut stems usually lodge and are not harvested [19]-[21].

Although mobile within a wheat stem, a WSS larva is not capable of switching hosts so maternal choices during host plant selection are important determinants of progeny fitness and survival. Thus, female choices for oviposition are expected to be under strong selective forces to optimize host selection and utilization [22]. Not surprisingly, host selection by WSS is a multi-step process orchestrated by several plant cues [23]. Pre-alighting behaviors are controlled by visual and long-range chemical cues [24], [25], while post-alighting behavioral displays are mainly driven by contact cues [23]. Utilization of plant cues for host selection by gravid females suggests that antixenosis mechanisms can influence WSS-host plant interaction. Alteration of plant traits used as cues could reduce infestation and consequently decrease insect damage in agroecosystems.

The selection of stems that are suitable for oviposition by female WSS is likely to be influenced by genes in the host plant. The ability to genetically differentiate the factors controlling this interaction exists in wheat due to the availability of molecular tools that do not exist for the native grasses. In this regard, Weaver et al. [25] reported that spring wheat cultivars grown in Montana vary in attractiveness to ovipositing WSS females. A cross between a cultivar attractive to females, ‘Reeder’ (PI 613586), and an unattractive cultivar, ‘Conan’ (WestBred, LLC), revealed three QTLs controlling the number of eggs

laid in the stem [7]. These QTLs are designated *Qwss.msub-2D*, *Qwss.msub-4A.1*, and *Qwss.msub-4A.2*. The Reeder alleles at two QTLs were associated with greater oviposition, while *Qwss.msub-4A.2* was not.

Researchers have discovered significant diversity within wheat cultivars for susceptibility to damage caused by WSS larvae. An important early discovery was that while all wheat cultivars grown in the Great Plains had hollow stems, some wheat landrace accessions had pith-filled or solid stems [26]. Larvae of WSS are unable to thrive in solid stem wheat [27], resulting in less damage and decreased overwintering success, as well as reduced fecundity in subsequent years [28]. Thus, the solid stem trait was quickly adopted as the primary control measure for WSS. This trait has been found to be under simple genetic control with a single QTL on chromosome 3B, *Qss.msub-3BL*, controlling most of the variation [7], [11], [29]-[31]. The solid stem allele commonly used for resistance derived from the Portuguese landrace S-615 [26]. Subsequently, research showed that a different allele at *Qss.msub.3BL* from the cultivar Conan not only affected stem solidness and decreased larval survival, but also reduced the number of stems selected for oviposition [32].

The existence of molecular markers for the QTLs affecting oviposition preference in wheat allowed the development of near-isogenic lines (NILs) for *Qss.msub-3BL*, *Qwss.msub-2D*, and *Qwss.msub-4A.1*. These NILs were used to assess the role of specific wheat QTLs in the oviposition behavior of WSS. Interactions between WSS females and specific host genes may provide an opportunity to develop pest management strategies to mitigate damage caused by WSS. The integration of knowledge from the fields of plant

quantitative genetics, chemical ecology, and insect behavioral ecology allowed to gain insights into the genetic basis of plant intraspecific variations that have important ecological effects on a phytophagous insect species. The approach used here can be broadly applicable to other plant and insect species and may lead to the identification of plant quantitative trait that have important community consequences.

## Methods

### Insects

Wheat stem sawfly larvae in overwintering diapause were collected from wheat stubble that was heavily-infested by WSS in a field near Amsterdam, MT, USA (45°45'29.85"N, 111°22'49.32"W). Larvae were maintained in cold storage (0-4°C) for 3-6 months to facilitate the completion of diapause. The overwintering 'stubs' were later transferred to plastic Tupperware boxes (70 by 35 by 20 cm) and held at room temperature (22-27°C) for 4 to 5 weeks until adult emergence. Newly-emerged adults were held in 2 L Mason glass jars until experiments were conducted. Narrow wooden skewers were placed inside the jars to allow the insects to perch, rest and climb. Adults used in this study were 24-48 h old and were provisioned with water *ad libitum* before experimentation.

### Near-Isogenic Line (NIL) Development

The NILs used in this study were developed using recombinant inbred line (RIL) populations derived from crosses between the spring wheat lines Reeder/Conan, 'Scholar' (PI 607557)/Conan, and 'Choteau' (PI633974)/Conan. Polymerase chain reaction was

conducted using microsatellite primer sets to identify NILs for each of three QTLs based on the procedure of Pumphrey et al.[33]. Primer sets included wmc161, linked to the 4A QTL (*Qwss.msub-4A.1*), identified by Sherman et al.[7]. The marker gwm539 was used for the 2D QTL *Qwss.msub-2D* [7], and gwm340 was used for *Qss.msub-3BL* [29]. The target populations used to identify NILs were F<sub>8</sub> RIL derived from F<sub>5</sub> plants.

Heterozygous individuals for each locus are expected to occur in the F<sub>5</sub> generation at a frequency of 6.25%. Three generations of self-pollination of the heterozygous individuals resulted in generation of primarily homozygous individuals. This procedure allowed for the identification of homozygous resistant (unfavorable hereafter) and homozygous susceptible (favorable hereafter) NILs for each one of the QTLs. A pair of unfavorable and favorable NILs derived from a heterozygous F<sub>8</sub> plants derived from F<sub>5</sub> heterozygous RIL are expected to be approximately 97% identical at loci unlinked to the target QTL. In total, four, three, and four NIL pairs polymorphic for *Qss.msub-3BL*, *Qwss.msub-2D*, and *Qwss.msub-4A.1*, respectively, were tested under controlled conditions, while 12, four, and six NIL pairs for *Qss.msub-3BL*, *Qwss.msub-2D*, and *Qwss.msub-4A.1*, respectively, were tested under field conditions. The NILs polymorphic for the *Qwss.msub-2D* were all derived from the cross of favorable Reeder and unfavorable Conan, because the other parental lines shared the same marker alleles on the 2D locus. The QTL alleles designated ‘a’ for *Qwss.msub-2D* and *Qwss.msub-4A.1* indicate alleles previously associated with preference of the WSS for oviposition, while alleles designated ‘b’ were associated with decreased preference [7], [11], [32]. The QTL allele designated ‘a’ for *Qss.msub-3BL* is the allele for hollow stems from Reeder , the allele designated ‘b’ is the

allele for solid stems from Choteau and Scholar [29], and the allele designated ‘c’ is the solid stem allele derived from Conan [32].

### Plant Culture

Experiments to monitor WSS oviposition behavior using the NIL pairs were performed in a greenhouse at the Montana State University Plant Growth Center (MSU-PGC, Bozeman, MT, USA). Three plants per pot were grown in tapered square pots (13 by 13 by 13.5 cm) under natural and artificial light (GE Multivapor Lamps model MVR1000/C/U, GE Lighting, General Electric Company, Cleveland, OH), photoperiod of 14:10 (L:D) h,  $22 \pm 2^{\circ}\text{C}$ , and 20 - 40% RH. Experiments were conducted from May through August in 2013 and in 2014. Plants were watered regularly and fertilized twice each week with Peters General Purpose Fertilizer (J.R. Peters, Allentown, PA) at 100 ppm in aqueous solution. Soil used consisted of equal parts of MSU-PGC soil mix (equal parts of sterilized Bozeman silt loam soil and washed concrete sand with Canadian sphagnum peat moss incorporated) and Sunshine Mix 1 (Canadian sphagnum peat moss, perlite, vermiculite, and Dolmitic lime; Sun Gro Horticulture, Bellevue, WA, USA) [34]. All plants used in choice tests were at Zadok's growth stage 33 to 34 with two to three nodes detectable [35].

### Host Preference Test

To test the effect of QTLs on WSS host plant selection for oviposition, two pots containing a pair of favorable and unfavorable NILs were placed inside screened cages (91.4 by 66.7 by 91.4 cm) with 530  $\mu\text{m}$  mesh openings. In the summer of 2013, choice

tests for each pair of NIL and the associated parental lines were conducted with 4 replications (cages). At 0900 hours in day one of the experiment, 15 WSS females and five males were released inside each cage and oviposition was allowed for two consecutive days. Oviposition behavior was observed each day from 1000 hours to 1300 hours, when WSS adults are most active. The duration of events, including females walking on the leaves and walking on stems, were recorded. The number of ovipositor insertions on the stem were recorded. At the end of day two of the experiment, pots were removed from cages and stems were dissected to assess the number of eggs laid. In the summer of 2014, choice tests for each pair of NIL and the associated parental lines were conducted with 12 replications (cages). At 1000 hours of each test day, 15 WSS females and five males were released inside each cage and oviposition was allowed for 2 consecutive hours. Oviposition behavior was observed and number of ovipositor insertions on the stem was recorded. Immediately after the behavioral observations, pots were removed from cages and stems were dissected to assess the number of eggs laid. This procedure quantified the number of times females inserted the ovipositor into stems without laying eggs into the stem.

#### Y-Tube Olfactometer

A closed-system Y-tube olfactometer similar to that described by Piesik et al.[28] was used to test whether QTLs had an effect on behaviorally active volatiles released by wheat plants. Humidified air was fed through a purifier-charcoal filter and then split into two air streams using a threaded 24/410 (inner diameter 24 mm) cap with a Teflon liner coupled to a 0.64-cm Swagelok union to deliver air to a pair of glass chambers (40 mm

diameter x 800 mm long). Each glass chamber enclosed a single wheat plant as the odor source. Plants were illuminated using an enhanced spectrum LED grow light (SunShine System Grow UFO Light SS-Gu90w) to provide light intensity comparable to field conditions. A flexible Teflon sleeve was tape-sealed around the base of the plant's stems to prevent unfiltered-air from entering the system. Teflon tubing then delivered air from the odor source chambers to each arm of the Y-tube. A male ground joint on the stimulus-delivery tube was connected to a female ground-glass joint on each arm of the Y-tube, yielding a consistent airtight fit. A flowmeter was used to set the airflow at 0.1 L/min. Tests were conducted in a 28-mm diameter x 300-mm long Corning glass tubing that branched at 20 cm and had an interior angle of the "Y" of 120°. Diverging arms extended for 4 cm in each direction before becoming parallel for the final 10 cm. The olfactometer was illuminated with a fiber optic illuminator (T-Q/FOI-1, Techni-Quip Corp, El Segundo, CA 90245, USA), centered between the Y-tube arms and 30 cm upwind of the bifurcation. This enhanced insect movement as WSS have strong positive phototaxis [23]. A pair of unfavorable and favorable NILs or a pair of parental lines were used as test stimuli for each bioassay. Females were individually placed at the basal unbranched section of the Y-tube at approximately 2 cm from the outlet. To facilitate insect movement towards the junction of the 'Y', it was necessary to place a long wire in the bottom of the Y-tube, extending 10 cm from the introduction point. Each female was observed for 5 min or until they occupied one of the two arms of the olfactometer. Between 31 and 50 females were used to test each NIL pair, and in successive testing, the stimulus from unfavorable or favorable NILs was alternately offered in a different arm of

the Y-tube. In total, two pairs of the 2D and 3B NILs, and 3 pairs of the 4A NILs were tested. All bioassays were conducted at room temperature.

#### Wheat Stem Sawfly Infestation under Field Condition

To test the effect of the QTLs on WSS infestation under natural field conditions, the NILs were planted in late April 2013 and 2014 at a traditionally WSS-infested site near Loma, MT, USA (48° 04' 21.96" N, 110° 27' 41.84" W) and in 2014 and 2015 at a second site with a history of WSS infestation near Amsterdam, MT, USA (45° 45' 29.85" N, 111° 22' 49.32" W). Experiments were established adjacent to stubble from a previous WSS-infested wheat crop as unreplicated plots in 2013 and in a randomized complete block design with two replications in 2014 and 2015. Each block included 12, 4, and 6 NIL pairs for *Qss.msub-3BL*, *Qwss.msub-2D*, and *Qwss.msub-4A.1*, respectively, along with parental lines. Plots consisted of 10 seeds per entry planted in individual hills with spacing of 0.8 m between adjacent hills. Stems were collected at maturity and dissected to determine the presence of WSS larvae (infestation).

#### Statistical Analysis

Results from host preference and host attraction bioassays were analyzed using a chi-square test for small sample sizes [36]. Data on the duration of specific female behaviors were analyzed using mixed model analysis of variance with maximum likelihood estimation using Proc MIXED in SAS v 9.3 [37]. Fixed effects included allele, day, and the interaction, and cage within allele was a random effect. Data were transformed before analysis using a Box-Cox transformation [38], but untransformed data

are presented. Field data on WSS infestation were analyzed using Proc MIXED in SAS. Families and locations were considered random effects while entry and allele types were considered fixed effects.

## Results

### Host Preference for Oviposition and Oviposition Behavior

The number of eggs laid on the unfavorable parental line (Conan) was significantly lower than that laid on the two favorable parental lines (Reeder and Scholar) ( $P < 0.05$ ) (Table 1). Significant differences between these parental lines were also observed for the number of ovipositor insertions on the stem ( $P < 0.05$ ). Females that had alighted on stems inserted their ovipositor in stems from the favorable lines (Reeder and Scholar) at double the frequency for stems from the unfavorable line (Conan) (Table 1). Ovipositor insertions into stems of this unfavorable line (Conan) often did not result in egg deposition (Table 2). Females spent more time ( $P < 0.05$ ) exploring the stem of the more favorable parent, Reeder, by walking up and down its length (mean  $\pm$  SE of walk time/per female  $20.59 \pm 2.31$ s; n of events = 37) than they did on the unfavorable parent (Conan) (mean  $\pm$  SE of walk time/female  $8.92 \pm 1.24$ s; n of events = 14), and host examination while walking on stem was always associated with rapid antennal movements of “tapping” the stem (data not shown) as described in [23]. There were no differences in the duration of time that females walked on stems from the favorable line Scholar (mean  $\pm$  SE of walk time/female  $20.04 \pm 3.66$ s; n of events = 22) or the unfavorable line (Conan) (mean  $\pm$  SE of walk time/female  $7.60 \pm 1.99$ s; n of events = 5).

Similarly, no differences between any of the parental lines were observed for the duration of walking on the leaves (data not shown).

Preference tests using pairs of NILs confirmed the effects of *Qss.msub-3BL* on host preference for oviposition and probing (ovipositor insertions on stem). The *Qss.msub-3BLc* (derived from the parental line Conan) at the 3B locus significantly reduced the number of ovipositor insertions and eggs laid in the stem for three of the four *Qss.msub-3BL* NIL pairs ( $P < 0.05$ ) (Table 1). An effect for the number of eggs laid was evident for *Qwss.msub-2D*, with the *Qwss.msub-2Db* (derived from the parental line Conan) reducing oviposition in two of three NIL pairs. The *Qwss.msub-2Db* also reduced probing behavior in one of three NIL pairs for *Qwss.msub-2D*. Probing behavior often did not result in egg deposition on NILs with the *Qwss.msub-2Db* ( $P < 0.05$ ) relative to NIL with *Qwss.msub-2Da* (Table 2). An effect of the *Qwss.msub-4A.1* on number of ovipositor insertions on the stem was observed for the three NIL pairs derived from the Scholar/Conan cross. One of these NIL pairs, SC-3 (Table 2), also differed at the 3B QTL, and thus it is not possible to determine whether the 3B or 4A QTL caused the difference. No effect of *Qwss.msub-4A.1* was observed for the NIL pair derived from the Reeder/Conan cross. No preference in egg deposition was observed between *Qwss.msub-4A.1* NIL pairs (Table 1). The cumulative outcomes for individual QTLs on WSS oviposition are shown in Figure 1. The NIL pairs did not differ for the time females spent walking on the leaf nor for the time they spent walking on the stems (data not shown).

### Y-Tube Olfactometer Behavioral Assays

These bioassays tested the attractiveness of volatile compounds released by NIL pairs polymorphic for three QTLs associated with WSS oviposition preference. More than 65% of the females preferred volatiles released by plants of the favorable parental line Reeder over those from the unfavorable (Conan) plants ( $P < 0.05$ ), but no difference in attraction was observed for volatiles released by plants from the favorable line Scholar and those from the unfavorable plants ( $P > 0.05$ ) (Table 3). Females were significantly attracted ( $P < 0.05$ ) to volatiles emitted by NIL with the favorable allele *Qwss.msub-4A.1a* for the two NIL pairs derived from the Scholar/Conan cross (Table 3). Bioassays testing the attraction of one pair of *Qwss.msub-4A.1* derived from a Reeder/Conan cross showed no differences between the NILs ( $P > 0.05$ ). Females showed no preference for volatiles released by NIL pairs polymorphic for the *Qss.msub-3BL* or *Qwss.msub-2D* QTLs ( $P > 0.05$ ) (Table 3).

Near isogenic lines that were shown to be less attractive to WSS females (designated SC-1-*Qwss.msub-4A.1b* and SC-2-*Qwss.msub-4A.1b*) (Table 3) were tested against pure air to ensure that females were not repelled by plant volatiles. Females preferred volatiles coming from SC-2- *Qwss.msub-4A.1b* over the pure air ( $P < 0.05$ ;  $\chi^2 = 8.01$ ;  $df = 1$ ), but there was no difference in choices between the plant and the pure air for SC-1- *Qwss.msub-4A.1b* ( $P > 0.05$ ;  $\chi^2 = 0.51$ ;  $df = 1$ ) (Table 3). The effect of QTLs on different steps of WSS host selection process is illustrated in Figure 2. The QTL *Qwss.msub-4A.1* affects the ability of WSS females to recognize a suitable host from a distance and the number of times a female inserts the ovipositor inside the stem, while the

*Qwss.msub-2D* and *Qss.msub-3BL* QTLs affect the number of ovipositor insertions and egg deposition once the female is in contact with the host. The QTL *Qss.msub-3BL* had the greatest effect (Table 1).

#### Wheat Stem Sawfly Infestation under Field Conditions

The favorable parental lines Reeder and Scholar were significantly ( $P > 0.05$ ) more infested than the unfavorable Conan (Table 4). The *Qss.msub-3BL* had an effect on the percent infestation for NIL pairs derived from the Scholar/Conan cross, with NILs carrying the *Qss.msub-3BLc* (derived from the parental line Conan) showing reduced levels of infestation ( $P > 0.05$ ). No effect was observed for NILs derived from Reeder/Conan or Choteau/Conan crosses (Table 4). Also, no effect on WSS field infestation was observed for NIL pairs for *Qwss.msub-2D* or *Qwss.msub-4A.1* (Table 4).

### Discussion

The oviposition behavior of WSS was first described by Ainslie [13]. In this early description, two main factors were identified as important determinants of oviposition, the plant growth stage and the environmental conditions during host plant selection. Subsequent studies showed that physical (e.g. stem diameter and length) and chemical (e.g. attractive volatiles) characteristics of host plants also influence female choice for oviposition [24], [25], [39]. A general functional organization of the WSS oviposition process was presented by Buteler et al. [23] based on measurements of the transitional frequencies between different behaviors displayed during host plant selection. Briefly,

WSS host selection starts with external host evaluation, characterized by walking on leaves and stems, followed by internal host evaluation through ovipositor insertions into the stem, and a final decision of host acceptance or rejection when choosing to oviposit. Our study describes the effect of three host plant QTLs on female choices during oviposition. This was made possible by the development of NILs for specific wheat QTLs impacting WSS resistance. Each NIL pair differed primarily only for the gene of interest at the specific QTL. This allowed the NIL pairs to be tested side-by-side on the same day, avoiding confounding issues such as amount of sunshine on any given day. The latter is especially important for WSS which exhibits strong phototaxis [23].

Results presented herein confirmed that host selection by WSS occurs in a stepwise fashion, with plant cues modulating different behavioral sequences. Allelic variations in individual host plant QTLs are sufficient to affect female choice and to determine host preference hierarchy. Because at least one of these QTLs is associated with larval mortality [32], female decisions to oviposit suggest an adaptation to optimize progeny performance and survival [22], [40]. To our knowledge, this is the first time that allelic variations on a host plant QTL affecting larval survival are shown to also influence female oviposition behavior and host preference.

#### *Qwss.msub-4A.1*: a QTL for Host Plant Attractiveness

Host selection by WSS begins before landing, with females evaluating long distance cues emitted by host plants. One of these cues is at least partially controlled by the *Qwss.msub-4A.1*, which was shown to affect host plant attractiveness to foraging

females. Wheat plants have a characteristic ‘green’ odor due to eight volatile compounds of 6-carbon aldehydes and alcohols [41]. Two of these compounds, (Z)-3-hexenyl acetate and (Z)-3-hexen-1-ol, are attractive to foraging WSS females, as is the terpenoid (E)- $\beta$ -ocimene [24]. The parental lines Reeder (favorable) and Conan (unfavorable) have been shown to differ for the emission of (Z)-3-hexenyl acetate, with the unfavorable line being less attractive and releasing a smaller amount of the attractive compound [25]. As pointed out by Weaver et al. [25], quantitative variation in the release of this chemical signal might explain differences in attraction between these two parental lines. In these experiments, differences in attraction were observed between all the *Qwss.msub-4A.1* NIL pairs derived from the Scholar/Conan cross but not from the one NIL pair derived from Reeder/Conan (Table 3). This indicates that genes other than *Qwss.msub-4A.1* may also play a role in host plant attractiveness to foraging females. It is important to notice that bioassays were not designed to quantify the emission of individual volatile compounds. Thus, it is unclear whether or not NILs differed for the emission of (Z)-3-hexenyl acetate, (Z)-3-hexen-1-ol, (E)- $\beta$ -ocimene or other unknown chemical signal.

*Qss.msub-3BL* and *Qwss.msub-2D*:  
QTLs for Oviposition Preference

While in contact with the host plant, female WSS assess external information about the plant by walking on leaves and stems [23], but none of the QTLs affected this stage of the host selection process. Internal information about the host plant is assessed by repeatedly inserting and withdrawing the saw-like ovipositor inside the stems. Similar behavioral displays were also reported for *Cephus pygmaeus* (Hymenoptera: Cephidae)

[42] and for the gall-inducing sawfly, *Euura lasiolepis* (Hymenoptera: Tenthredinidae) [43]. As reported for *E. lasiolepis*, WSS females inserted their ovipositor more often in preferred host plants, yet ovipositor insertions did not necessarily result in egg deposition (Table 2). Thus, plant cues, perhaps including puncture resistance at the stem wall and solidity of the stem, assessed in this step of the host selection process appear to be important determinants of host acceptance. All three QTLs studied here affected probing behavior, so multiple plant cues are evaluated by WSS females in this specific step of the selection process. Once females examined internal characteristics of the host stem, allelic differences in the *Qws.msub-4A1* locus did not affect the decision of accepting or rejection a host. However, allelic variations at *Qwss.msub-2D* and *Qss.msub.3BL* influenced female choices for oviposition made when probing (Table 1).

The *Qss.msub-3BL* affected ovipositor insertions and egg deposition in the stem. The effect of this locus on females host selection process was also confirmed in the field, under natural WSS infestation conditions for Scholar/Conan NILs (Table 4). Low levels of natural WSS infestation during the study years may have precluded the observation of QTL effect for other populations of NILs. Infestation data from Sherman et al. [7] and Talbert et al. [32] which suggested the effect of 3B, 4A [7], [32] and 2D [7] QTLs on Reeder/Conan and Scholar/Conan RILs were higher than the ones observed in this study. Previous studies have shown that lines containing the favorable allele from Reeder have hollow stems [7], while lines containing the *Qss.msub-3BLc* or *Qss.msub-3BLb* alleles have intermediate levels of stem solidness [32]. Despite the similarities in the degree of stem solidness conferred by the Conan and Scholar alleles, the *Qss.msub-3BLc* allele

causes higher levels of larval mortality [32]. Interestingly, NILs with the *Qss.msub-3BLc* allele were also significantly less preferred than NILs containing either the *Qss.msub-3BLa* or the *Qss.msub-3BLb* alleles. Taken together, these results suggest that WSS host selection appear to be in accordance with the preference-performance hypothesis, which postulates that females preference for oviposition are adapted to optimize progeny performance [22], [40]. Furthermore, *Qss.msub-3BL* seems to determine the proximate mechanistic explanations for both larval mortality and the ability of females to detect suitable stems for oviposition. Ultimately, it appears that these decisions are linked to the overall favorability of the host because multiple females following the behavioral sequence outlined in [23] can select the same stem even if it is already infested with WSS eggs or feeding larvae. However, this experiment evaluated oviposition decisions among stems from a single favorable line of wheat. Subsequent oviposition events have increased internecine risk for the offspring, but assessment of host suitability is decisive, with previous infestation having no influence on the outcome.

An issue that may have affected WSS reaction to the NIL pairs was background alleles at the other QTLs. In one case, females exposed to the *Qwss.msub-2D* NIL pair RC-2 (Table 1) displayed a different behavioral response than those exposed to other *Qwss.msub-2D* NIL pairs. The pair RC-2-*Qwss.msub-2D* had their stems intensively probed by females, but only a single egg was laid (Table 1). This NIL pair had a *Qwss.msub-4A.1a* allele at the 4A locus, which would have made both plants attractive to females; but they also had a *Qss.msub-3BLc* allele at the 3B locus, which would have made both plants less preferred for oviposition. In general, NIL pairs that had the allele

derived from the parental line Conan at the *Qss.msub-3BL* (e.g. RC-2-*Qwss.msub-2D*, SC-1-*Qwss.msub-4A.1*.) (Table 1) tended to have very few eggs. This might have precluded detection of differences between NILs with different alleles at either *Qwss.msub-2D* or *Qwss.msub-4A.1*. These observations suggest that plant cues might be processed by females synergistically, with the effect of different cues combining to determine host suitability for oviposition.

### Conclusions

This study provides repeated evidence that host discrimination by phytophagous insects can occur in a fine scale, with ovipositing females being able to discriminate variation in plant cues resulting from allelic variations within individual plant QTLs. In the case of WSS, maternal choices during oviposition appear to reflect adaptation to optimize progeny performance and survival. This study represents a step toward understanding the genetic mechanisms modulating the complex process of host selection by WSS. From a practical perspective, QTLs shown to affect WSS oviposition preference and host attraction may provide a complement to the current use of solid stems as an antibiosis mechanism to improve pest management in wheat fields.

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Table 2.1. Summary statistics of WSS eggs and ovipositor insertion on wheat stems of near isogenic lines.

| Plant                            | Allele                         | Number of Eggs |      |          |         | Number of Insertions |      |          |         |
|----------------------------------|--------------------------------|----------------|------|----------|---------|----------------------|------|----------|---------|
|                                  |                                | Obs            | Exp  | $\chi^2$ | P value | Obs                  | Exp  | $\chi^2$ | P value |
| Parental lines                   |                                |                |      |          |         |                      |      |          |         |
| Reeder                           |                                | 24             | 13.5 | 16.333   | < 0.001 | 25                   | 18.0 | 5.444    | 0.020   |
| Conan                            |                                | 3              | 13.5 |          |         | 11                   | 18.0 |          |         |
| Scholar                          |                                | 5              | 2.5  | 5.000    | 0.025   | 16                   | 10.0 | 7.200    | 0.007   |
| Conan                            |                                | 0              | 2.5  |          |         | 4                    | 10.0 |          |         |
| NIL                              |                                |                |      |          |         |                      |      |          |         |
| Pair <sup>1</sup>                | 2D QTL ( <i>Qwss.msub-2D</i> ) |                |      |          |         |                      |      |          |         |
| RC-1                             | <i>Qwss.msub-2Da</i>           | 31             | 22.0 | 7.364    | 0.007   | 37                   | 32.5 | 1.246    | 0.264   |
|                                  | <i>Qwss.msub-2Db</i>           | 13             | 22.0 |          |         | 28                   | 32.5 |          |         |
| RC-2                             | <i>Qwss.msub-2Da</i>           | 0              | 0.5  | 1.000    | 0.317   | 28                   | 19.5 | 7.410    | 0.006   |
|                                  | <i>Qwss.msub-2Db</i>           | 1              | 0.5  |          |         | 11                   | 19.5 |          |         |
| RC-3                             | <i>Qwss.msub-2Da</i>           | 11             | 5.5  | 11.000   | < 0.001 | 22                   | 18.5 | 1.324    | 0.250   |
|                                  | <i>Qwss.msub-2Db</i>           | 0              | 5.5  |          |         | 15                   | 18.5 |          |         |
| 3B QTL ( <i>Qss.msub-3BL</i> )   |                                |                |      |          |         |                      |      |          |         |
| RC-1                             | <i>Qss.msub-3BLa</i>           | 16             | 10.5 | 5.762    | 0.016   | 20                   | 13.0 | 7.538    | 0.006   |
|                                  | <i>Qss.msub-3BLc</i>           | 5              | 10.5 |          |         | 6                    | 13.0 |          |         |
| SC-1                             | <i>Qss.msub-3BLb</i>           | 3              | 4.5  | 1.000    | 0.317   | 6                    | 8.5  | 1.471    | 0.225   |
|                                  | <i>Qss.msub-3BLc</i>           | 6              | 4.5  |          |         | 11                   | 8.5  |          |         |
| SC-2                             | <i>Qss.msub-3BLb</i>           | 21             | 11.5 | 15.695   | < 0.001 | 57                   | 30.5 | 46.05    | < 0.001 |
|                                  | <i>Qss.msub-3BLc</i>           | 2              | 11.5 |          |         | 4                    | 30.5 |          |         |
| SC-3                             | <i>Qss.msub-3BLb</i>           | 70             | 46.5 | 23.753   | < 0.001 | 42                   | 31.5 | 7.000    | 0.008   |
|                                  | <i>Qss.msub-3BLc</i>           | 23             | 46.5 |          |         | 21                   | 31.5 |          |         |
| 4A QTL ( <i>Qwss.msub-4A.1</i> ) |                                |                |      |          |         |                      |      |          |         |
| RC-1                             | <i>Qwss.msub-4A.1a</i>         | 0              | 0.5  | 1.000    | 0.317   | 11                   | 11.0 | 0        | 1.000   |
|                                  | <i>Qwss.msub-4A.1b</i>         | 1              | 0.5  |          |         | 11                   | 11.0 |          |         |
| SC-1                             | <i>Qwss.msub-4A.1a</i>         | 2              | 1.0  | 2.000    | 0.157   | 14                   | 8.0  | 9.00     | 0.003   |
|                                  | <i>Qwss.msub-4A.1b</i>         | 0              | 1.0  |          |         | 2                    | 8.0  |          |         |
| SC-2                             | <i>Qwss.msub-4A.1a</i>         | 19             | 23.0 | 1.391    | 0.238   | 26                   | 17.5 | 8.257    | 0.004   |
|                                  | <i>Qwss.msub-4A.1b</i>         | 27             | 23.0 |          |         | 9                    | 17.5 |          |         |
| SC-3                             | <i>Qwss.msub-4A.1a</i>         | 21             | 11.5 | 15.695   | < 0.001 | 57                   | 30.5 | 46.05    | < 0.001 |
|                                  | <i>Qwss.msub-4A.1b</i>         | 2              | 11.5 |          |         | 4                    | 30.5 |          |         |

<sup>1</sup> RC: source of NIL pair is Reeder/Conan, SC: source of NIL pair is Scholar/Conan;  
Obs: Observed value, Exp: Expected value.

Table 2.2. Summary statistics of WSS ovipositor insertion on wheat main stems where no egg was found.

| Plant                            | Allele                 | Number of insertions that did not yield eggs |      |          |                |
|----------------------------------|------------------------|----------------------------------------------|------|----------|----------------|
|                                  |                        | Obs                                          | Exp  | $\chi^2$ | P value        |
| Parental lines                   |                        |                                              |      |          |                |
| Reeder                           |                        | 2                                            | 9.5  | 11.842   | < <b>0.001</b> |
| Conan                            |                        | 17                                           | 9.5  |          |                |
| Scholar                          |                        | 2                                            | 5.0  | 3.600    | <b>0.057</b>   |
| Conan                            |                        | 8                                            | 5.0  |          |                |
| NIL Pair <sup>1</sup>            |                        | 2D QTL ( <i>Qwss.msub-2D</i> )               |      |          |                |
| RC-1                             | <i>Qwss.msub-2Da</i>   | 0                                            | 3.0  | 6.000    | <b>0.014</b>   |
|                                  | <i>Qwss.msub-2Db</i>   | 6                                            | 3.0  |          |                |
| RC-2                             | <i>Qwss.msub-2Da</i>   | 5                                            | 14.0 | 11.571   | < <b>0.001</b> |
|                                  | <i>Qwss.msub-2Db</i>   | 23                                           | 14.0 |          |                |
| 3B QTL ( <i>Qss.msub-3BL</i> )   |                        |                                              |      |          |                |
| RC-1                             | <i>Qss.msub-3BLa</i>   | 5                                            | 6.0  | 0.333    | 0.564          |
|                                  | <i>Qss.msub-3BLc</i>   | 7                                            | 6.0  |          |                |
| SC-3                             | <i>Qss.msub-3BLb</i>   | 1                                            | 9.5  | 15.211   | < <b>0.001</b> |
|                                  | <i>Qss.msub-3BLc</i>   | 18                                           | 9.5  |          |                |
| 4A QTL ( <i>Qwss.msub-4A.1</i> ) |                        |                                              |      |          |                |
| RC-1                             | <i>Qwss.msub-4A.1a</i> | 11                                           | 12.5 | 0.360    | 0.548          |
|                                  | <i>Qwss.msub-4A.1b</i> | 14                                           | 12.5 |          |                |
| SC-2                             | <i>Qwss.msub-4A.1a</i> | 1                                            | 4.0  | 4.500    | <b>0.034</b>   |
|                                  | <i>Qwss.msub-4A.1b</i> | 7                                            | 4.0  |          |                |

<sup>1</sup> RC: source of NIL pair is Reeder/Conan, SC: source of NIL pair is Scholar/Conan; Obs: Observed value, Exp: Expected value. Bioassays were conducted in 2014 using parental lines and near isogenic lines polymorphic for different resistance quantitative trait loci.

Table 2.3. Responses of female WSS to volatiles released by wheat near-isogenic lines in a Y-tube olfactometer.

| Plant                            | Allele                 | Behavioral Responses           |      |          |              |
|----------------------------------|------------------------|--------------------------------|------|----------|--------------|
|                                  |                        | Obs                            | Exp  | $\chi^2$ | P value      |
| Parental Lines                   |                        |                                |      |          |              |
| Reeder                           |                        | 28                             | 21.5 | 3.930    | <b>0.047</b> |
| Conan                            |                        | 15                             | 21.5 |          |              |
| Scholar                          |                        | 15                             | 17.5 | 0.714    | 0.398        |
| Conan                            |                        | 20                             | 17.5 |          |              |
| NIL Pair <sup>1</sup>            |                        | 2D QTL ( <i>Qwss.msub-2D</i> ) |      |          |              |
| RC-1                             | <i>Qwss.msub-2Da</i>   | 27                             | 22.5 | 1.800    | 0.179        |
|                                  | <i>Qwss.msub-2Db</i>   | 18                             | 22.5 |          |              |
| RC-2                             | <i>Qwss.msub-2Da</i>   | 16                             | 20   | 1.600    | 0.206        |
|                                  | <i>Qwss.msub-2Db</i>   | 24                             | 20   |          |              |
| 3B QTL ( <i>Qss.msub-3BL</i> )   |                        |                                |      |          |              |
| RC-1                             | <i>Qss.msub-3BLa</i>   | 16                             | 15.5 | 0.032    | 0.179        |
|                                  | <i>Qss.msub-3BLc</i>   | 15                             | 15.5 |          |              |
| SC-3                             | <i>Qss.msub-3BLb</i>   | 27                             | 23.5 | 1.042    | 0.307        |
|                                  | <i>Qss.msub-3BLc</i>   | 20                             | 23.5 |          |              |
| 4A QTL ( <i>Qwss.msub-4A.1</i> ) |                        |                                |      |          |              |
| RC-1                             | <i>Qwss.msub-4A.1a</i> | 22                             | 20   | 0.400    | 0.527        |
|                                  | <i>Qwss.msub-4A.1b</i> | 18                             | 20   |          |              |
| SC-1                             | <i>Qwss.msub-4A.1a</i> | 31                             | 23.5 | 4.787    | <b>0.029</b> |
|                                  | <i>Qwss.msub-4A.1b</i> | 16                             | 23.5 |          |              |
| SC-2                             | <i>Qwss.msub-4A.1a</i> | 35                             | 25   | 8.000    | <b>0.005</b> |
|                                  | <i>Qwss.msub-4A.1b</i> | 15                             | 25   |          |              |

<sup>1</sup> RC: source of NIL pair is Reeder/Conan, SC: source of NIL pair is Scholar/Conan; Obs: Observed value, Exp: Expected value.

Table 2.4. Summary statistics of field infestation of WSS in pairs of near-isogenic lines.

| Pairing Source of NILs                | Allele Type            | Infestation (%)        |
|---------------------------------------|------------------------|------------------------|
| <b>Parental lines</b>                 |                        |                        |
| <b>Reeder</b>                         |                        | 31.18 ± 7.44 <b>A</b>  |
| <b>Scholar</b>                        |                        | 28.93 ± 7.89 <b>A</b>  |
| <b>Conan</b>                          |                        | 13.02 ± 3.56 <b>B</b>  |
| <b>Choteau</b>                        |                        | 11.62 ± 4.15 <b>B</b>  |
| <b>2D QTL (<i>Qwss.msub-2D</i>)</b>   |                        |                        |
| <b>Reeder/Conan</b>                   | <i>Qwss.msub-2Db</i>   | 16.96 ± 2.40 <b>A</b>  |
|                                       | <i>Qwss.msub-2Da</i>   | 17.34 ± 2.19 <b>A</b>  |
| <b>3B QTL (<i>Qss.msub-3BL</i>)</b>   |                        |                        |
| <b>Reeder/Conan</b>                   | <i>Qss.msub-3BLc</i>   | 18.25 ± 3.19 <b>A</b>  |
|                                       | <i>Qss.msub-3BLa</i>   | 17.94 ± 4.208 <b>A</b> |
| <b>Scholar/Conan</b>                  | <i>Qss.msub-3BLc</i>   | 18.23 ± 2.10 <b>A</b>  |
|                                       | <i>Qss.msub-3BLb</i>   | 24.08 ± 2.46 <b>B</b>  |
| <b>Choteau/Conan</b>                  | <i>Qss.msub-3BLc</i>   | 19.39 ± 2.74 <b>A</b>  |
|                                       | <i>Qss.msub-3BLb</i>   | 18.71 ± 3.09 <b>A</b>  |
| <b>4A QTL (<i>Qwss.msub-4A.1</i>)</b> |                        |                        |
| <b>Reeder/Conan</b>                   | <i>Qwss.msub-4A.1b</i> | 19.43 ± 4.21 <b>A</b>  |
|                                       | <i>Qwss.msub-4A.1a</i> | 18.63 ± 6.76 <b>A</b>  |
| <b>Scholar/Conan</b>                  | <i>Qwss.msub-4A.1b</i> | 16.07 ± 2.46 <b>A</b>  |
|                                       | <i>Qwss.msub-4A.1a</i> | 14.78 ± 1.94 <b>A</b>  |

Mean values with different letters within a pairing source denote significant differences according to t-test (LSD) at  $P < 0.05$ . <sup>1</sup> RC: source of NIL pair is Reeder/Conan, SC: source of NIL pair is Scholar/Conan. Mean averaged over four environments, Loma 2013, Loma 2014, Amsterdam 2014, and Amsterdam 2015.

Figure 2.1. Wheat stem sawfly preference for oviposition on near isogenic lines polymorphic for resistance QTLs. Resistance QTLs are located on chromosomes 3B, 2D, and 4A. Number of eggs are combined over all pairs of NILs tested.\*\*\*significant differences ( $P < 0.001$ ) based on chi-squared test analysis. Favorable alleles are defined as those that lead to increased oviposition by the wheat stem sawfly (*Qwss.msub-2Da*, *Qss.msub-3BLa* and *Qss.msub-3BLb*, and *Qwss.msub-4A.1a*, respectively), while unfavorable alleles are defined as those that lead to decreased oviposition (*Qwss.msub-2Db*, *Qss.msub-3BLc*, and *Qwss.msub-4A.1b*, respectively).

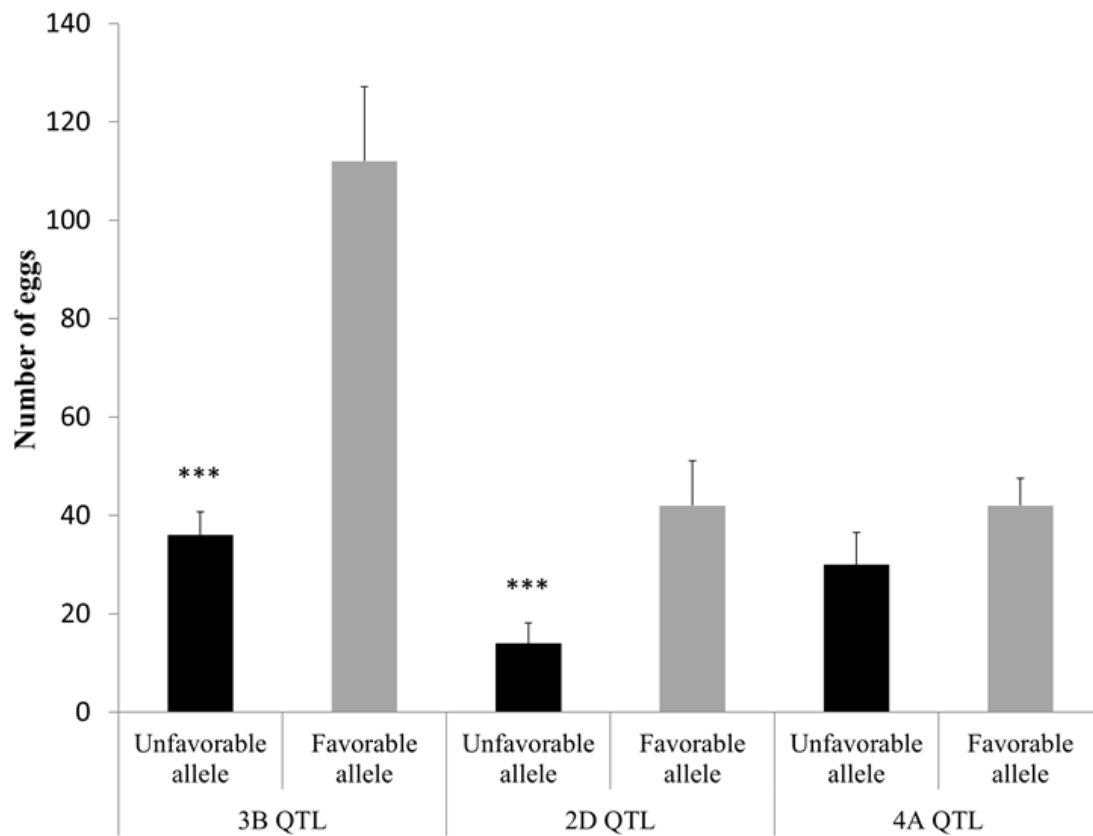
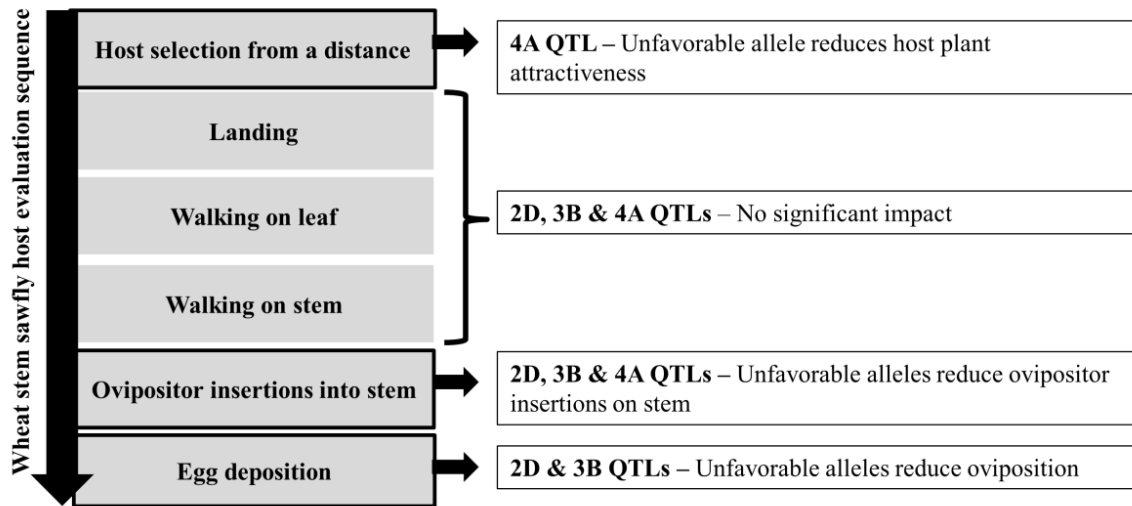


Figure 2.2. Wheat QTLs associated with host selection and oviposition behavior by the wheat stem sawfly. Favorable alleles are defined as those that lead to increased oviposition by the wheat stem sawfly (*Qwss.msub-2Da*, *Qss.msub-3BLa* and *Qss.msub-3BLb*, and *Qwss.msub-4A.1a*, respectively), while unfavorable alleles are defined as those that lead to decreased oviposition ((*Qwss.msub-2Db*, *Qss.msub-3BLc*, and *Qwss.msub-4A.1b*, respectively).



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## CHAPTER THREE

TEMPORAL PATTERNS OF PITH EXPRESSION AND RETRACTION IN WHEAT  
STEMS AND ITS EFFECT ON RESISTANCE TO THE WHEAT STEM SAWFLYContribution of Authors and Co-AuthorsManuscript in Chapter 3

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Contribution: Performed cage trials to assess insect preference for oviposition and reviewed the manuscript.

Co-Author: Luther E. Talbert

Contribution: Conceived and designed the experiments, developed near-isogenic lines for experimentation, provided critical input at all stages of experiment including interpretation of results and manuscript preparation.

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Manuscript Information Page

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## CHAPTER THREE

## TEMPORAL PATTERNS OF PITH EXPRESSION AND RETRACTION IN WHEAT STEMS AND ITS EFFECT ON RESISTANCE TO THE WHEAT STEM SAWFLY

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Abstract

Structural and temporal variations in pith expression in wheat stems may affect levels of wheat stem sawfly (WSS) resistance and influence efficiency of pest control; however little is known about temporal patterns of stem solidness expression in wheat. A study has been carried out using spring wheat genotypes that differed for the degree of stem solidness. Results showed that in one genotype, Conan, the solid pith undergoes rapid retraction during the stem maturation period, resulting in significantly less solidness at maturity. In other solid-stemmed genotypes including the standard WSS-resistant cultivar Choteau, the amount of pith in the stem remains mostly unchanged throughout plant's development. Conan plants also showed increased degree of early solidness, pith moisture, and stem density. In cage trials, Conan plants were shown to be less preferred for oviposition by the WSS when compared to Choteau. Much of the variation for stem solidness and resistance was associated with the *Qss.msub-3BL* locus for solid stems. Bioassays using recombinant inbred lines and near-isogenic lines polymorphic for

*Qss.msub-3BL* showed that the Conan allele provides high levels of early stem solidness and rapid pith retraction during stem maturation. Genotypes carrying the Conan allele at *Qss.msub-3BL* showed increased WSS neonatal mortality in field trials. Collectively, results provided evidence for allele-specific variation in temporal patterns of solidness expression and WSS resistance. Phenotypic selection should consider temporal variations in pith expression for best results. Molecular markers linked to *Qss.msub-3BL* allow differentiation of alleles at *Qss.msub-3BL* and may help breeders improve selection for early solidness expression.

### Introduction

Most species of the genus *Triticum* have hollow stems, except for a few tetraploid and hexaploid genotypes in which the stem is filled with a solid pith (Bozzini and Avanzi 1962). The potential of solid stems to reduce damage caused by the wheat stem sawfly (WSS), *Cephus cinctus* Norton, may be expected based on the biology of the WSS. Female adults lay eggs in wheat stems during early summer and larvae feed inside the stem until the end of the growing season, when they cut the base of the plant in preparation for overwintering diapause. The solid pith acts as a physical barrier reducing egg deposition, larval movement, and survival (Holmes and Peterson 1962; Wallace and McNeal 1966), while excessive pith moisture in solid-stemmed genotypes reduces egg hatch (Holmes and Peterson 1964). Since its introduction into North American wheat germplasm (Platt et al. 1948), the solid stem trait has become the most effective control mechanism used to manage damage caused by WSS (Beres et al. 2011).

Variation in stem solidness in wheat is controlled by a major locus on chromosome 3B, *Qss.msub-3BL* (synonymously *Sst1*) (Cook et al. 2004; Houshmand et al. 2007; Sherman et al. 2010; Kalous et al. 2011; Varella et al. 2015), and other minor loci scattered throughout the genome (Larson and MacDonald 1962; Lanning et al. 2006; Varella et al. 2015). Solidness can also be adversely affected by environmental factors such as light intensity, photoperiod, humidity, and air temperature (Platt et al. 1941; Holmes et al. 1959). Pith expression inconsistency has been one of the major challenges in breeding for stem solidness. Other challenges associated with WSS resistance breeding involve the low yield potential of solid-stemmed genotypes (McNeal et al. 1965; Weiss & Morrill 1992), and the necessity of screening nurseries at WSS-infested sites to select for resistance mechanisms that are not associated with a morphological trait. Genetic studies have been a great asset in overcoming these challenges. Quantitative trait loci (QTLs) for larval mortality, decreased host plant attractiveness, and reduced stem cutting have been identified and allow for marker-assisted selection (MAS) (Sherman et al. 2010; Varella et al. 2015). Poor yield potential of some current solid-stemmed cultivars has been determined to be due to poor genetic backgrounds instead of an effect of *Qss.msub-3BL* (Hayat et al. 1995; Cook et al. 2004; Lanning et al. 2006; Sherman et al. 2015). Nevertheless, despite genetic advances, WSS resistance breeding is still highly dependent on the ability to select solid-stemmed genotypes.

The expression of stem solidness in wheat has both structural and temporal components. The structural component describes the amount of pith filling the stem, while the temporal component refers to variation in pith filling over time. Variation in

either of these components affects levels of resistance and influence efficiency of pest control (O'Keeffe et al. 1960; Varella et al. 2015). Often, the degree of stem solidness is used as a measure of resistance to the WSS (McNeal et al. 1959) and little attention is given to temporal variations in solidness expression. However, timing is a key element in plant-insect interactions. In particular, wheat plants are most vulnerable to WSS infestation during stem elongation and booting stages, when adult females are searching for a host plant to lay eggs (Buteler et al. 2009). For this reason, genotypes expressing high degrees of stem solidness earlier in the season are less vulnerable to WSS infestation than those expressing solidness at maturity (Varella et al. 2015).

Contrary to expectations, solid-stemmed genotypes from North America do not all share the *Qss.msub-3BL* allele derived from S-615, the Portuguese landrace that is the source of stem solidness in many of North American wheat genotypes (Talbert et al. 2014; Varella et al. 2015). The spring wheat cultivar 'Conan' (PI607549) (WestBred, LLC) is one such genotype. The Conan allele at *Qss.msub-3BL* provides an intermediate degree of stem solidness, yet causes high levels of WSS resistance (Talbert et al. 2014). The fact that distinct solid stem alleles providing similar degrees of solidness can cause different levels of WSS resistance reinforces the argument that phenotypic selection based solely on the degree of stem solidness is not sufficient to select the most resistant genotypes. The success of phenotypic selection lies on the ability to identify traits that are indicative of intended performance (Fiorani and Schurr 2013). In the case of stem solidness, both the structural and temporal components of the trait determine levels of resistance (Varella et al. 2015). Thus, a better understanding of temporal variations in

pith expression might improve our ability to select for solid-stemmed genotypes that better control WSS infestations.

In the present study, a comparative characterization of stem solidness in different spring wheat genotypes allowed for the identification of distinct patterns of early stem solidness expression and pith retraction during stem elongation and maturation. Sets of recombinant inbred lines (RILs) and near-isogenic lines (NILs) were then used to determine if previously identified QTL for resistance controlled variations in early stem solidness and its effect on plant resistance to the WSS. Implications for phenotypic and marker-assisted selection in wheat breeding for WSS resistance are discussed.

## Material & Methods

### Plant Material

Bioassays were conducted using spring wheat genotypes known to differ for stem solidness at maturity. These included solid-stemmed ‘Choteau’ (PI633974), semi-solid-stemmed Conan and ‘Scholar’ (PI 607557), and hollow-stemmed ‘Reeder’ (PI613586), as well as populations of RILs and NILs. Three populations of F<sub>5</sub>-derived RILs were developed by single-seed descent beginning with F<sub>2</sub> seeds from crosses between Reeder/Conan, Scholar /Conan, and Choteau /Conan. Previous results have shown that three alleles at *Qss.msub-3BL* with different effects on WSS resistance exist among these lines, including an allele shared by Scholar and Choteau, a distinct allele in Conan, and an allele for hollow stems in Reeder (Cook et al. 2004; Talbert et al. 2014). The RIL populations were used to derive NILs by selecting heterozygous individuals in the F<sub>5</sub>

generation on the procedure of Pumphrey et al. (2007) as modified by Blake et al. (2011) and Varella et al. (submitted). Polymerase chain reaction was conducted using microsatellite primer sets including GWM340 linked to *Qss.msub-3BL* (Cook et al. 2004) to identify lines that were homozygous for different alleles at *Qss.msub-3BL*. This procedure allowed for the identification of 12 pairs of NILs for *Qss.msub-3BL*.

#### Comparative Characterization of Stem Solidness under Controlled Conditions

Bioassays assessing morphological variations associated with stem solidness during stem maturation were performed in a greenhouse at the Montana State University Plant Growth Center (MSU-PGC, Bozeman, MT, USA) in the summers of 2008, 2011, and 2014. The wheat cultivars tested were Choteau, Conan, and Reeder. Forty-eight square pots (13 by 13 by 13.5 cm) of each plant genotype were planted with 3 plants per pot in a staggered planting regime under natural and artificial light (GE Multivapor Lamps model MVR1000/C/U, GE Lighting, General Electric Company, Cleveland, OH), photoperiod of 14:10 (L:D) h,  $22 \pm 2^{\circ}\text{C}$ , and 20 - 40% RH. Plants were watered regularly and fertilized twice each week with Peters General Purpose Fertilizer (J.R. Peters, Allentown, PA) at 100 ppm in aqueous solution. Soil consisted of equal parts of MSU-PGC soil mix (equal parts of sterilized Bozeman silt loam soil and washed concrete sand with Canadian sphagnum peat moss incorporated) and Sunshine Mix 1 (Canadian sphagnum peat moss, perlite, vermiculite, and Dolmitic lime; Sun Gro Horticulture, Bellevue, WA, USA). Morphological evaluations started approximately 35 days after planting and were done once a week for four consecutive weeks. In total, twelve pots of

each genotype were sampled for each evaluation date every year. Evaluations consisted of individually collecting plant main stems and measuring height, diameter, solidness score, and weight of each internode. Internodes were rated for stem solidness using a 1 (completely hollow) to 5 (completely solid) scale, as described in Varella et al. (2015). To give a final score of stem solidness, the ratings of the five internodes were summed to provide scores between 5 (hollow) and 25 (solid) for each stem (Sherman et al. 2010). In 2014, after each evaluation, stems were placed inside a drying room for seven days after which dry weight measurements were taken. Values of internode height and diameter were used to calculate the volume of each internode, which was then summed to obtain the total volume of a wheat stem. Stem density was calculated as dry weight over wet volume, while pith moisture was calculated as fresh weight minus dry weight over wet volume. In 2008 and 2011, dry weight measurements were not collected.

#### Assessing the Genetic Basis Underlying Variations in Early Stem Solidness Expression and Pith Retraction

To test whether allelic variation at *Qss.msub-3BL* influenced patterns of early stem solidness and pith retraction during stem maturation, NILs were planted in late April 2013 and 2014 near Bozeman, MT, USA, at the Arthur H. Post Research Farm (45°41'N, 111°00'W). Experiments were conducted using a randomized complete block design with three replications. Each block included 12 NIL pairs along with parental lines. The entries were planted in single-row 3m plots at a seeding rate of 8 to 10 g per plot. To assess early stem solidness, the main stems of three plants of each plot were individually collected in five collection dates, starting 35 days after planting when plants were at

Zadok 31 (one internode detectable) and ending when plants were at Zadok 49 (5 internodes detectable; when the awns of the developing head are first visible). To assess late stem solidness, the main stems of three plants of each plot were individually collected at maturity in late August. For each sampling event, stems were dissected and each internode was rated for stem solidness as described above.

#### Effect of Distinct Patterns of Early Stem Solidness Expression on WSS Resistance

To assess the effect of early stem solidness expression on WSS resistance, two bioassays were conducted. First, cage trials tested the effect of temporal variation in solidness expression on WSS oviposition preference (infestation). For that, two pots containing Conan and Choteau plants were placed inside four screened cages (91.4 by 66.7 by 91.4 cm) with 530  $\mu\text{m}$  mesh openings. Fifteen WSS adult females and five males were released inside each cage and oviposition was allowed for 48 hours, after which pots were removed from cages and stems were dissected to assess the number of eggs laid. To account for temporal variation in pith expression trials were conducted when plants were at Zadok stage 32 (two internodes detectable; approximately 37 days after planting) and again at Zadok stage 49 (when the awns of the developing head are first visible; approximately 44 days after planting) (Zadoks et al. 1974). Experiments were conducted under controlled conditions at the MSU-PGC as described above. The second bioassay tested the effect of early solidness expression on neonate larval mortality. Scholar/Conan RILs were planted in late April 2011 and 2012 at a traditionally WSS-infested site near Amsterdam, MT, USA (45°45'29.85"N, 111°22'49.32"W) and in 2012

at a second site with a history of WSS infestation near Loma, MT, USA (48°04'21.96"N, 110°27'41.84"W). Experiments were established adjacent to stubble from a previous WSS-infested wheat crop in a randomized complete block design with two replications in 2011, and three replications in 2012. Each block included 91 RILs along with parental lines. Plots consisted of 10 seeds per entry planted in individual hills with spacing of 0.8 m between adjacent hills. Stems were collected at maturity and dissected to determine the presence of dead neonate larvae ('early' mortality). A neonate was considered as a larva that had tunneled the length of one internode or less. Data are reported as percent mortality (dead neonate/infested stems). Results from this trial showing different levels of resistance as determined by stem cutting were previously reported (Talbert et al. 2015).

### Statistical Analysis

Temporal variation for pith moisture, stem density, and stem solidness were analyzed using the lme function from the nlme package (Pinheiro et al., 2013) in R (R Development Core Team 2010). Pith moisture and stem density data were averaged over replications for each genotype and were regressed over time. Greenhouse and field data on early stem solidness were averaged over replications and years for each genotype and were regressed over time. Differences among slopes and intercepts were tested by an analysis of covariance (ANCOVA) model, including a classifying variable (genotype or internode), time, and the interaction. To test for differences in pith moisture and stem density within each sampling event, data were analyzed using Proc MIXED in SAS v 9.3 (SAS Institute 2012). Mean values were averaged over replications prior to analysis. Genotypes were used as fixed effect and plant cohorts were used as random effect.

Results from cage trials were analyzed using a chi-square test for small sample sizes (Sokal and Rohlf 1995). Neonatal mortality data was analyzed using mixed model analysis of variance with maximum likelihood estimation using Proc MIXED in SAS. Fixed effects included percentage mortality and allele, while location and blocks were used as random effects.

### Results

The comparative characterization of stem solidness during stem elongation and maturation revealed distinct patterns of early solidness expression and pith retraction among wheat cultivars (Figure 1 and Supplemental Table S1). At 35 d after planting, Conan and Choteau had similar levels of stem solidness and were significantly (ANCOVA intercept,  $P < 0.05$ ) more solid than Reeder (Figure 1a). Over the course of the experiment, Conan stems showed a rapid pith retraction that differed (ANCOVA slope,  $P < 0.05$ ) from that of Reeder or Choteau (Figure 1a). Early solidness expression varied across the stem, with each individual internode showing different amounts of pith (Figure 1b-d). The range of variation for early solidness across the stem was wider for Conan plants (Figure 1c).

Morphological measurements revealed that Conan stems were denser and had higher pith moisture than Reeder stems ( $P < 0.05$ ) during all evaluation dates (Table 1). When compared to Choteau, Conan stems had higher ( $P < 0.05$ ) pith moisture at the initial period of stem elongation. As time progressed, Conan stems lost more moisture than Choteau's, so that at the final evaluation date, stems from both genotypes had similar

levels of pith moisture (Table 1). Also, Conan stems had significantly higher density than Choteau stems in two evaluation dates ( $P < 0.05$ ) (Table 1). The comparison of density and pith moisture of each internode across individual stems showed that internodes four and five contained less moisture and were less dense than other internodes in all three genotypes (Figure 2a-f and Supplemental Table S2 and S3). Internodes two and three of Conan stems contained significantly ( $P < 0.05$ ) more moisture than the same internodes in Choteau and Reeder stems at the beginning of the stem elongation period (Supplemental Table S2).

An additional study confirmed the distinct patterns of early stem solidness expression and pith retraction during stem elongation and maturation under natural field conditions (Figure 3 and Supplementary Table 4). At 35 d after planting, Conan stems showed significantly (ANCOVA intercept,  $P < 0.05$ ) higher solid scores than Choteau, Scholar, and Reeder stems in both 2014 and 2015, and Reeder stems had significantly (ANCOVA intercept,  $P < 0.05$ ) lower solidness scores than all other genotypes (Figure 3a). Conan stems showed a rapid pith retraction that differed (ANCOVA slope,  $P < 0.05$ ) from that of Reeder, Scholar and Choteau (Figure 3a).

The use of NILs allowed determination of the effect of the *Qss.msub-3BL* on early stem solidness expression and pith retraction (Figures 3b-d and Supplementary Table 4). The NILs with the Conan allele at *Qss.msub-3BL* locus showed significantly (ANCOVA intercept,  $P < 0.05$ ) higher early solidness scores when compared to Reeder or Scholar-type NILs (Figures 3c-d and Supplementary Table 4). Also, NILs with the Conan allele at *Qss.msub-3BL* showed rapid pith retraction in Scholar/Conan, Choteau/Conan, and

Reeder/Conan genetic backgrounds (ANCOVA slope,  $P < 0.05$ ) (Figures 3c-d and Supplemental Table S4). Cultivars showed considerable variation for late stem solidness score, with Choteau having the highest solidness mean (18.45), followed by Conan (11.74), Scholar (11.58), and Reeder (7.70). The Conan allele at *Qss.msub-3BL* was shown to confer semi-solidness scores for late stem solidness regardless of the genetic background of the NILs (data not shown).

The distinct patterns of early stem solidness expression were shown to be related to resistance to the WSS. When plants were at Zadok stage 32, the number of eggs laid on Conan main stems and tillers were significantly lower than that laid on Choteau stems ( $P < 0.05$ ) (Table 2). At Zadok stage 49, fewer eggs were laid on stems of either cultivars. No differences were observed in the number of eggs laid in main stems, but females laid significantly more eggs on Choteau tillers than in Conan's ( $P < 0.05$ ) (Table 2). In the field, RILs with the Conan allele at *Qss.msub-3BL* showed higher levels of neonatal mortality when compared with lines containing the Scholar allele at the 3B locus (percent neonate larval mortality: RIL with the Conan allele = 32.0%, RIL with the Scholar allele = 23.31%,  $F = 17.93$ ,  $P < 0.0001$ ). The Conan genotype also showed higher levels of WSS neonatal mortality than Scholar (percent neonate mortality: Conan = 51.67%, Scholar = 11.18%,  $F = 7.38$ ,  $P = 0.02$ ).

### Discussion

Several QTLs for WSS resistance exists in wheat (Cook et al. 2004; Lanning et al. 2006; Sherman et al. 2010; Joukhadar et al. 2013; Varella et al. 2015). A single locus,

*Qss.msub-3BL*, has the strongest impact on stem solidness and is most often targeted for selection in breeding programs in WSS-infested regions (Beres et al. 2011). Solid stem alleles confer different levels of resistance depending on the degree and timing of pith expression (Varella et al. 2015); however, little is known about temporal variation in solidness expression in wheat. In the present study, wheat genotypes differing for alleles at *Qss.msub-3BL* were used to characterize temporal patterns of solidness expression and pith retraction and its effect on WSS resistance.

Two patterns of temporal variation in solidness expression were identified. In genotypes carrying the solid stem allele at *Qss.msub-3BL* derived from S-615 (e.g. Choteau and Scholar), the amount of pith filling the stem remained mostly unchanged throughout plant's development. Conversely, in Conan, which has a distinct solid stem allele as indicated by markers at *Qss.msub-3BL* (Talbert et al. 2014), pith expression was characterized by high levels of early stem solidness followed by a rapid rate of pith retraction during stem maturation. A possible explanation for pith retraction is pith autolysis, a physiological phenomenon in which parenchymal cells from a solid stem are eliminated by autolysis creating a longitudinal cavity (hollowness) inside the stem (Farage-Barhom et al. 2008). One of the consequences of pith retraction is the expression of intermediate degree of stem solidness at maturity, when phenotypic selection for this trait is traditionally conducted. The effect of distinct alleles at *Qss.msub-3BL* on temporal patterns of early solidness expression and pith retraction was confirmed using NILs. The effect of the Conan allele at *Qss.msub-3BL* was consistent in all genetic backgrounds tested and under different environmental conditions.

The high scores of early solidness expressed in Conan plants were accompanied by increased pith moisture and stem density. Moisture content of plant tissue impacts the use of the tissue by insects. High tissue moisture inhibits WSS egg-hatching (Holmes and Peterson 1964), while low tissue moisture leads to larval weight loss, desiccation, and death (Holmes 1960). Conan stems have high pith moisture early in the season during WSS oviposition period, especially in internodes two and three, which are most frequently chosen for oviposition by the WSS (Holmes and Peterson 1960). Conversely, later in the season when larvae might be mining the stems, Conan plants have low levels of pith moisture. Thus, temporal variation in pith moisture in Conan stems might allow for increased immature mortality.

Two sets of bioassays tested the effect of early solidness expression on WSS resistance. In cage trials, females showed an increased preference for egg laying in Choteau stems, especially during stem elongation, when Choteau stems were less solid than Conan's. Although stem solidness is an important determinant of host plant selection by the WSS, host selection is actually a complex process that involves several other plant cues, such as stem length and diameter, plant volatiles, and plant growth stage (Holmes and Peterson 1960; Piesik et al. 2008; Buteler et al. 2009; Weaver et al. 2009). It has been hypothesized that plant cues are processed by WSS females synergistically, with the effect of different cues being combined to determine host suitability for oviposition (Varella et al. 2016 -submitted). Thus, female choices observed in cage trials might have been influenced by a combination of cues rather than being determined exclusively by differences in stem solidness. Nevertheless, cage trials using NILs for *Qss.msub-3BL*

derived from a Scholar/Conan cross have shown that WSS females indeed prefer to oviposit in Scholar-type NILs (Scholar and Choteau share the same solid stem allele) rather than in Conan-type NILs (Varella et al. 2016 - submitted).

Experiments assessing temporal variation of solidness expression and its effect on WSS mortality had to be conducted in different field locations because it is not possible to accurately measure solidness in developing stems when WSS larval tunneling is ongoing. Genotypes carrying the Conan allele at *Qss.msub-3BL* locus showed higher levels of neonatal mortality than those expressing the Choteau-type solid stem allele at *Qss.msub-3BL*. Neonatal mortality is desirable, because it ensures that minimal injury is caused to the stem. Stem-mining injures vascular tissues, and reduces photosynthetic capacity and the transport of nutrients and water to grains (Morril et al. 1992; Macedo et al., 2007; Delaney et al. 2010). The effect of stem solidness includes egg and neonatal mortality. The solid pith is a significant source of larval mortality (Buteler et al. 2015), with genotypes carrying the Conan allele at *Qss.msub-3BL* causing significantly higher in-stem mortality than genotypes expressing the Choteau-type (Talbert et al. 2014).

Taken together, these bioassays show that allelic variation at *Qss.msub-3BL* affects both the degree of early stem solidness and the pattern of pith retraction during stem maturation, with important consequences for WSS resistance. Phenotypic selection of solid-stemmed genotypes conducted prior to anthesis may help breeders account for temporal variation in stem solidness expression. Molecular markers linked to the *Qss.msub-3BL* can be used to complement phenotypic selection of this trait.

### Acknowledgements

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Table 3.1. Mean  $\pm$  SE of stem length, pith moisture, and density of wheat main stems evaluated over four sampling events during stem maturation period.

| <b>Sampling Events<br/>(DAP<sup>a</sup>)</b> | <b>Plant<br/>Genotypes</b> | <b>Pith moisture (g/mm<sup>3</sup>)</b> | <b>Stem Density (g/mm<sup>3</sup>)</b> |
|----------------------------------------------|----------------------------|-----------------------------------------|----------------------------------------|
| 1 (35)                                       | Choteau                    | 1.33E-03 $\pm$ 4.1E-05 <b>B</b>         | 1.46E-04 $\pm$ 1.5E-05 <b>B</b>        |
|                                              | Conan                      | 1.50E-03 $\pm$ 3.8E-05 <b>A</b>         | 1.99E-04 $\pm$ 1.3E-05 <b>A</b>        |
|                                              | Reeder                     | 1.25E-03 $\pm$ 4.5E-05 <b>B</b>         | 1.56E-04 $\pm$ 1.5E-05 <b>B</b>        |
| 2 (42)                                       | Choteau                    | 1.19E-03 $\pm$ 2.6E-05 <b>B</b>         | 2.20E-04 $\pm$ 1.5E-05 <b>A</b>        |
|                                              | Conan                      | 1.47E-03 $\pm$ 3.8E-05 <b>A</b>         | 2.40E-04 $\pm$ 1.1E-05 <b>A</b>        |
|                                              | Reeder                     | 1.06E-03 $\pm$ 4.6E-05 <b>C</b>         | 1.90E-04 $\pm$ 1.1E-05 <b>B</b>        |
| 3 (49)                                       | Choteau                    | 9.83E-04 $\pm$ 4.5E-05 <b>B</b>         | 2.17E-04 $\pm$ 1.20E-05 <b>B</b>       |
|                                              | Conan                      | 1.12E-03 $\pm$ 5.5E-05 <b>A</b>         | 2.58E-04 $\pm$ 1.42E-05 <b>A</b>       |
|                                              | Reeder                     | 8.11E-04 $\pm$ 3.6E-05 <b>C</b>         | 2.12E-04 $\pm$ 0.80E-05 <b>B</b>       |
| 4 (56)                                       | Choteau                    | 8.98E-04 $\pm$ 1.6E-05 <b>A</b>         | 2.95E-04 $\pm$ 1.32E-05 <b>A</b>       |
|                                              | Conan                      | 8.64E-04 $\pm$ 2.9E-05 <b>A</b>         | 2.98E-04 $\pm$ 1.36E-05 <b>A</b>       |
|                                              | Reeder                     | 6.67E-04 $\pm$ 2.3E-05 <b>B</b>         | 2.22E-04 $\pm$ 0.98E-05 <b>B</b>       |

Mean values with different letters within a sampling event denote significant differences according to t-test (LSD) at  $P < 0.05$ .

\*DAP days after planting.

Table 3.2. Chi-square test, observed and expected ratios of wheat stem sawfly oviposition on stems of wheat cultivars differing for temporal patterns of pith expression.

| Plant Stage<br>(DAP*) | Stem<br>Types | Plant Genotypes | Number of Eggs |      |          |          |
|-----------------------|---------------|-----------------|----------------|------|----------|----------|
|                       |               |                 | Obs            | Exp  | $\chi^2$ | P value  |
| Zadok 32 (37)         | Main stem     | Choteau         | 35             | 18.5 | 29.43    | < 0.0001 |
|                       |               | Conan           | 2              | 18.5 |          |          |
|                       | Tillers       | Choteau         | 40             | 20.5 | 37.10    | < 0.0001 |
|                       |               | Conan           | 1              | 20.5 |          |          |
| Zadok 49 (44)         | Main stem     | Choteau         | 7              | 5.5  | 0.82     | 0.3657   |
|                       |               | Conan           | 4              | 5.5  |          |          |
|                       | Tillers       | Choteau         | 9              | 5.0  | 6.40     | 0.0114   |
|                       |               | Conan           | 1              | 5.0  |          |          |

Obs: Observed value, Exp: Expected value. \*DAP days after planting.

Figure 3.1. Linear regression for early stem solidness per internode over time (sampling events) for three wheat genotypes, Choteau, Conan, and Reeder. Data averaged over three years (2008, 2011, 2014). Sampling events started at Zadoks 31 and finish at Zadoks 59. Internodes were numbered from the bottom to the top of the stem. (A) Early solidness per internode for Choteau, Conan, and Reeder stems, (B) Early solidness for each individual internode in Choteau stems, (C) Early solidness for each individual internode in Conan, (D) Early solidness for each individual internode in Reeder. Sampling events are 35 (1), 42 (2), 49 (3), and 56 (4) days after planting.

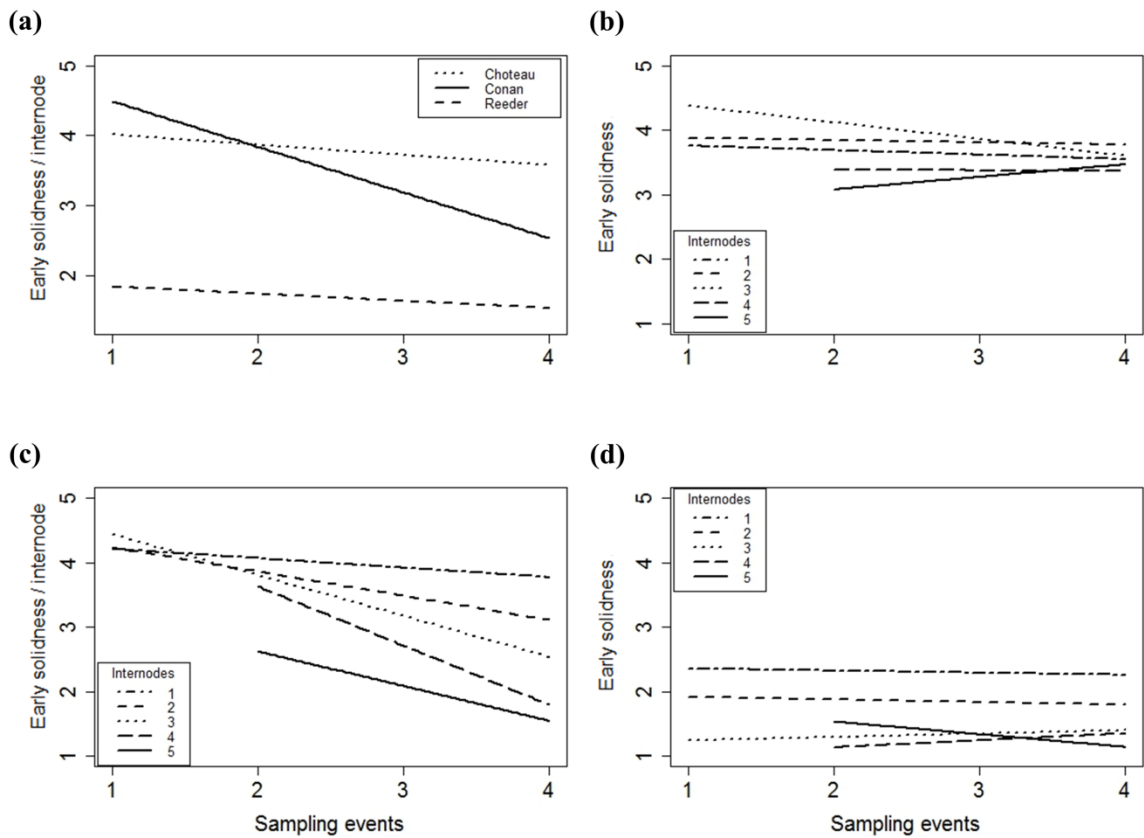


Figure 3.2. Linear regression for pith moisture ( $\text{g}/\text{mm}^3$ ) and stem density ( $\text{g}/\text{mm}^3$ ) per internode over time (sampling events) for three wheat genotypes, Choteau, Conan, and Reeder. Sampling events started at Zadoks 31 and finish at Zadoks 59. Internodes were numbered from the bottom to the top of the stem. Pith moisture for each individual internode in Choteau (A), Conan (B), and Reeder (C) stems. Stem density for each individual internode in Choteau (D), Conan (E), and Reeder (F) stems. Sampling events are 35 (1), 42 (2), 49 (3), and 56 (4) days after planting.

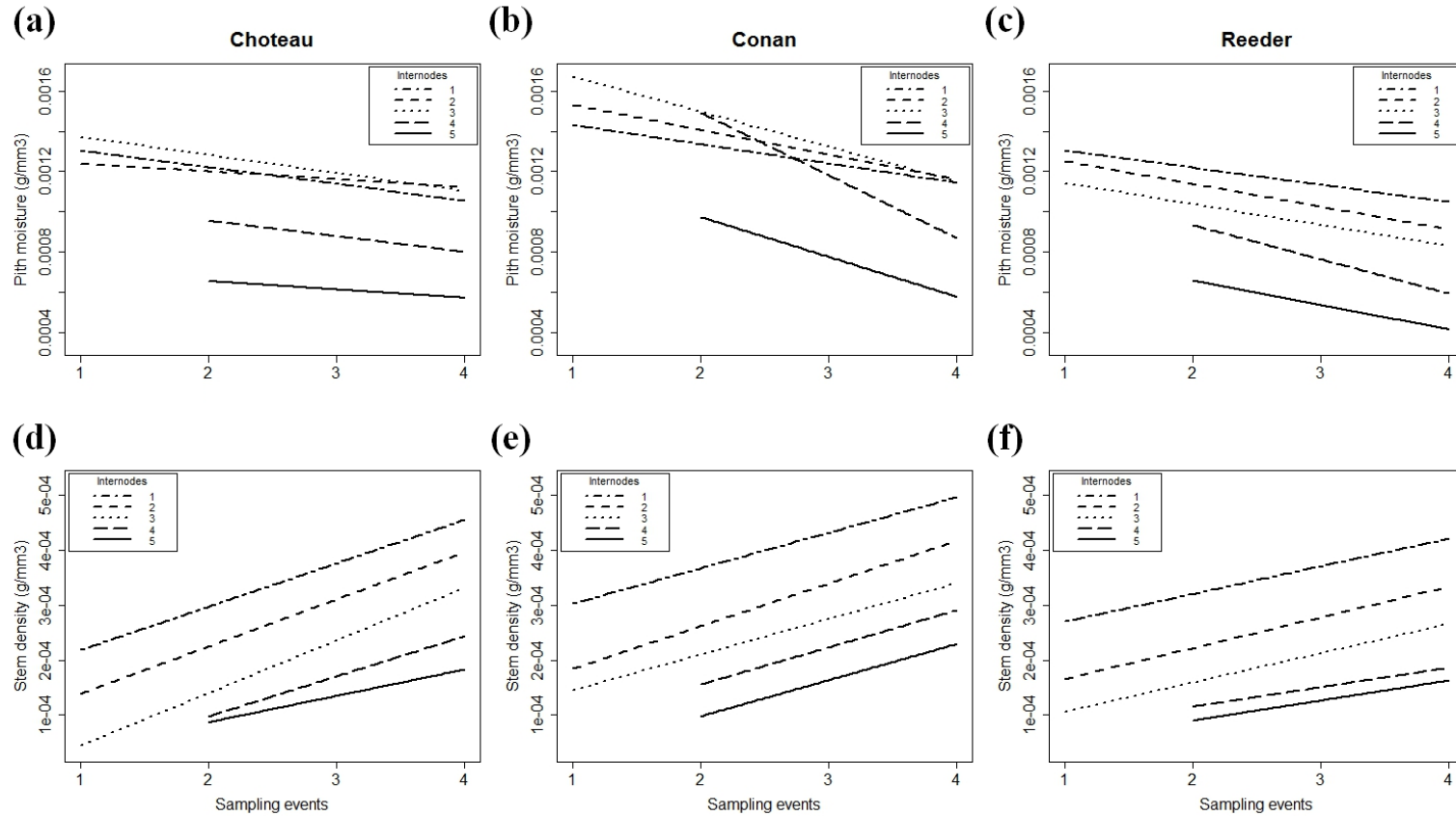
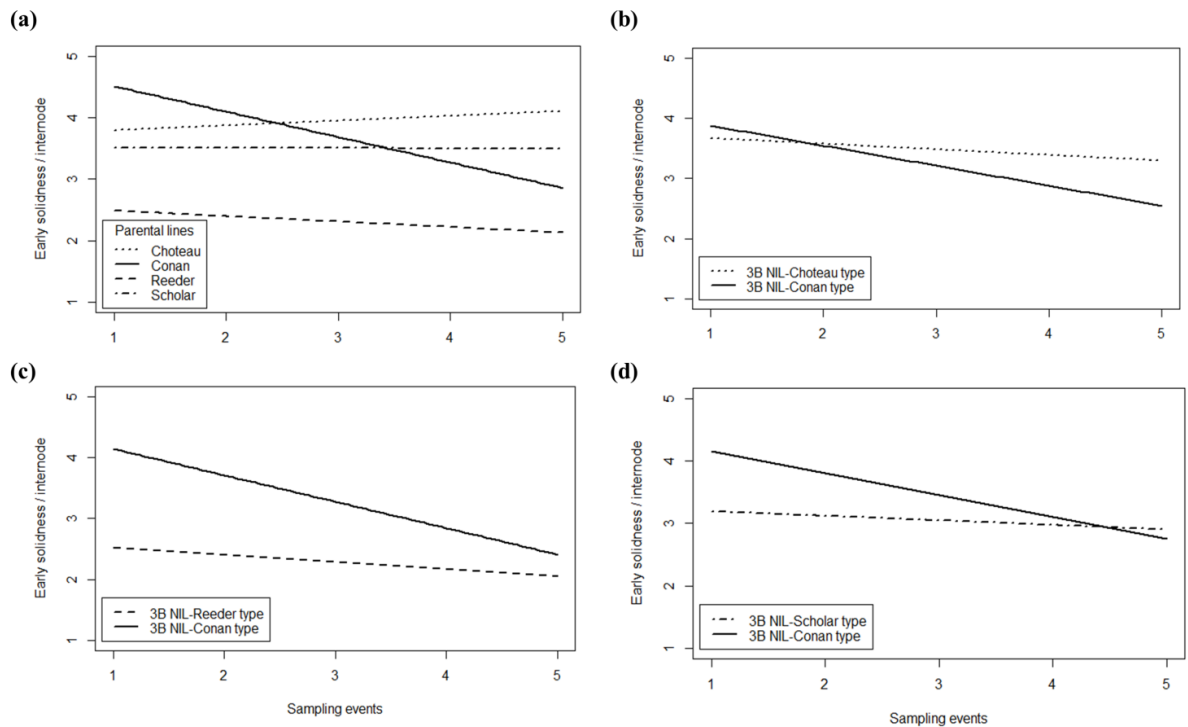


Figure 3.3. Effect of alleles at *Qss.msub-3BL* on early stem solidness per internode in NILs derived from three different genetic backgrounds along with parental lines. Sampling events occurred in Bozeman 2014 and 2015 when plants developmental stage was between Zadoks 31 to 49. Internodes were numbered from the bottom to the top of the stem. (A) Parental lines Scholar, Choteau, Conan, and Reeder, (B) Choteau/Conan genetic background, (C) Reeder/Conan genetic background, (D) Scholar/Conan genetic background. Sampling events are 35 (1), 40 (2), 45 (3), 50 (4), and 55 (5) days after planting



Supplemental Material

Table S3.1. Slope  $\pm$  SE and intercept  $\pm$  SE of regression lines modeling temporal patterns of pith expression in genotypes expressing different levels of stem solidness. Data averaged over replications and years.

| Plant Genotype | Internode | Slope              | Intercept         |
|----------------|-----------|--------------------|-------------------|
| Choteau        |           | $-0.145 \pm 0.097$ | $4.160 \pm 0.290$ |
| Conan          |           | $-0.652 \pm 0.093$ | $5.144 \pm 0.270$ |
| Reeder         |           | $-0.103 \pm 0.045$ | $1.947 \pm 0.198$ |
| Choteau        | 1         | $-0.070 \pm 0.062$ | $3.901 \pm 0.319$ |
|                | 2         | $-0.035 \pm 0.070$ | $3.925 \pm 0.274$ |
|                | 3         | $-0.263 \pm 0.089$ | $4.622 \pm 0.294$ |
|                | 4         | $0.009 \pm 0.170$  | $3.261 \pm 0.571$ |
|                | 5         | $0.215 \pm 0.261$  | $2.527 \pm 0.913$ |
| Conan          | 1         | $-0.146 \pm 0.057$ | $4.463 \pm 0.314$ |
|                | 2         | $-0.378 \pm 0.060$ | $4.668 \pm 0.272$ |
|                | 3         | $-0.633 \pm 0.077$ | $5.038 \pm 0.269$ |
|                | 4         | $-0.949 \pm 0.161$ | $5.507 \pm 0.525$ |
|                | 5         | $-0.644 \pm 0.223$ | $3.974 \pm 0.816$ |
| Reeder         | 1         | $-0.033 \pm 0.056$ | $2.443 \pm 0.215$ |
|                | 2         | $-0.041 \pm 0.057$ | $1.956 \pm 0.263$ |
|                | 3         | $0.037 \pm 0.047$  | $1.201 \pm 0.311$ |
|                | 4         | $0.105 \pm 0.076$  | $0.920 \pm 0.323$ |
|                | 5         | $-0.165 \pm 0.118$ | $1.860 \pm 0.422$ |

Table S3.2. Mixed model analysis and mean  $\pm$  SE for pith moisture and stem density of wheat genotypes expressing different levels of stem solidness. Data averaged over replications.

| Sampling Events (DAP <sup>a</sup> ) | Internode | Plant Genotype | Pith Moisture (g/mm <sup>3</sup> ) | Stem Density (g/mm <sup>3</sup> ) |
|-------------------------------------|-----------|----------------|------------------------------------|-----------------------------------|
| 1 (35)                              | 1         | Choteau        | 1.37E-03 $\pm$ 9.9E-05 A           | 1.77E-04 $\pm$ 2.9E-05 B          |
|                                     |           | Conan          | 1.44E-03 $\pm$ 6.1E-05 A           | 2.91E-04 $\pm$ 3.3E-05 A          |
|                                     |           | Reeder         | 1.32E-03 $\pm$ 6.3E-05 A           | 2.33E-04 $\pm$ 3.0E-05 AB         |
|                                     | 2         | Choteau        | 1.21E-03 $\pm$ 1.8E-05 B           | 1.14E-04 $\pm$ 1.5E-05 A          |
|                                     |           | Conan          | 1.54E-03 $\pm$ 2.9E-05 A           | 1.55E-04 $\pm$ 1.6E-05 A          |
|                                     |           | Reeder         | 1.25E-03 $\pm$ 5.4E-05 B           | 1.41E-04 $\pm$ 1.75E-05 A         |
| 2 (42)                              | 1         | Choteau        | 1.14E-03 $\pm$ 3.5E-05 B           | 3.68E-04 $\pm$ 3.04E-05 A         |
|                                     |           | Conan          | 1.33E-03 $\pm$ 6.5E-05 A           | 3.77E-04 $\pm$ 3.4E-05 A          |
|                                     |           | Reeder         | 1.18E-03 $\pm$ 4.6E-05 B           | 3.63E-04 $\pm$ 2.7E-05 A          |
|                                     | 2         | Choteau        | 1.24E-03 $\pm$ 4.3E-05 B           | 2.65E-04 $\pm$ 1.8E-05 AB         |
|                                     |           | Conan          | 1.46E-03 $\pm$ 4.4E-05 A           | 3.02E-04 $\pm$ 2.3E-05 A          |
|                                     |           | Reeder         | 1.12E-03 $\pm$ 4.6E-05 C           | 2.44E-04 $\pm$ 2.0E-05 B          |
|                                     | 3         | Choteau        | 1.29E-03 $\pm$ 3.1E-05 B           | 1.46E-04 $\pm$ 9.6E-06 B          |
|                                     |           | Conan          | 1.60E-03 $\pm$ 4.6E-05 A           | 1.98E-04 $\pm$ 1.2E-05 A          |
|                                     |           | Reeder         | 1.08E-03 $\pm$ 4.2E-05 C           | 1.48E-04 $\pm$ 1.5E-05 B          |
|                                     | 4         | Choteau        | 9.4E-04 $\pm$ 9.6E-05 B            | 1.01E-04 $\pm$ 7.3E-06 B          |
|                                     |           | Conan          | 1.50E-03 $\pm$ 1.2E-04 A           | 1.54E-04 $\pm$ 1.3E-05 A          |
|                                     |           | Reeder         | 9.32E-04 $\pm$ 6.6E-05 B           | 1.08E-04 $\pm$ 5.5E-06 B          |
|                                     | 5         | Choteau        | 6.48E-04 $\pm$ 2.8E-05 B           | 9.00E-05 $\pm$ 1.7E-05 A          |
|                                     |           | Conan          | 9.06E-04 $\pm$ 3.2E-05 A           | 9.96E-05 $\pm$ 7.4E-06 A          |
|                                     |           | Reeder         | 6.34E-04 $\pm$ 4.1E-05 B           | 8.91E-05 $\pm$ 3.5E-06 A          |
| 3 (49)                              | 1         | Choteau        | 1.11E-03 $\pm$ 8.1E-05 A           | 3.60E-04 $\pm$ 3.6E-05 B          |
|                                     |           | Conan          | 1.21E-03 $\pm$ 5.9E-05 A           | 4.49E-04 $\pm$ 3.2E-05 A          |
|                                     |           | Reeder         | 1.17E-03 $\pm$ 5.6E-05 A           | 3.99E-04 $\pm$ 2.1E-05 AB         |
|                                     | 2         | Choteau        | 1.15E-03 $\pm$ 6.1E-05 AB          | 3.02E-04 $\pm$ 2.2E-05 A          |
|                                     |           | Conan          | 1.22E-03 $\pm$ 5.0E-05 A           | 3.47E-04 $\pm$ 2.2E-05 A          |
|                                     |           | Reeder         | 1.02E-03 $\pm$ 3.5E-05 B           | 3.07E-04 $\pm$ 1.5E-05 A          |
|                                     | 3         | Choteau        | 1.17E-03 $\pm$ 4.8E-05 A           | 2.25E-04 $\pm$ 1.8E-05 B          |
|                                     |           | Conan          | 1.26E-03 $\pm$ 5.3E-05 A           | 2.79E-04 $\pm$ 1.7E-05 A          |
|                                     |           | Reeder         | 9.28E-04 $\pm$ 3.3E-05 B           | 2.35E-04 $\pm$ 1.2E-05 B          |
|                                     | 4         | Choteau        | 8.86E-04 $\pm$ 6.6E-05 B           | 1.67E-04 $\pm$ 1.6E-05 B          |
|                                     |           | Conan          | 1.17E-03 $\pm$ 8.6E-05 A           | 2.20E-04 $\pm$ 1.7E-05 A          |
|                                     |           | Reeder         | 7.51E-04 $\pm$ 5.5E-05 B           | 1.64E-04 $\pm$ 1.4E-05 B          |
|                                     | 5         | Choteau        | 6.17E-04 $\pm$ 7.3E-05 B           | 1.48E-04 $\pm$ 1.1E-05 B          |
|                                     |           | Conan          | 8.33E-04 $\pm$ 3.6E-05 A           | 1.75E-04 $\pm$ 1.6E-05 A          |
|                                     |           | Reeder         | 5.41E-04 $\pm$ 2.8E-05 B           | 1.36E-04 $\pm$ 7.4E-06 B          |
| 4 (56)                              | 1         | Choteau        | 1.10E-03 $\pm$ 2.4E-05 AB          | 4.43E-04 $\pm$ 1.7E-05 A          |
|                                     |           | Conan          | 1.16E-03 $\pm$ 4.8E-05 A           | 4.83E-04 $\pm$ 3.2E-05 A          |
|                                     |           | Reeder         | 1.04E-03 $\pm$ 4.0E-05 B           | 3.89E-04 $\pm$ 2.4E-05 B          |

Table S3.2 Continued

|   |         |                       |                       |
|---|---------|-----------------------|-----------------------|
| 2 | Choteau | 1.12E-03 ± 2.5E-05 A  | 3.89E-04 ± 1.45E-05 A |
|   | Conan   | 1.19E-03 ± 3.3E-05 A  | 3.99E-04 ± 2.4E-05 A  |
|   | Reeder  | 9.17E-04 ± 3.2E-05 B  | 3.07E-04 ± 1.6E-05 B  |
| 3 | Choteau | 1.11E-03 ± 3.8E-05 A  | 3.38E-04 ± 1.5E-05 A  |
|   | Conan   | 1.15E-03 ± 4.3E-05 A  | 3.42E-04 ± 2.0E-05 A  |
|   | Reeder  | 8.14E-04 ± 3.1E-05B   | 2.56E-04 ± 1.3E-05 B  |
| 4 | Choteau | 7.94E-04 ± 6.1E-05 A  | 2.45E-04 ± 1.9E-05 B  |
|   | Conan   | 8.76E-04 ± 5.8E-05 A  | 2.93E-04 ± 2.0E-05 A  |
|   | Reeder  | 5.97E-04 ± 5.58E-05 B | 1.79E-04 ± 9.7E-06 C  |
| 5 | Choteau | 5.86E-04 ± 4.1E-05 A  | 1.93E-04 ± 1.3E-05 B  |
|   | Conan   | 5.46E-04 ± 2.7E-05 A  | 2.25E-04 ± 1.0E-05 A  |
|   | Reeder  | 4.16E-04 ± 2.7E-05 B  | 1.50E-04 ± 7.6E-06 C  |

Mean values with different letters within a sampling event and internode number denote significant differences ( $P < 0.05$ ).

<sup>a</sup> DAP days after planting.

Table S3.3. Slope  $\pm$  SE and intercept  $\pm$  SE of regression lines modeling temporal patterns of pith moisture and stem density in genotypes expressing different levels of stem solidness.

| Plant Genotype       | Internode | Slope                     | Intercept                 |
|----------------------|-----------|---------------------------|---------------------------|
| <b>Pith Moisture</b> |           |                           |                           |
| Choteau              | 1         | -8.303E-05 $\pm$ 2.50E-05 | 1.386E-03 $\pm$ 6.99E-05  |
|                      | 2         | -3.873E-05 $\pm$ 1.79E-05 | 1.277E-03 $\pm$ 5.61E-05  |
|                      | 3         | -8.999E-05 $\pm$ 2.78E-05 | 1.461E-03 $\pm$ 8.70E-05  |
|                      | 4         | -7.467E-05 $\pm$ 5.18E-05 | 1.099E-03 $\pm$ 1.65E-04  |
|                      | 5         | -2.990E-05 $\pm$ 3.92E-05 | 7.037E-04 $\pm$ 1.22E-04  |
| Conan                | 1         | -9.599E-05 $\pm$ 2.01E-05 | 1.527E-03 $\pm$ 8.93E-05  |
|                      | 2         | -1.231E-04 $\pm$ 1.32E-05 | 1.654E-03 $\pm$ 6.28E-05  |
|                      | 3         | -1.832E-04 $\pm$ 2.96E-05 | 1.873E-03 $\pm$ 1.01E-04  |
|                      | 4         | -3.117E-04 $\pm$ 5.89E-05 | 2.115E-03 $\pm$ 1.93E-04  |
|                      | 5         | -1.944E-04 $\pm$ 1.97E-05 | 1.356E-03 $\pm$ 7.40E-05  |
| Reeder               | 1         | -8.549E-05 $\pm$ 1.92E-05 | 1.390E-03 $\pm$ 7.65E-05  |
|                      | 2         | -1.132E-05 $\pm$ 1.67E-05 | 1.364E-03 $\pm$ 5.88E-05  |
|                      | 3         | -1.020E-04 $\pm$ 1.95E-05 | 1.237E-03 $\pm$ 5.90E-05  |
|                      | 4         | -1.672E-04 $\pm$ 4.15E-05 | 1.262E-03 $\pm$ 1.30E-04  |
|                      | 5         | -1.092E-04 $\pm$ 2.26E-05 | 8.587E-04 $\pm$ 7.12E-05  |
| <b>Stem Density</b>  |           |                           |                           |
| Choteau              | 1         | 7.911E-05 $\pm$ 1.27E-05  | 1.393E-04 $\pm$ 4.24E-05  |
|                      | 2         | 8.573E-05 $\pm$ 7.91E-06  | 5.386E-05 $\pm$ 2.74E-05  |
|                      | 3         | 9.588E-05 $\pm$ 8.23E-06  | -5.134E-05 $\pm$ 3.10E-05 |
|                      | 4         | 7.684E-05 $\pm$ 9.75E-06  | -6.235E-05 $\pm$ 3.48E-05 |
|                      | 5         | 5.999E-05 $\pm$ 7.48E-06  | -3.666E-05 $\pm$ 2.58E-05 |
| Conan                | 1         | 6.470E-05 $\pm$ 1.23E-05  | 2.382E-04 $\pm$ 4.71E-05  |
|                      | 2         | 7.767E-05 $\pm$ 9.32E-06  | 1.068E-04 $\pm$ 3.17E-05  |
|                      | 3         | 6.465E-05 $\pm$ 9.04E-06  | 8.165E-05 $\pm$ 3.33E-05  |
|                      | 4         | 6.859E-05 $\pm$ 9.95E-06  | 1.688E-05 $\pm$ 2.52E-05  |
|                      | 5         | 6.320E-05 $\pm$ 7.15E-06  | -2.510E-05 $\pm$ 2.52E-05 |
| Reeder               | 1         | 5.043E-05 $\pm$ 9.55E-06  | 2.198E-04 $\pm$ 4.21E-05  |
|                      | 2         | 5.614E-05 $\pm$ 7.06E-06  | 1.098E-04 $\pm$ 2.83E-05  |
|                      | 3         | 5.179E-05 $\pm$ 6.90E-06  | 5.806E-05 $\pm$ 2.35E-05  |
|                      | 4         | 3.553E-05 $\pm$ 7.27E-06  | 4.392E-05 $\pm$ 2.36E-05  |
|                      | 5         | 3.067E-05 $\pm$ 4.67E-06  | 3.260E-05 $\pm$ 1.57E-05  |

Table S3.4. Slope  $\pm$  SE and intercept  $\pm$  SE of regression lines modeling temporal patterns of pith expression in NILs derived from three different genetic backgrounds along with parental lines. Data averaged over replications and years.

| <b>Plant Genotype</b>             | <b>Slope</b>       | <b>Intercept</b>  |
|-----------------------------------|--------------------|-------------------|
| Choteau                           | $0.078 \pm 0.060$  | $3.716 \pm 0.200$ |
| Conan                             | $-0.413 \pm 0.072$ | $4.923 \pm 0.236$ |
| Reeder                            | $-0.089 \pm 0.041$ | $2.577 \pm 0.136$ |
| Scholar                           | $-0.002 \pm 0.094$ | $3.510 \pm 0.311$ |
| <b>Choteau/Conan derived NILs</b> |                    |                   |
| 3B NIL-Choteau type               | $-0.093 \pm 0.037$ | $3.761 \pm 0.125$ |
| 3B NIL-Conan type                 | $-0.332 \pm 0.130$ | $4.208 \pm 0.043$ |
| <b>Reeder/Conan derived NILs</b>  |                    |                   |
| 3B NIL-Reeder type                | $-0.117 \pm 0.057$ | $2.641 \pm 0.190$ |
| 3B NIL-Conan type                 | $-0.433 \pm 0.012$ | $4.575 \pm 0.038$ |
| <b>Scholar/Conan derived NILs</b> |                    |                   |
| 3B NIL-Scholar type               | $-0.073 \pm 0.047$ | $3.267 \pm 0.159$ |
| 3B NIL-Conan type                 | $-0.349 \pm 0.039$ | $4.501 \pm 0.131$ |

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CHAPTER FOUR

ASSOCIATION ANALYSIS OF STEM SOLIDNESS AND WHEAT STEM SAWFLY  
RESISTANCE IN A PANEL OF NORTH AMERICAN SPRING WHEAT  
GERMPLASM

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## CHAPTER FOUR

ASSOCIATION ANALYSIS OF STEM SOLIDNESS AND WHEAT STEM SAWFLY  
RESISTANCE IN A PANEL OF NORTH AMERICAN SPRING WHEAT  
GERMPLASM

The following chapter has been published in the journal Crop Sciences and appears in this thesis with the journal's written expressed permission.

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Abstract

The expanding geographical range of wheat stem sawfly (WSS) damage, coupled with the limited number of effective control measures, calls for a need to better characterize and explore the genetic variability for resistance in wheat (*Triticum aestivum* L.) germplasm from North America. An association-mapping analysis for stem solidness and wheat stem sawfly resistance was conducted using a set of 244 elite spring wheat lines from 10 North American breeding programs. Three previously identified quantitative trait loci (QTL) for resistance were confirmed, including QTL for stem solidness, heading date, and stem cutting. Three new QTL on chromosomes 2A, 3A, and 5B were identified to be associated with larval mortality and a QTL associated with early stem solidness was identified on chromosome 5D. Genetic variation for late stem

solidness is mainly determined by the solid-stem QTL on chromosome 3B, while variation for early solidness is affected by chromosomal regions on 1B, 3B, and 5D. Potential sources of a novel resistance mechanism causing larval mortality were identified among the panel lines. Favorable alleles for stem solidness and other resistance traits are available within North American wheat germplasm, but only Montana and South Dakota breeding programs appear to have favorable alleles for all QTLs detected in this study. Improvements in wheat stem sawfly management, for both historically impacted areas and for regions first experiencing damage, may be obtained using alleles that already exist in elite germplasm.

### Introduction

The wheat stem sawfly, *Cephus cinctus* Norton, is historically a pest of major economic importance in wheat in the Northern Great Plains of North America. Damage resulting from WSS has recently expanded southward into parts of Wyoming, Nebraska, and Colorado, increasing concerns about wheat susceptibility to this pest over a large vulnerable area. Plant damage is caused by the stem-mining larvae that feed on the parenchyma tissue and vascular bundles of wheat, reducing photosynthetic capacity (Macedo et al., 2005), kernel weight (Morrill et al., 1994), grain quality, and yield (Morrill et al., 1992; 1994). The amount of grain that can be harvested is also reduced by mature larvae that cut the base of the stems, causing them to lodge when exposed to rain or wind (Criddle, 1922). More than \$350 million are lost every year because of this pest in Canada and the United States (Beres et al., 2011b). Despite decades of research on

chemical, biological, and cultural methods of control (reviewed in Beres et al., 2011b), limited success has been achieved in mitigating losses caused by WSS. Host plant resistance has proven to be most consistently effective and is the foundation for integrated pest management of WSS (Beres et al., 2011a).

Host-plant resistance due to solid or pith-filled stems impedes larval development and movement, reducing survival (Wallace and McNeal, 1966). The solid-stem trait was first observed in a landrace from Portugal, S-615, which was used as the source of resistance in the first North American solid-stemmed cultivar in the 1940s, Rescue (CITR1235) (Platt et al., 1948). The genetic basis of solid stem variation in wheat is mostly controlled by the solid-stem QTL, *Qss.msub-3BL* (Cook et al., 2004), where two distinct favorable alleles provide different levels of resistance to WSS (Talbert et al., 2014). Other minor genes, including *Qssmsub-3DL*, modify the degree of stem solidness provided by *Qss.msub-3BL* (Lanning et al., 2006).

One factor that has led to the widespread use of solid stems for WSS resistance is the ease of selection for this trait in breeding populations. Selection for stem solidness can be accomplished in the absence of WSS pressure, allowing efficient screening of segregating materials at managed research sites that have no WSS. The ability to rapidly select for solidness in the absence of the pest limits the potential to make new entomological observations during the selection for the most solid stems. Conversely, resistance mechanisms that do not involve an easily visible morphological trait require screening of nurseries in WSS impacted sites. There are several challenges with this approach, which include the year-to-year variability in the degree of WSS infestation at

any given site (Morrill et al., 1992) and the confounding influence of variable numbers of parasitoids that kill the wheat stem larvae before stem cutting (Runyon et al., 2002). Despite their overall value in WSS suppression (Weaver et al., 2004), assessing host-plant resistance in the presence of varying numbers of parasitoids is difficult (Talbert et al., 2014).

Efforts to breed for WSS resistance have long neglected exploitation of other types of resistance mechanisms. Resistance based on stem solidness is not always reliable, partially because the degree of solidness is influenced by photoperiod and light intensity during stem elongation (Beres et al., 2011b). Control efficiency is reduced if solidness appears later in the growing season, missing most of the WSS oviposition window. Hence, additional resistance mechanisms can potentially improve WSS control in wheat fields. In this regard, there has been a trend toward investigating host-plant attractiveness that targets ovipositing adults rather than the mining larvae. Weaver et al. (2009) reported differences in the attractiveness of two wheat cultivars to female WSS and suggested that this could be due to differences in a known attractant for adult females that was identified by characterizing volatiles from growing wheat plants (Piesik et al., 2008).

In addition to the solid stem QTLs, other QTLs for resistance to WSS have been identified on chromosomes 1B, 1D, 2D, 4A, 5BL, 6BS, and 7AS (Sherman et al., 2010; Joukhadar et al., 2013). The QTLs on chromosomes 1B, 2D, 4A and 5B were identified in a recombinant inbred line population derived from a cross between the spring wheat lines Conan (PI607549) (WestBred, LLC) and Reeder (PI613586) (Sherman et al., 2010),

which are the parent cultivars described in Weaver et al. (2009). Quantitative trait loci on chromosomes 1B and 5BL were both associated with heading date. Lines that head late in the season escape infestation because their stems are not suitable for egg-laying during the WSS oviposition period. The QTLs on chromosomes 2D and 4A reduced WSS cutting and did not cosegregate with heading date or stem solidness. The remaining QTLs were identified in an association-mapping panel comprised of wheat landraces, synthetic hexaploid wheat, and elite lines from West Asia (Joukhadar et al., 2013). In that study, analysis of gene ontology for the molecular marker associated with the 1D QTL showed that this locus is linked to genes associated with cell defense response and apoptosis. Other alleles known to influence plant susceptibility to the WSS are found at the *Rht-B1* and *Rht-D1* loci controlling plant height on chromosomes 4BS and 4DS, respectively. Taller plants are preferred by sawfly females for oviposition (Buteler et al., 2009).

Quantitative trait loci discovery is possible either through biparental QTL mapping or association mapping (Mackay et al., 2009). Among the advantages of an association-mapping approach are the ability to sample a large amount of allelic variation and the high resolution in QTL localization. These advantages may increase the chances of finding favorable alleles for traits of interest and reveal closely linked markers that can be effectively used in marker-assisted selection (MAS). Resistance alleles identified in germplasm from regions devoid of WSS damage may be useful in historically impacted areas such as Montana and may also help breeders select favorable lines as the range of WSS damage expands. Better characterization of resistance genes in historically

impacted areas will provide additional tools for breeders throughout the entire impacted region.

This study used an elite panel of spring wheat lines from North America to, first, characterize the genetic variation for stem solidness and WSS resistance and, second, identify marker–trait associations for traits related to WSS resistance. Although elite lines may represent a small part of the genetic diversity of wheat, favorable alleles present in these lines can be readily explored by plant breeders. Our results suggest that improvements to WSS control in both historically impacted areas and regions experiencing new outbreaks can be obtained using alleles that already exist in elite germplasm.

## Materials and Methods

### Wheat Germplasm

The spring wheat association-mapping (SW-AM) panel used in this study consisted of 244 elite lines from the North America breeding programs of Montana (MSU;  $n = 24$ ); Washington (WAS;  $n = 26$ ); Idaho (UIA;  $n = 32$ ); California (UCD;  $n = 34$ ); Minnesota (UMN;  $n = 26$ ); South Dakota (SDK;  $n = 29$ ); Alberta, Canada (ALB;  $n = 9$ ); Saskatchewan, Canada (SSK;  $n = 26$ ); Manitoba, Canada (MTB;  $n = 13$ ); and CIMMYT, Mexico ( $n = 25$ ). Lines in the panel included both cultivars and elite breeding germplasm. Panel lines were genotyped using the wheat 90K iSelect single nucleotide polymorphism (SNP) assay (Wang et al., 2014) and 25,728 polymorphic markers were detected. Further information about the genotypic data and the panel lines used in this

study are available online at the Triticeae Toolbox (T3, [triticeaetoolbox.org](http://triticeaetoolbox.org) [verified 9 Oct. 2014]). The estimated chromosomal positions of 2753 markers were based on the consensus WSNP map developed by Cavanagh et al. (2013). Genotypic data on *Rht-B1*, *Rht-D1* (reduced -height loci), and *Ppd-D1* (photoperiod-insensitive locus) were obtained using the protocols of Ellis et al. (2002) and Beales et al. (2007), respectively.

#### Early and Late Stem Solidness

The SW-AM panel was planted in late April 2011, 2012, and 2013 near Bozeman, MT, USA, at the Arthur H. Post Research Farm (45°41' N, 111°00' W) and in 2013 at the Southern Agricultural Research Center near Huntley, MT, USA (45°92' N, 108°24' W). Experiments were conducted using an augmented design in which entries were unreplicated and checks were replicated in each block. Entries were divided into five blocks with 50 entries per block. Five check entries were included in each block. Check cultivars were AC Barrie (PI593658), Thatcher (PI168659), Hollis (PI632857), Choteau (PI633974), and Clear White 515. The entries were planted in four-row plots at a seeding rate of 40 g per plot at both locations. The main stems of five plants of each plot were individually collected 5 wk after planting to assess early solidness and at maturity in late August to assess late solidness. For each sampling event, stems were dissected and each internode was rated for stem solidness. The scale was 1 to 5, with 1 being a completely hollow internode and 5 being a completely solid internode. To give a final score of late stem solidness, the ratings of the five internodes were summed to provide scores between 5 (hollow) and 25 (solid) for each stem (Sherman et al., 2010). Early solidness was

measured in Bozeman and Huntley 2013, while late solidness was measured in all locations and years.

#### Wheat Stem Sawfly Cutting and Infestation

The SW-AM panel was planted in late April 2013 and 2014 at a site with a history of WSS infestation near Loma, MT, USA (48°04'21.96" N, 110°27'41.84" W) and in 2014 at a second traditionally WSS-infested site near Amsterdam, MT, USA (45°45'29.85" N, 111°22'49.32" W). Experiments were established adjacent to stubble from a previous WSS-infested wheat crop. Trials were conducted as a randomized complete block design with two replications. Plots consisted of 10 seeds per entry planted in individual hills with spacing of 0.8 m between adjacent hills. A visual evaluation of percentage cut stems was performed at maturity in late August 2013 and early September 2014, after which, stems of each plot were collected. Stems collected in 2013 at Loma were dissected to determine the presence of WSS larvae (infestation), larval cadavers (mortality), and percentage parasitism. Because parasitism was a confounding factor in assessing the impact of host-plant resistance, the data on larval mortality were corrected for parasitoids as described by Talbert et al. (2014). Host-plant resistance was indicated if larval mortality was not caused by external factors such as parasitoids or pathogens.

#### Statistical Analysis

Stem solidness data from trials grown as augmented designs were subjected to analysis of variance for each location. The model was that for an augmented design where the check entries were assigned randomly within blocks using methods described

in Wolfinger et al. (1997) using PROC MIXED in SAS version 9.3 (SAS Institute, 2012). The blocks and entries were considered random effects. Best linear unbiased predictors (BLUPs) were obtained for all entries in each location. An analysis of variance was combined over locations using the BLUP values for the entries from each location. Stem cutting and infestation data were subjected to analysis of variance for a randomized block combined over locations using PROC GLM.

#### Association Mapping Analysis

Markers used in this study had a minor allele frequency  $\geq 5\%$  and  $<10\%$  missing data. Before analysis, the population mean was used to impute genotype data for 5% of the markers that had missing data. Basically, each data point at a given marker was replaced with the population mean at that marker. This imputation method is sufficient when  $< 20\%$  of genotypes are missing (Rutkoski et al., 2013). Population structure of the SW-AM panel was assessed by principal components analysis using the eigenvalue decomposition of the covariance matrix and data from all SNP markers (Supplemental Fig. S1). The percentage variance accounted for by the first principal component was  $< 8\%$  of the total variance. Therefore, models incorporated kinship, but integration of population structure and kinship models was unnecessary. Association analysis was performed using a mixed linear model that included random effect for genetic background with covariance proportional to kinship. An average over all locations for each trait was used as described above. Association mapping analysis was done using the function GWAS in rrBLUP package (Endelman, 2011) in R (R Development Core Team, 2010). To control for false positive associations, a false discovery rate of 0.05 was used.

## Results

### Characterization of Genetic Variation for Stem Solidness and Cutting

Significant variation for early stem solidness per internode among all lines was observed (Table 1). The mean value for early stem solidness per internode was 2.04 (Table 1) and data showed a skewed distribution toward small values (hollowness). The range of variation for early stem solidness ranged from 1.00 (hollow) to 4.31 (solid) (Table 1). Lines from Montana and CIMMYT had the highest early stem solidness per internode scores in the panel (data not shown). There was also significant phenotypic variation ( $P < 0.05$ ) for late stem solidness in the spring wheat AM panel (Table 1). Solid scores ranged from 6.95 (hollow) to 19.17 (solid). Lines from Montana, which historically faced severe WSS pressure, included the 10 most solid lines when data were averaged over all sites (data not shown). The correlation between early and late stem solidness was 0.85 ( $P < 0.001$ ) (data not shown). A broad range of variation was observed for stem cutting (Table 1). The average value for this trait was 15.30% (Table 1). All breeding programs represented in the panel had lines that were not cut by the WSS (Fig. 1). Interestingly, stem cutting was not always associated with late solidness, and many lines with low stem solidness scores had low percentages of cutting (Fig. 2).

### Association Mapping

Marker–trait associations were found for most of the traits studied (Table 2). Association analysis for early and late stem solidness, stem cutting, larval mortality, and parasitism was conducted on the entire SW-AM panel. When marker–trait associations

involved unmapped markers, genetic maps (Cabral et al., 2014; Cyrille et al., 2013) available online at the Triticeae Toolbox were used in an attempt to physically localize markers. A complete list of significant associated markers for all traits can be found in Supplemental Table S1.

Several markers were significantly associated with early stem solidness (Fig. 3a). Most were located on chromosome 3B near position 144 cM; two markers were located on chromosome 1B, position 64.4 cM; one marker was located on chromosome 5D, position 67.49 cM; and a few markers remained unmapped. The logarithm of odds (LOD) scores of markers associated with early solidness ranged from 13 to 4.6 (Fig. 3a). Lines from CIMMYT, Montana, Manitoba, South Dakota, Saskatchewan, California, and Minnesota contained favorable alleles for the 1B QTL (Fig. 4a), and lines from the three Canadian breeding programs (Alberta, Manitoba, and Saskatchewan) had only unfavorable alleles for the 5D QTL (Fig. 4b). Markers associated with late stem solidness also mapped to chromosome 3B near position 144 cM (Fig. 3b). The LOD scores for markers associated with this trait ranged from 19.8 to 5.1 (Fig. 3b). Favorable alleles for the two most significant markers associated with the solid stem QTL on 3B (*Qss.msub-3BL*) were seen in lines from all breeding programs except those from Idaho, Minnesota, and Washington (Fig. 4c,d).

A significant marker–trait association was observed for stem cutting on chromosome 4A, position 134.6 cM (Table 2). The LOD scores for markers associated with this trait ranged from 11.0 to 10.6 (Fig. 3c). The favorable allele for the 4A QTL was highly represented in the SW-AM panel and was found in nine of the 10 breeding

programs (Fig. 4e). Endemic parasitoids were responsible for varying levels of parasitism (0 to 22%) inside the stem, but no marker–trait associations were detected for level of parasitism (Fig. 3d). Larval mortality because of host-plant resistance, which was determined by stem dissection done to assess the cause of mortality, was associated with markers on chromosomes 5B, location 61.0 cM; 2A, location 187.3 cM; 3A, location 0.5; and with markers of unknown locations (Supplemental Table S1). The LOD scores for markers associated with stem cutting ranged from 7.2 to 4.5 (Fig. 3e). Favorable alleles for the 2A, 5B, and 3A QTLs were present in 25, 21, and 14% of the panel lines, respectively (Table 2) and were distributed among several breeding programs (Fig. 4f–h). Wheat stem sawfly resistance was not associated with major growth habit loci *Rht-B1*, *Rht-D1*, or *Ppd-D1*. A complete list of panel lines and their allele types for markers significantly associated with stem solidness and WSS resistance traits can be found in Supplemental Table S2.

### Discussion

The WSS has been a serious pest of wheat in the Northern Great Plains of North America for decades. Limitations constraining other potential control measures have driven pest management efforts toward resistance breeding. Selection for resistance has mainly targeted stem solidness because of this trait’s effectiveness and ease of phenotyping, but the genetic architecture of WSS resistance goes beyond the stem solidness QTL. The advent of molecular markers may allow breeders to improve WSS control by selecting for these other resistance mechanisms without having to rely on

complicated and often unreliable field-based screening. In this study, the genetic architecture of WSS resistance in wheat germplasm from North America was assessed and a series of molecular markers was identified.

Among the SW-AM panel, six chromosomal regions associated with WSS resistance were detected, three of which were not related to solidness. The highest diversity of favorable alleles was found in spring wheat breeding programs from Montana and South Dakota, both located in traditionally impacted areas. Regions experiencing new outbreaks, and even those with no WSS damage, also harbor alleles for resistance. Because of the importance of stem solidness in WSS control, stem solidness was traditionally assessed at maturity. However, it was also assessed during the early growth stage of stem elongation, during the flight period of adult WSS. This was based on a long-standing observation that, in Montana, the WSS flight coincides with the early development of spring wheat plants. This results in frequent oviposition in the lower, newly formed internodes. Eggs can be concentrated in these internodes because female WSS cannot discriminate internodes that are already infested (Buteler et al., 2009). Both early and late stem solidness associate with the solid-stem QTL (*Qss.msub-3BL*) on chromosome 3B. Early stem solidness was also impacted by alleles at loci on 1B and 5D. A further distinction between early and late stem solidness is also relevant because lines that become solid later in the growing season are likely to be less effective against WSS than those that develop pith early. Therefore, breeding programs that rely on stem solidness to breed for WSS resistance should consider its temporal and structural components for best results. Despite the significant selection pressure for stem solidness

within historically infested areas, no favorable allele has been selected to fixation. Stem solidness was only observed in high frequencies in programs with germplasm specifically selected to be resistant to WSS. This is likely due to the introduction of stem solidness into germplasm of the Northern Great Plains in the middle of the 20th century and subsequent introgression of the genes for stem solidness into lines targeted specifically for WSS-impacted areas. A recent genetic study using near-isogenic lines for the *Qss.msub-3BL* showed that the allele for solid stems did not have a negative effect on grain yield (Sherman et al., 2015) based on yield trials conducted over nine environments. However, there was also no advantage to the allele for solid stems in the absence of WSS, thus, the solid stem allele is absent or present at low frequencies in most germplasm.

There were 24 lines that showed stem solidness scores equal to or greater than Rescue, the donor of the solid stem trait to Northern Great Plains germplasm. Based on analysis of five linked SNP markers (IWA1756, IWA2148, BobWhite\_c45118\_495, BS00063160\_51, and BS00079029\_51) (Supplemental Table S1), a total of 22 of the 24 lines shared the Rescue haplotype for the SNPs. The two exceptional lines, CIMMYT 9253 and 9263, had haplotypes that differed from that of Rescue. This may indicate a different origin of the solid-stem alleles in these lines.

A measure of resistance to the WSS is reduced stem cutting at maturity. In other studies, the solid-stem QTL on 3B was associated with reduced stem cutting (Beres et al., 2011b; Cook et al., 2004; Sherman et al., 2010). The solidness QTL on 3B was not associated with cutting in the SW-AM panel. This likely results from the fact that so few

of the lines carried the alleles for solidness. Other mechanisms of host-plant resistance to the WSS are available within elite lines of North America in the SW-AM panel. A previously identified QTL on chromosome 4A was associated with stem cutting in the SW-AM panel (Sherman et al., 2010). The high frequency of the favorable allele at this locus in germplasm from areas lacking any history of WSS outbreaks contrasts with the pattern observed for the 3B QTL. The 4A QTL could potentially be involved with antixenosis rather than antibiosis, because the favorable allele reduces infestation and stem cutting without increased insect mortality inside the stem. Further experiments will be necessary to determine whether alleles at this locus play a role in host attractiveness to ovipositing females. Association analysis revealed that the favorable allele of the 4A QTL is present in a high frequency among germplasm from WSS infested and uninfested areas. This suggests that this chromosomal region is either under selection throughout North America for reasons other than WSS resistance or, at least, does not confer a cost in terms of other agronomic traits. Resistance in elite germplasm not known to be under selection pressure has also been identified for the orange wheat blossom midge, *Sitodiplosis mosellana* Gehin (Thomas et al., 2005). The presence of the favorable alleles in elite lines provides opportunity for their rapid introduction into cultivar development programs.

Several hollow-stemmed lines with reduced cutting were identified (Fig. 2). These lines represent candidates for introduction of new mechanisms of resistance into currently available germplasm to augment solid stems. Indeed, stem dissection revealed dead WSS eggs, neonates, and larvae inside the stem. The number of dead neonates and eggs was

small compared with the number of dead larvae (data not shown). Some of the larvae were killed by parasitoids, but plant dissection revealed that most of the mortality was caused by host-plant resistance. Based on genotypic and phenotypic data, genotypes that have the best potential to be used as source of novel resistance to the wheat stem sawfly are SD4264 (South Dakota), MT0945 (Montana), MN01333-A (Minnesota), and Hollis (PI632857; Washington).

The use of an elite panel limited the array of allelic variation assessed in this study and, as expected, failed to detect a few QTLs that have been previously shown to be associated with WSS resistance (Sherman et al., 2010; Joukhadar et al., 2013). Nevertheless, the use of this panel allowed us to determine the genetic basis of WSS resistance available within North American breeding programs. Based on these results, improvements on WSS management in newly impacted regions can be readily achieved by collaboration with breeding programs from historically impacted areas, which apparently harbor a greater diversity of favorable alleles. Marker-assisted selection can help breeders introgress alleles associated with resistance into regionally adapted germplasm. Additionally, breeders may be able to identify existing cultivars with favorable alleles at resistance QTL for planting in newly infested regions. For instance, the 4A QTL conferring resistance is present at high frequency in several of the North American breeding programs. There is an opportunity to improve WSS control in breeding programs from the Northern Great Plains. In fact, these results indicate improvement is possible in all programs through MAS pyramid of favorable alleles into a single genotype.

### Conclusions

Association mapping analysis offers potential to explore natural genetic variation and to uncover the genetic basis of complex traits such as insect resistance. In this study, the extent of genetic variation associated with WSS resistance within 10 North American wheat breeding programs was investigated. Favorable alleles for stem solidness and other resistance traits were available among most of the breeding programs represented in this research. New significant marker–trait associations for larval mortality and early stem solidness were identified. The genetic diversity for traits associated with WSS resistance uncovered in this study can be exploited by breeders through marker-assisted selection and will potentially benefit both historically impacted areas and regions vulnerable to future outbreaks.

### Acknowledgments

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(Agriculture and Agri-Food Canada), and the International Maize and Wheat Improvement Center (CIMMYT).

Table 4.1. *P*-values from analysis of variance (ANOVA), range, mean, standard deviation, and coefficient of variance for traits associated with wheat stem sawfly resistance in the spring wheat association-mapping panel from North America.

| Traits                             | <i>P</i> -values |          | Range                   | Mean  | Standard deviation | Coefficient of variation |
|------------------------------------|------------------|----------|-------------------------|-------|--------------------|--------------------------|
|                                    | Lines            | Location |                         |       |                    |                          |
| Early stem solidness per internode | 0.001            | <0.0001  | 1.00–4.31 <sup>†</sup>  | 2.04  | 0.46               | 22.54                    |
| Late stem solidness                | <0.0001          | <0.0001  | 6.95–19.17 <sup>‡</sup> | 9.22  | 1.56               | 17.75                    |
| Cutting                            | <0.0001          | <0.0001  | 0–46.67 <sup>§</sup>    | 15.30 | 9.91               | 64.77                    |

<sup>†</sup>Ranges from 1.00 to 5.00,

<sup>‡</sup>Ranges from 5.00 to 25.00.

<sup>§</sup>Ranges from 0.00 to 100.00.

Table 4.2. Significant markers associated with resistance to the wheat stem sawfly in the spring wheat association-mapping panel from North America.

| Chromosome | Marker               | Freq <sup>†</sup> | LOD <sup>‡</sup> | Trait                                | Coincident QTL <sup>§</sup>            |
|------------|----------------------|-------------------|------------------|--------------------------------------|----------------------------------------|
| 1B         | RAC875_c8662_762     | 0.12              | 4.95             | Early stem solidness or heading date | Sherman et al. (2010)                  |
| 2A         | Excalibur_c3454_1674 | 0.25              | 4.69             | Larval mortality                     | Novel                                  |
| 3A         | Kukri_rep_c89183_306 | 0.21              | 4.56             | Larval mortality                     | Novel                                  |
| 3B         | BS00065603_51        | 0.16              | 13.06            | Early stem solidness                 | Stem solidness ( <i>Qss.msub-3BL</i> ) |
| 3B         | BS00074345_51        | 0.16              | 19.80            | Late stem solidness                  | Stem solidness ( <i>Qss.msub-3BL</i> ) |
| 4A         | BS00064494_51        | 0.61              | 11.07            | Cutting                              | Sherman et al. (2010) <sup>¶</sup>     |
| 5D         | BobWhite_c8092_726   | 0.08              | 5.80             | Early stem solidness                 | Novel                                  |
| 5B         | RFL_Contig4751_1197  | 0.14              | 7.21             | Larval mortality                     | Novel                                  |

<sup>†</sup>Freq, frequency of favorable allele.

<sup>‡</sup>LOD, logarithm of odds.

<sup>§</sup>QTL, quantitative trait loci.

<sup>¶</sup>Sherman et al. (2010) identified two QTLs on chromosome 4A, the QTL identified here was flanked for markers wmc760 and wPt1743.

Figure 4.1. Percentage and origin of genotypes not cut by the wheat stem sawfly in the spring wheat association panel from North America. Breeding programs origins are Montana (MSU), Washington (WAS), Idaho (UIA), California (UCD), Minnesota (UMN), South Dakota (SDK), Alberta, Canada (ALB), Saskatchewan, Canada (SSK), Manitoba, Canada (MTB), and CIMMYT, Mexico.

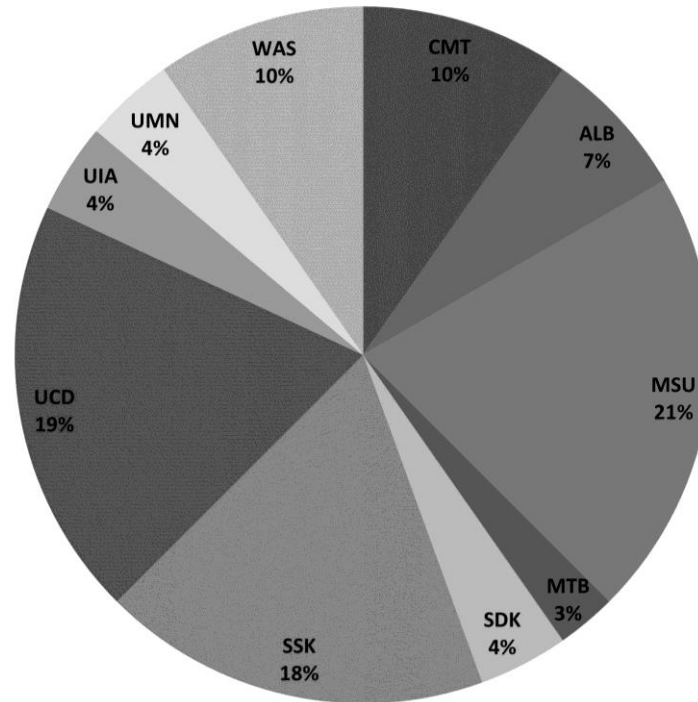


Figure 4.2. Scatter plot of wheat stem sawfly cutting and late stem solidness in the spring wheat association-mapping panel from North America. Breeding programs origins are Montana (MSU), Washington (WAS), Idaho (UIA), California (UCD), Minnesota (UMN), South Dakota (SDK), Alberta, Canada (ALB), Saskatchewan, Canada (SSK), Manitoba, Canada (MTB), and CIMMYT, Mexico.

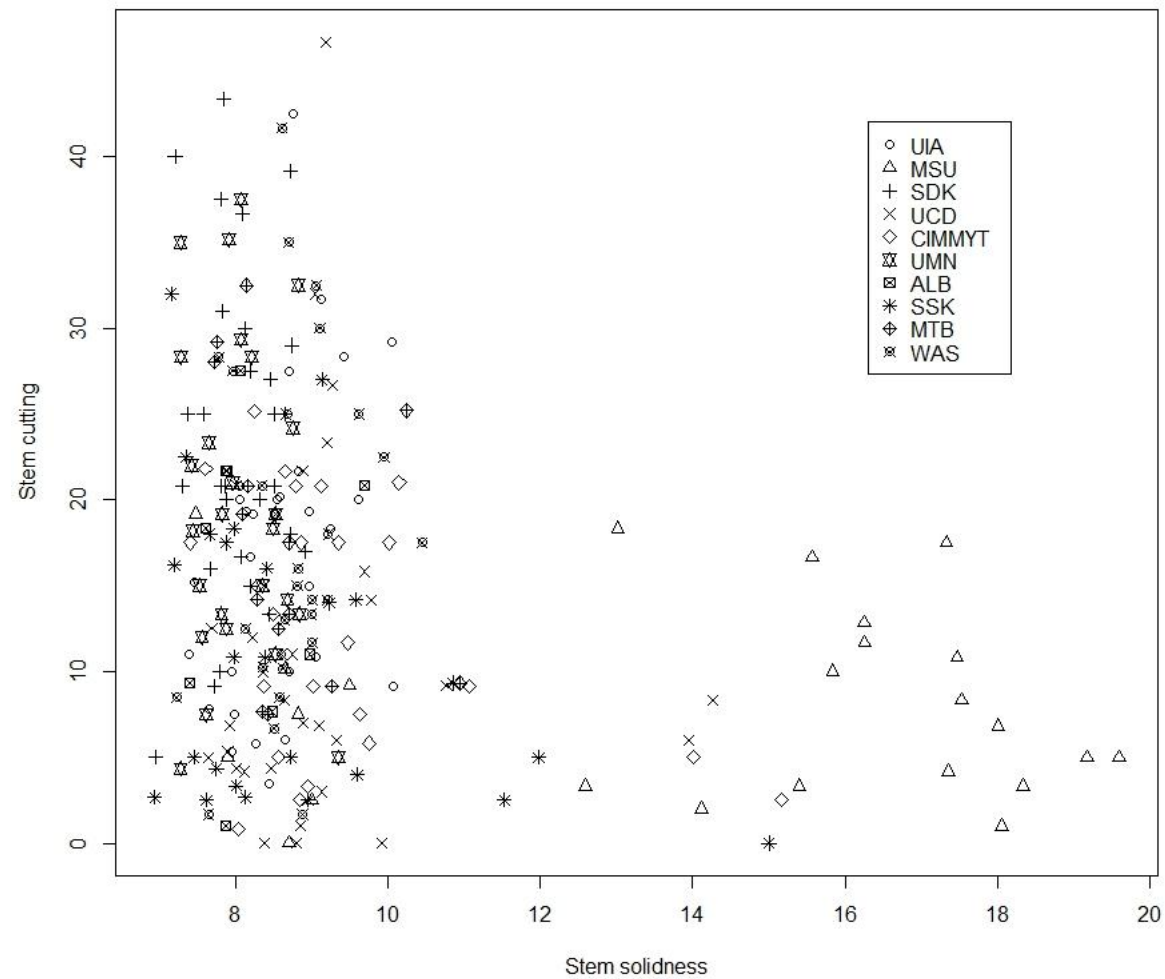


Figure 4.3. Manhattan plots for traits associated with wheat stem sawfly resistance: (A) early stem solidness; (B) late stem solidness; (C) percentage of cutting; (D) percentage of parasitism; and (E) larval mortality (corrected for parasitism). Wheat's 21 chromosomes are shown on the horizontal axis. Markers on the right-most column are not mapped and therefore were not assigned to any chromosome (horizontal lines correspond to a false discovery rate of 0.05. Dashed line is omitted when there are no significant associations).

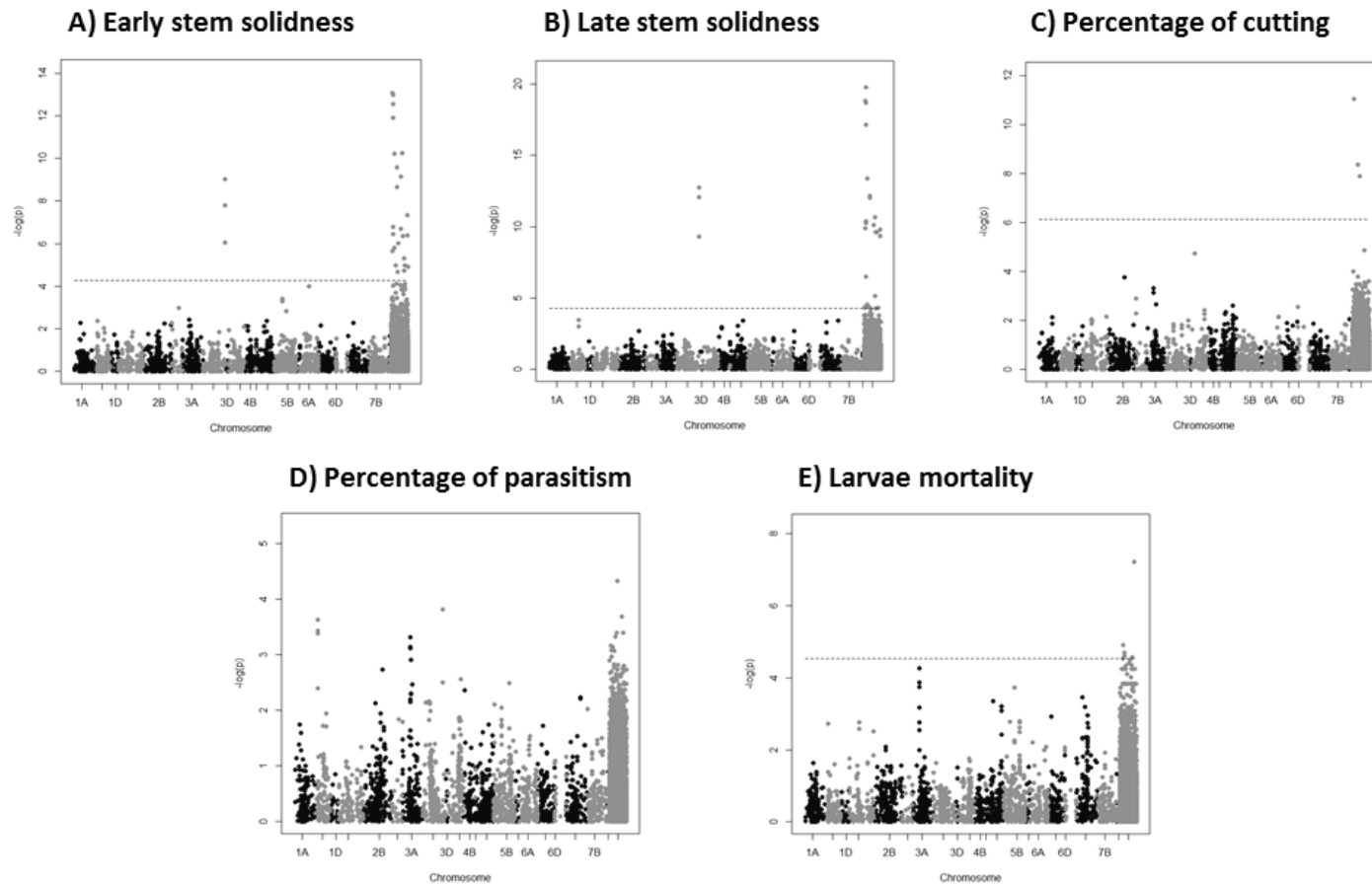
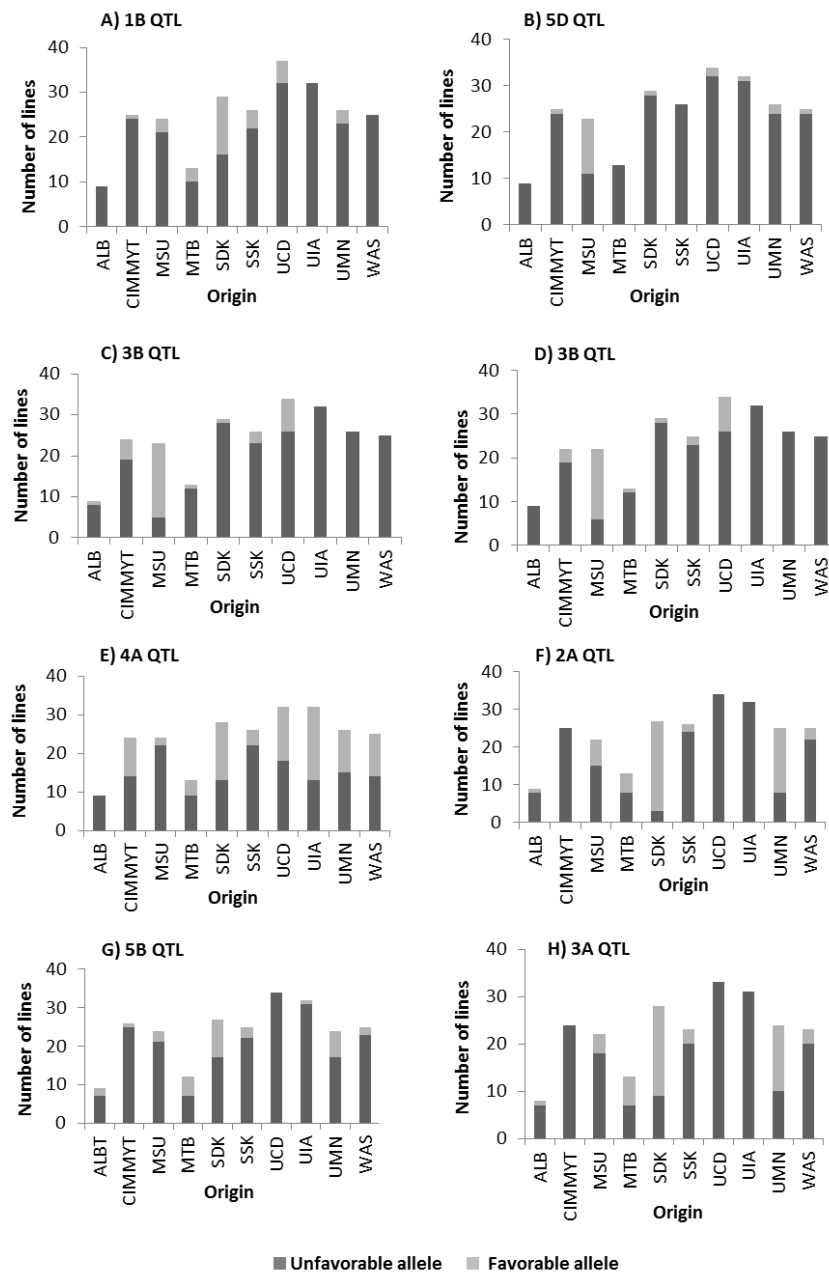


Figure 4.4. Distribution of alleles of some of the significant markers associated with wheat stem sawfly resistance in the spring wheat association-mapping panel from North America breeding programs. (A) Marker RAC875\_c8662\_762; (B) marker BobWhite\_c8092\_726; (C) marker BS00065603\_51; (D) marker BS00074345\_51; (E) marker BS00064494\_51; (F) marker Excalibur\_c3454\_1674; (G) marker RFL\_Contig4751\_1197; and (H) marker Kukri\_rep\_c89183\_306. Breeding programs origins are Montana (MSU), Washington (WAS), Idaho (UIA), California (UCD), Minnesota (UMN), South Dakota (SDK), Alberta, Canada (ALB), Saskatchewan, Canada (SSK), Manitoba, Canada (MTB), and CIMMYT, Mexico.



Supplemental Material

Table S4.1. Markers significantly associated with stem solidness and wheat stem sawfly resistance traits in the association mapping panel from North America.

| Marker                      | Chromosome | Position | Source of map position   |
|-----------------------------|------------|----------|--------------------------|
| <b>Early stem solidness</b> |            |          |                          |
| BS00065603_51               | 3B         | 144.74   | Cyrille et al., (2013)   |
| BS00071183_51               | 3B         | 144.74   | Wang et al. (2014)       |
| BS00074345_51               | 3B         | 144.74   | Cyrille et al., (2013)   |
| BS00073411_51               | 3B         | 144.74   | Wang et al. (2014)       |
| Kukri_c37097_1849           | UNK†       | -        | -                        |
| BobWhite_c45118_495         | 3B         | 140.50   | Cyrille et al., (2013)   |
| GENE-1910_358               | UNK        | -        | -                        |
| Jagger_c3292_145            | 3B         | 139.61   | Cavanaugh et. al. (2013) |
| IWA1756                     | 3B         | 143.44   | Cyrille et al., (2013)   |
| GENE-1925_118               | 3B         | 142.14   | Cyrille et al., (2013)   |
| IWA2148                     | 3B         | 140.50   | Cyrille et al., (2013)   |
| Tdurum_contig59566_2309     | UNK        | -        | -                        |
| BS00079029_51               | 3B         | 140.50   | Cavanaugh et. al. (2013) |
| Ku_c16949_1230              | UNK        | -        | -                        |
| BS00091257_51               | 3B         | 140.50   | Cyrille et al., (2013)   |
| Tdurum_contig59566_4435     | 3B         | 143.29   | Cyrille et al., (2013)   |
| Tdurum_contig59566_1534     | 3B         | 143.29   | Wang et al. (2014)       |
| Kukri_c55981_194            | UNK        | -        | -                        |
| IWA5892                     | 3B         | 143.29   | Cavanaugh et. al. (2013) |
| IACX1477                    | UNK        | -        | -                        |
| BobWhite_c8092_726          | 5D         | 67.49    | Wang et al. (2014)       |
| BS00063160_51               | 3B         | 143.29   | Cyrille et al., (2013)   |
| RAC875_c37741_218           | 3B         | 142.14   | Cyrille et al., (2013)   |
| RAC875_c8662_762            | 1B         | 64.46    | Cyrille et al., (2013)   |
| Excalibur_c27675_1815       | 1B         | 64.46    | Cyrille et al., (2013)   |
| wsnp_BF473138A-Ta_2_3       | UNK        | -        | -                        |
| RAC875_c3925_1043           | UNK        | -        | -                        |
| GENE-3471_298               | UNK        | -        | -                        |
| <b>Late stem solidness</b>  |            |          |                          |
| BS00074345_51               | 3B         | 144.74   | Wang et al. (2014)       |
| BS00065603_51               | 3B         | 144.74   | Cyrille et al., (2013)   |
| BS00071183_51               | 3B         | 144.74   | Wang et al. (2014)       |
| BS00073411_51               | 3B         | 144.74   | Wang et al. (2014)       |
| BobWhite_c45118_495         | 3B         | 140.50   | Cyrille et al., (2013)   |
| IWA1756                     | 3B         | 143.44   | Cavanaugh et. al. (2013) |
| GENE-1910_358               | 3B         | 144.74   | Cavanaugh et. al. (2013) |
| IWA2148                     | 3B         | 140.50   | Cyrille et al., (2013)   |
| GENE-1925_118               | 3B         | 142.14   | Cyrille et al., (2013)   |
| Kukri_c37097_1849           | UNK        | -        | -                        |
| BS00079029_51               | 3B         | 140.50   | Wang et al. (2014)       |
| BS00091257_51               | 3B         | 140.50   | Cyrille et al., (2013)   |
| Jagger_c3292_145            | 3B         | 139.61   | Wang et al. (2014)       |
| BS00063160_51               | 3B         | 143.29   | Cyrille et al., (2013)   |
| Tdurum_contig59566_2309     | 3B         | 143.29   | Wang et al. (2014)       |

Table S4.1 Continued

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| Kukri_c55981_194                                   | 3B  | 143.29 | Cavanaugh et. al. (2013) |
| RAC875_c37741_218                                  | 3B  | 142.14 | Cyrille et al., (2013)   |
| Tdurum_contig59566_4435                            | 3B  | 143.29 | Cyrille et al., (2013)   |
| Tdurum_contig59566_1534                            | 3B  | 143.29 | Wang et al. (2014)       |
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| RFL_Contig4751_1197                                | 5B  | 61.0   | Cabral et al., (2014)    |
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Table S4.2. Elite lines allele types for some of the markers significantly associated with stem solidness and wheat stem sawfly resistance traits. Elite lines are organized by breeding program origin.

| Elite Lines           | Early<br>Solidness<br>3B QTL† | Early<br>Solidness<br>1B QTL‡ | Early<br>Solidness<br>5D QTL§ | Late<br>Solidness<br>3B QTL¶ | Stem<br>Cutting<br>4A QTL# | Larvae<br>Mortality<br>5B QTL†† | Larvae<br>Mortality<br>2A QTL‡‡ |
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| <b>ALBERTA/CANADA</b> |                               |                               |                               |                              |                            |                                 |                                 |
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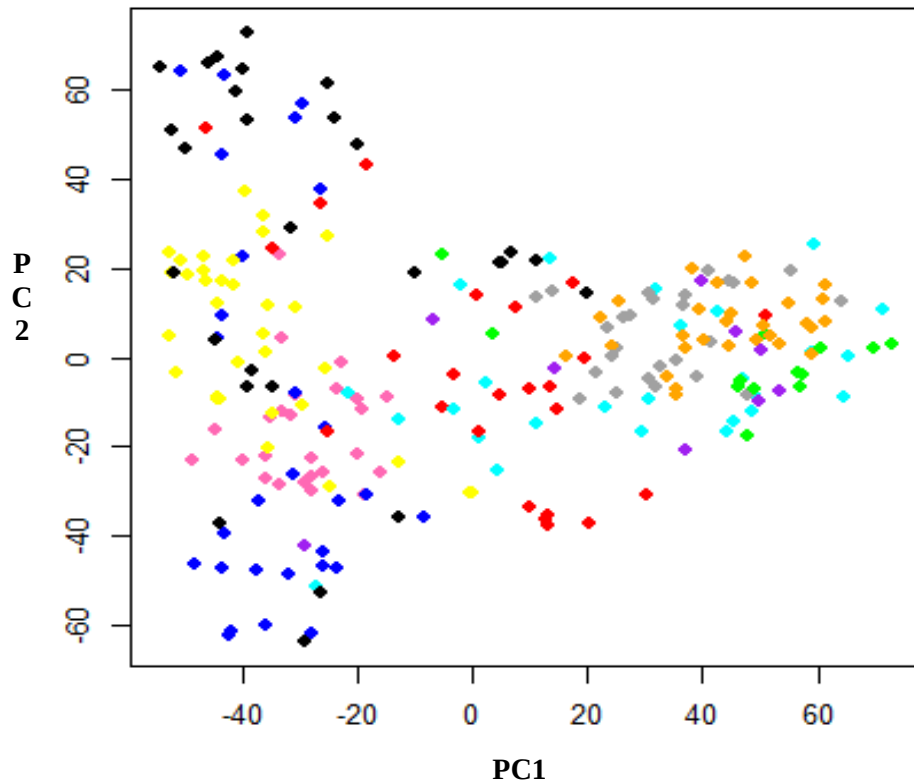
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## References

- Beales, J., A. Turner, S. Griffith, J.W. Snape, and D.A. Laurie. 2007. A *Pseudo-Response Regulator* is misexpressed in the photoperiod insensitive *Ppd-D1a* mutant of wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* 115:721–733.
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## CHAPTER FIVE

## CONCLUSION

Plant breeding for insect resistance is an integral component of pest management in agroecosystems due to its economical and environmental advantages. However, breeding for insect resistance can be challenging if (i) it is difficult to establish stable field screening nurseries with high levels of pest infestation, (ii) phenotypic selection is expensive and time-consuming, (iii) natural enemy populations are confounding factors for the assessment of host plant resistance, (iv) the insect pest cannot be reared in the laboratory, (v) genetic resistance has a quantitative basis, and (vi) environmental factors influence the expression of resistance.

Genomic tools offer great potential to facilitate and increase the efficiency of plant breeding for insect resistance. The use of molecular markers allows for (i) the identification of genomic regions associated with resistance, (ii) the characterization of genetic variation associated with resistance within a particular germplasm, (iii) genomic selection, (iv) selection of parental lines for crossing, (v) and marker-assisted pyramiding of resistance genes. Furthermore, the combination of plant genetics and high-throughput phenotyping techniques allows for a better understanding of the mechanisms underlying plant resistance. However, none of this is possible without integrating the fields of plant breeding, molecular biology, genetics, entomology, and statistics. In the research presented herein, we integrated multiple competencies to develop both knowledge- and application-oriented research to characterize the genetic

basis of WSS resistance in wheat and to enhance the repertoire of genetic resistance to WSS available for exploitation by breeders.

Our collaborative effort allowed us to bridge the gap between plant genetics and host plant selection by insects. We developed a new framework for assessing QTL effects on insect behavior using near-isogenic lines (NIL) under controlled conditions. As shown in chapter two, a series of greenhouse and laboratory bioassays done with wheat NILs for *Qss.msub-3B*, *Qwss.msub-2D*, and *Qwss.msub-4A.1* demonstrated the effect of QTLs on WSS host selection process. Favorable alleles were shown to affect specific behavioral sequences in oviposition by the WSS and to reduce host attraction and host preference for oviposition. Furthermore, maternal choices during oviposition appeared to reflect an adaptation to optimize progeny performance and survival, because females preferred to lay eggs in plants where the progeny would have higher chances of surviving. These controlled bioassays were fundamental to improve our understanding of the genetic mechanisms modulating the complex process of WSS host plant selection.

In our next chapter, we determined if previously identified QTL for resistance controlled variation in temporal patterns of stem solidness expression and pith retraction in wheat and its effect on WSS resistance. Our results provided evidences for allele-specific variation in early solidness expression and pith retraction. Near-isogenic lines carrying the Conan allele for the solid stem locus, *Qss.msub-3BL*, showed high degrees of early stem solidness and a rapid rate of pith retraction during stem maturation. Conversely, the amount of pith in the stem for lines containing the solid stem allele derived from S-615 remained mostly unchanged throughout plant development. Also, Conan plants showed an increased degree of early stem solidness,

pith moisture, and stem density when compared to Choteau and Reeder plants. Complementary cage trials and field experiments revealed that early stem solidness expression is related to decreased WSS infestation and increased neonatal mortality. Therefore, wheat breeders may consider temporal variations in pith expression for best results in selecting resistant solid-stemmed genotypes.

Studies presented in chapters two and three characterized the effect of three QTLs on WSS resistance. This information will help breeders and entomologists to better integrate management strategies to reduce damage caused by the WSS. For example, host preference for oviposition explored in chapter two is relative rather than absolute. This fact has important implications for the utilization of antixenosis QTLs for WSS management. Females could potentially lay more eggs than would be expected on resistant plants, providing more suitable hosts were rare or not available. Thus, in wheat monoculture, the use of less preferable/attractive varieties might not have the desirable effect on pest management. Varieties with antixenic properties may be better employed in the context of trap cropping. This is a behavioral manipulation method that reduces pest abundance via the integration of a push-pull strategy with a subsequent pest mortality caused either by trap crop destruction or antibiosis mechanisms of resistance. Marker assisted selection would be a key tool for the development of such varieties and the implementation of push-pull strategies in wheat fields.

The fourth chapter reports a characterization of elite spring wheat germplasm of North America for both previously identified and potentially new QTLs for WSS resistance. Our association mapping study revealed that the genetic architecture of WSS resistance goes beyond the stem solidness QTL. The identification of new QTLs

associated with WSS resistance is exciting because it is the first step towards incorporating new mechanisms of resistance into wheat varieties. For instance, larval mortality was shown to be associated with QTLs on chromosomes 2A, 3A, and 5B. Previously identified QTLs for resistance were confirmed, including QTLs for heading date and stem cutting on chromosomes 1B and 4A, respectively. Also, three QTLs associated with early stem solidness expression were identified. Our results showed that improvements in WSS management may be obtained using alleles that already exist in elite germplasm. Further research will be necessary to validate the effect on newly-identified QTLs under different environmental conditions.

Collectively, our research provided wheat breeders with a better tool kit for improving resistance to the WSS. Furthermore, these studies demonstrate the power of integrating multiple competencies in broadening our understanding of plant-insect interactions and their genetic basis. Increased interest in multidisciplinary team work, along with the rapid pace in the advance of molecular technologies and high throughput phenotyping promises an exciting future for plant breeding for insect resistance.

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