THESIS

CHARACTERIZATION AND QUANTITATIVE TRAIT LOCI (QTL) ANALYSIS FOR WHEAT STEM SAWFLY (HYMENOPTERA: CEPHIDAE) RESISTANCE IN WINTER WHEAT

Submitted By

Bradley Pakish

Department of Soil and Crop Sciences

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Master's Committee:

Advisor: Scott D. Haley

Maria Muñoz-Amatriaín
Frank B. Peairs

ABSTRACT

CHARACTERIZATION AND QUANTITATIVE TRAIT LOCI (QTL) ANALYSIS FOR WHEAT STEM SAWFLY (HYMENOPTERA: CEPHIDAE) RESISTANCE IN WINTER WHEAT

The wheat stem sawfly (*Cephus cinctus*) (WSS) has quickly become a major pest of Colorado wheat production over the past ten years. Prior resistant cultivars have relied on the expression of a solid-stemmed trait (*Qss.msub*-3BL) to decrease damage from sawfly infestations, however environmental factors (sun, rain, etc.) may result in inconsistent pith expression. The limitations of solid-stemmed varieties have aided in the recent identification of novel quantitative trait loci (QTL) for reducing WSS infestation and stem cutting by host-plant preference. In this light, crosses between 'Denali'/'Hatcher' and 'Avery'/'CO11D1397' were completed in the greenhouse during Fall 2014 and Spring 2015 to create two doubled haploid (DH) populations for discovery of QTL associated with non solid-stemmed resistance. Each population was grown under naturally occurring sawfly pressure at two different northeastern Colorado locations during the 2018-19 field season, however only the Avery/CO11D1397 population was selected for planting in the 2019-20 field season due to resource limitations. Entries were evaluated for plant height, heading date, physiological maturity, cutting score, and kernel weight. Next generation sequencing data were generated through genotyping-by-sequencing and resulted in 776 single-nucleotide polymorphisms (SNP) markers in the final genetic map for Avery/CO11D1397. Quantitative trait loci analysis identified a total of 11 QTL, seven major-effect and four minor-effect, in the Avery/CO11D1397 DH population for reduced WSS cutting in multiple environments. Two QTL were associated on the same chromosomal arms as photoperiod genes *Ppd-D1* (*Qwss.csu*-2DS) and *Ppd-B1* (*Qwss.csu*-2BS). The *Qwss.csu*-1BL was also associated on the long arm of chromosome 1B with the earliness per se gene *Eps-B1*. *Qwss.csu*-7DS and *Qwss.csu*-5BS were the only two major-effect QTL identified that were not associated with major developmental genes, and thus could be associated with antixenosis. Results from

this study suggest that a relationship between lower cutting score and a later flowering date exists for genotypes within the Avery/CO11D1397 DH population. Introgression of *Qwss.csu*-7DS and *Qwss.csu*-5BS into cultivars with stem-solidness may help in developing new wheat varieties with durable WSS resistance.

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CHAPTER I – LITERATURE REVIEW

Wheat Stem Sawfly

Geographic Distribution in North America

The wheat stem sawfly (*Cephus cinctus Norton*), hereafter referred to as WSS, was first identified in North America almost 150 years ago. Edward Norton in 1872 was first to document the WSS infesting the native grasses of Colorado (Fulbright et al., 2017; Ivie, 2001; Lesieur et al., 2016), with similar observations in Nevada (1872), California (1890), Montana (1890), Saskatchewan and Manitoba (1895) (Fulbright et al., 2017; Ivie, 2001). Reports of wheat (*Triticum aestivum L.)* being damaged by WSS first occurred in New York State in 1889 (Beres et al., 2011a) and only six years later in the Canadian prairie provinces of Saskatchewan and Manitoba (Ivie, 2001). Further WSS infestations continued as more land was put into wheat production across the Canadian prairies and northern portions of the American Midwest (Beres et al., 2011a). By 1910, damage had been reported in spring wheat production of Montana and North Dakota (Beres et al., 2011a; Ivie, 2001).

Conflicting theories exist for the origins of WSS in North America. Ivie (2001) hypothesized that WSS was introduced from northeastern Asia based on six different criteria: 1) presence of Eurasian populations, 2) early collections, 3) host plant presence, 4) biogeography of northeast Asia, 5) lack of parasitoids, and 6) opportunity for introductions. These criteria were based on the discrepancies between WSS and interactions with native host plants and parasitoids in North America (Ivie, 2001). Stem diameter of native host plants seemed to be too small to support WSS, and parasitoids were not well synchronized with the life cycle of the WSS (Ivie, 2001). Lesieur et al. (2016) published the first paper showing that the WSS was indeed endemic to North America. Sequencing for two different loci (16S and CO1) in WSS samples from Canada and the United States concluded that North American *Cephus cinctus* were indeed genetically different from *Cephus cinctus* of Asia (Lesieur et al., 2016). Three distinct genetic clusters were identified in North America: the northern group (Canada and Montana), the southern group (Wyoming, Nebraska, and Colorado), and the mountain group (western Montana and

Idaho). The likely explanation for the different genetic groups is due to adaptation of local WSS populations to wheat from surrounding wildland areas (F. Peairs, personal communication).

Biology and Life Cycle

Cephus cinctus belongs to the family *Cephidae* and the order *Hymenoptera* - consisting of female insects with a saw-like ovipositor for inserting eggs into a host (Robertson et al., 2018). Adult WSS have a wasp-like appearance; with a total length of 12 mm and the presence of three yellow bands across the abdomen (Fisher, 2017; Irell & Peairs, 2010). Because males are haploids and females are diploids, unfertilized eggs produce male offspring with one set of chromosomes, while fertilized eggs produce females WSS with two sets of chromosomes (Beres et al., 2011a).

Adult WSS start emergence in late May or early June in Colorado – when field conditions are near 10 ºC and winds are calm (Fisher, 2017; Irell & Peairs, 2010). Male WSS typically emerge before females (Beres et al., 2011a) and live for approximately one week (Fulbright et al., 2017; Irell & Peairs, 2010). The flight period lasts for about three to six weeks, with only one generation being produced per year (Beres et al., 2011a). Oviposition occurs shortly after emergence, with eggs laid at the beginning of the flight period producing mostly female WSS in comparison to the end of emergence where most eggs produce male offspring (Beres et al., 2011a). The phenology of wheat provides a suitable host for oviposition due its larger stem diameter, stem hollowness, and stem greenness prior to spike emergence at Feekes scale 10 (Large, 1954). Each female carries approximately 50 eggs and will oviposit one egg per stem, between the 2nd to 4th internode (Beres et al., 2011a). Larval emergence occurs six to seven days after the egg has been laid (Beres et al., 2011a).

Multiple eggs can be laid into a single stem if an abundance of WSS are present due to females not being able to discern which stems have already been infested (Beres et al., 2011a). Only one larva will survive to maturity, however it is unclear if this is caused directly by cannibalism or indirectly through feeding activities (Beres et al., 2011a; Fisher, 2017; Irell & Peairs, 2010). Wheat stem sawfly has been

described as a weak flier and will only travel as far as needed to find a suitable host (Irell & Peairs, 2010). This behavior results in infestations starting on the edges of fields before moving inward (Ainslie, 1920).

Hatched larvae feed within the stem throughout the summer until environmental cues induce a mandatory diapause. These environmental cues include light penetration of the stem wall and a decrease in stem moisture concentration (Beres et al., 2011a). The maturation and drying of the host plant signal the larvae to start preparing for overwintering by moving down the stem where the creation of a hibernaculum is initiated (Holmes, 1975). Creating an overwintering chamber requires the larvae to cut a 'v' notch into the stem of the plant before plugging the stem with frass – resulting in the girdling of the wheat stem near the soil surface (Holmes, 1975). The newly formed cocoon sits just below the soil surface and protects the larvae from fluctuations in moisture and temperature levels (Ainslie, 1920). Once overwintering is complete, adult WSS emerge the following spring by chewing through the plugged stub - starting the cycle over again (Weiss & Morrill, 1992).

Economic Impact from Field Damage

Direct damage from WSS occurs in two main ways: 1) reduced kernel weight (and thus grain yield) and 2) lodging of wheat at harvest. Larval feeding disrupts translocation of carbohydrates, protein, and other minerals to developing wheat kernels (Morrill et al., 1994). Stem feeding activities also result in the flag leaf having a reduced photosynthetic rate (Delaney et al., 2010). Additionally, phosphorus stress and water stress can increase WSS damage by up to 50% (Delaney et al., 2010). Amount of feeding damage is positively correlated with amount of time larvae spend within the stem (Beres et al., 2011a). Feeding damage can cause yield losses of up to 20% (Weaver et al., 2004) and further economic damages can be occurred by the producer if the crop is sold for seed – due to reduced germination rates from shrunken seeds (Delaney et al., 2010) and increased "clean out" during the seed conditioning process from a greater numbers of shrunken kernels (C. Mertens, personal communication). Additional damage occurs at plant senescence, when larvae cut a notch in the stem for overwintering preparation (Beres et al., 2011a). Girdled stems are susceptible to lodging from wind and rain events – resulting in a 5-10% yield

loss due to a lower efficiency in harvesting lodged plants by having to combine slower, in only one direction, and the inability to pick cut stems off the ground (Fisher, 2017).

Damage from lodging and reduced kernel weight can cause yield loss greater than 25% (Beres et al., 2011a), however growers have reported damage of more than 50% in extremely susceptible varieties (C. Mertens, personal communication). Additional economic losses occur in wheat-based cropping systems, common in eastern Colorado, due to a reduction in crop residue persistence and soil moisture availability (C. Mertens, personal communication). The preserved wheat stubble is important for water conservation and soil erosion in these production systems to provide moisture for the following summer crop (Fisher, 2017). The economic cost from this has yet to be explored, but producers have commented that subsequent maize (*Zea mays*) crops can yield approximately 627 kg/ha lower. Together, lodging and kernel reduction causes $25 million in damages annually for Montana (Berzonsky et al., 2002), with an estimated economic loss of $350 million per year across North America (Beres et al., 2011a). However, the annual estimate of WSS damage for North American was calculated before WSS exploded further south in the Great Plans.

Transition to Winter Wheat

While WSS had historically been more of a problem in spring wheat, more recently a host-plant shift resulted in damage now occurring in winter wheat. Wheat stem sawfly was first observed damaging winter wheat in Montana during the 1980s when adult WSS started to emerge earlier in the spring to match the phenology of winter wheat (Morrill & Kushnak, 1996). Recent winter wheat infestations in Colorado are also a result of endemic WSS populations shifting host-plant preference (F. Peairs, personal communication). Damage in winter wheat occurred in Colorado in 2010 when the first WSS infestations were reported in Weld County, Colorado (Irell & Peairs, 2010). It is unknown how WSS in Colorado became adapted to winter wheat, but the most likely explanation is that WSS made a shift of host-plant preferences from native and non-native grass species to winter wheat (Lesieur et al., 2016). Stem diameter plays a role for WSS preference and wheat has a larger diameter stem than most non-cultivated grasses

(Morrill & Weaver, 2000). A study by Cockrell et al. (2017) showed that the Colorado WSS population prefers oviposition on winter wheat compared to the non-cultivated grass downy brome *(Bromus tectorum L.*).

Management Strategies

A combination of chemical, cultural, and biological management options has been explored for mitigating WSS.

Control Option - Chemical

The prolonged larval life cycle within the stem of wheat plants poses a challenge to growers in combatting WSS infestations. Approximately 97% of the WSS lifecycle occurs within the stem (Beres et al., 2011b), and effectiveness of an insecticide is dependent on direct contact with the target pest (Bekkerman & Weaver, 2018). Protection within the stem, combined with an extended flight period, has inhibited the effective use of chemical controls for combating WSS (Weiss & Morrill, 1992). There are no insecticides or seed treatments currently registered for controlling WSS (Beres et al., 2011b). The most effective pesticide against WSS larvae was heptachlor, an organochlorine compound, but this pesticide has been banned in the USA since 1998 due to prolonged residual effects in soil and plants (Beres et al., 2011a). A special pesticide registration of an organophosphate (phorate) was approved for wheat growers in Montana (Boswell, 2015), but this registration has also been removed due to concerns over environmental and human health risks (F. Peairs, personal communication).

Control Option – Cultural

Several cultural practices have been implemented to aid in control of WSS. Tillage of wheat stubble was the first control method implemented to combat WSS infestations (Beres et al., 2011b). Criddle (1922) suggested use of a moldboard plow to expose wheat roots to environmental conditions. Colder temperatures lead to increased mortality of overwintering larvae, however the effectiveness of this

practice doesn't affect a significant proportion of WSS to reduce damage (Weaver et al., 2004). Survival of parasitoids is also reduced due to their overwintering occurring aboveground in the wheat stubble left after harvest (Weaver et al., 2004). Recent adoption of no-till agriculture and emphasis on soil conservation has led to reduced interest in traditional tillage operations (Beres et al., 2011b). Burning of stubble has also been tried but was ineffective because of the high level of protection provided within the wheat stub (Ainslie, 1920).

Alteration of planting methods has been shown to be somewhat effective for decreasing WSS populations. Traditionally, wheat growers in Colorado have planted in long narrow strips to limit soil erosion (Fisher, 2017), but planting in larger blocks can reduce the total length of field edges (Weaver et al., 2004). This process has been widely adopted in Montana, but the only benefits seem to be from convenience of planting in larger blocks than a significant reduction in overall WSS damage (Weaver et al., 2004). Delayed planting of spring wheat can also allow wheat to escape WSS pressure (Weaver et al., 2004), however the planting window is already short for winter wheat in Colorado – making it impractical. Finally, changing row spacing has been shown to decrease cutting of hollow-stemmed varieties (Beres et al., 2011b). Narrower spacing combined with a high seeding rate was shown to decrease cutting in hollowed-stemmed genotypes, however this practice tends to have a negative effect on the amount of pith expression within the stem of solid-stem varieties (Beres et al., 2011b).

Because infestations start on field margins, planting trap crops along the field perimeter in tandem with swathing has been proposed as an effective control method (Beres et al., 2011a). Trap crops can either be a resistant solid-stem variety or other host crops such as barley (*Hordeum vulgare*), oats (*Avena sativa*), and triticale (× *Triticosecale*) (Criddel, 1922). These alternate hosts are less susceptible to WSS cutting and lead to a higher rate of larval mortality (Biyiklioglu et al., 2018), however trap crops need to be at the proper stem elongation stage for them to be a viable host for WSS (Weaver et al., 2004). Additional research has looked at removing the trap crop as hay (Beres et al., 2011a). This practice helps to reduce WSS damage, but it has been shown to be less effective at reducing the underlying WSS population since swathing occurs after WSS migrate down the stem after feeding damage has already

occurred (Bekkerman & Weaver, 2018). A study by Bekkerman and Weaver (2018) showed that either swathing or solid-stem planting result in similar damage reductions for the first 4–5 years, however the planting of a solid-stem variety decreased the WSS population to a lower infestation level, compared to swathing, after 5 years.

Control Option - Biological

Insect species within the *Hymenoptera* order can act as a biological control agent for WSS (Shanower & Hoelmer, 2004). Two species of Hymenoptera, *Bracon cephi* and *Bracon lissogaster*, are major parasitoids of WSS in wheat (Beres et al., 2011a). Both parasitoids may produce two generations of adults per year (Shanower & Hoelmer, 2004). The first emergence of *B. cephi* and *B. lissogaster* occurs alongside the flight period of WSS in late spring and early summer (Beres et al., 2011a), however the second generation of emergence can be affected by the wheat harvest in late summer (Portman et al., 2018). Because these parasitoids overwinter in higher internodes, cultural practices can affect subsequent parasitoid populations (Portman et al., 2018). Wheat stem sawfly biocontrol effects have been previously unsuccessful, because of issues with rearing, timing of release, and losses from shipping of parasitoids (Shanower & Hoelmer, 2004).

Control Option - Plant Resistance

Of all the control options, host-plant resistance has been the most reliable and effective method for controlling WSS (Sherman et al., 2010). Observations of European WSS (*C. pygmaeus*) in the 1920s showed that host-plants with a higher expression of pith within the stem inhibited the development of WSS larvae (Weiss & Morrill, 1992). These conclusions led Kemp (1934) to suggest the use of mechanical resistance offered by solid-stemmed landraces for use in wheat varieties to hinder larval development, resulting in the Canadian government evaluating wheat germplasm from around the world (New Zealand, Spain, Morocco, and Portugal) to identify landraces with increased pith expression (Beres et al., 2011a). One landrace, ‘S-615’ (CI 12157), was identified from Portugal that showed high levels of

stem solidness (Beres et al., 2011a). In 1948, 'Rescue' (CItr 12435) became the first WSS-resistant variety released by crossing S-615 with the hollow-stemmed variety 'Apex' (CItr 11636) (Beres et al., 2011a). Rescue helped to reduce damage by 95% in comparison to susceptible varieties (Weiss & Morrill, 1992), and breeding for WSS resistance has since been dominated by selecting for stem solidness. However, the success of Rescue was short lived, when high rates of WSS sawfly cutting was observed in Regina, SK, Canada (Beres et al., 2007) due to inconsistent pith expression.

Genetic Basis of Resistance

Three broad classes of insect resistance are currently used in wheat cultivar development: antibiosis, antixenosis, and tolerance (Berzonsky et al., 2002). Antibiosis resistance mechanisms for WSS reduce the growth or survival of larvae in the stem (Berzonsky et al., 2002), while antixenosis relates to the preference of female WSS during oviposition (Berzonsky et al., 2002). Tolerance allows plants to withstand damage from the mature larval feeding - without a significant loss in yield (Sherman et al., 2010). Traditionally, breeding for WSS resistance has relied on the antibiosis resistance provided by stem solidness (Berzonsky et al., 2002), however recent studies have identified new QTL with antixenosis resistance mechanisms provided by host-plant preference.

Expression of Stem Solidness

The most common source of stem solidness is conferred by the presence of a major QTL located on the long arm of chromosome 3B (Cook et al., 2004). Multiple alleles at *Qss.msub-3BL* account for more than 76% of the phenotypic variation in pith expression (Cook et al., 2004) . Stem solidness provides resistance by physically reducing the ability of larvae to move within the stem - resulting in larval mortality (Talbert et al., 2014). Because larvae die prematurely before migrating down into the stem base, the percentage of stem cutting is reduced (Sherman et al., 2010). Stem solidness also affects the ability of female WSS to oviposition since they are restricted to stems their ovipositor can penetrate

(Sherman et al., 2010). While the 3BL QTL may not always confer sufficient stem solidness (Cook et al., 2004), this QTL has been the primary source of stem solidness for breeding programs targeting this trait.

After Rescue was commercially released, the expression of stem solidness was discovered to be affected by environmental factors including photoperiod, temperature, moisture, and plant spacing (Beres et al., 2011b). The stem solidness trait introgressed from S-615 is influenced by 3 to 4 recessive genes - providing inconsistent pith expression (Beres et al., 2017). This results in varieties with resistance derived from S-615 to have varying pith expression when exposed to different photoperiods (Beres et al., 2011b). Stem elongation is greatly affected by sunlight and high light intensity will result in maximum pith expression, while prolonged cloudy conditions can cause a reduction in solidness (Beres et al., 2011b). Other factors also affect pith expression, with Nilsen et al. (2016) demonstrating higher sowing density having a negative effect on the expression of stem solidness, and Beres et al. (2017) reporting that timing of precipitation during the growing season influences stem solidness. While precipitation cannot be altered, a planting density below 350 plants per square meter can maximize pith expression - with a target planting density of 250-350 seeds m^{-2} (Beres et al., 2011b).

Besides inconsistency in the expression of the solid-stemmed trait, lower yield potential associated with stem solidness has also contributed to the reluctance of growers to plant solid-stemmed varieties derived from S-615 (Weiss & Morrill, 1992). Solid stemmed varieties may be lower yielding than their hollow-stemmed counterparts because of the introgression of genetically inferior alleles from S-615, the diversion of nutrients from developing wheat kernels to parenchyma cells for pith expression (Sherman et al., 2015), or both. Several studies have evaluated the effect of stem solidness and grain yield in offspring from crosses between solid and hollow-stemmed varieties and concluded there is no genetic correlation between the two traits (Hayat et al., 1995; Sherman et al., 2015). Newer solid-stem varieties still yield lower than their hollow-stemmed counterparts, however their yield under high WSS pressure is significantly greater (Beres et al., 2009; Beres et al., 2007; Bruckner & Berg, 2016).

Over the last 10 years, several QTL studies have identified new loci that contribute to stem solidness. Shortly after the discovery of the stem solidness locus on 3BL (Cook et al., 2004), Lanning et

al. (2006) reported a QTL on the long arm of chromosome 3D which also affected stem solidness, however this QTL was not consistently reported in a follow up study by Talbert et al. (2014). While evaluating a collection of recombinant inbred lines (RILs) developed using a landrace from Turkey (PI 41353), a novel QTL on 1B was identified as a source of stem solidness (Varella et al., 2019). Additional chromosomes identified with stem solidness QTL include 4B (Varella et al., 2019) and 2D (Sherman et al., 2010). Stacking of minor QTLs for stem solidness may be needed to achieve a high level of pith expression when developing a new variety (Varella et al., 2019).

Wheat Stem Sawfly Resistance in Durum Wheat

Evaluation of durum wheat (*Triticum turgidum* L. var *durum*) varieties has shown that stem solidness is also an important trait for decreasing WSS damage. Houshmand et al. (2007) evaluated two doubled haploid populations derived from the resistant parents 'Golden Ball' (CItr 11477) and 'Kyle*2/Biodur' (PI 591067). The discovered source of stem solidness in durum wheat population mapped to a similar genomic region as the *Qss.msub*-3BL allele found in hexaploid wheat. Microsatellite markers associated with stem solidness were located to the 3BL genomic region and the durum-derived allele was named *SSt1* (Houshmand et al., 2007). The difference in ploidy levels between durum and bread wheat has hindered the transfer of important genes (Lanning et al., 2008), resulting in this resistance allele not being identified outside of durum cultivars. A study by Beres et al. (2013) showed that varieties with *SSt1*-derived solidness had increased resistance to WSS.

Temporally-Expressed Stem-Solidness Resistance

A new allele variant for stem-solidness was recently discovered from evaluations of the wheat variety 'Conan' (Sherman et al., 2010). Conan is a hard red spring wheat developed by WestBred (Bayer Group, Germany) and released as a commercial cultivar in 1999. Sherman et al. (2010) used Conan in a QTL mapping study by creating a RIL population with the WSS susceptible variety 'Reeder' (PI 613586). The allele provided by Conan at the *Qss.msub*-3BL locus conferred less infestation and stem cutting,

despite only having a semi-solid stem, and it was concluded that Conan had a different allele variant at the 3BL locus (Talbert et al., 2014). Varella et al. (2017) showed that the resistance mechanism behind the Conan allele (*Qss.msub*-3BL.c) was caused by an early expression of pith during female WSS oviposition, followed by pith reduction at plant maturity. The higher pith expression was attributed to a greater moisture content within the stem at larval hatching – increasing the mortality rate before stem cutting (Varella et al., 2017). The *Qss.msub*-3BL.c allele has been shown to not have a negative effect on grain yield in studies by Talbert et al. (2014) and more recently by Cook et al. (2019). An association analysis of elite spring wheat cultivars from North America showed two other QTLs on 1B and 5D may also affect the expression of early stem solidness (Varella et al., 2015).

An allele with similar function as *QSS.msub*-3BL.c was reported by Varella et al. (2019) in a RIL population of durum wheat. The allele from the variety 'Pierce' (PI 632366) on chromosome 3A caused a reduction in cutting of 25%. Named *Qss.msub*-3AL, the allele promotes stem solidness early in the growing season and retraction of pith at plant maturity. Because this phenotype appears only during stem elongation, screening of offspring for early stem solidness expression needs to occur during stem elongation rather than at maturity (Varella et al., 2019).

Wheat Stem Sawfly Resistance in Barley

For over 70 years, barley has been known to have a higher level of resistance to WSS than wheat (Platt & Farstad, 1946), but few studies have been conducted to evaluate its effectiveness. Varella et al. (2018) was the first publication to evaluate a set of spring barley cultivars for resistance to WSS damage. A combination of resistance mechanisms was observed to be present for WSS resistance without the presence of a stem-solidness trait (Varella et al., 2018). Each of the six tested varieties had lower cutting than the susceptible hexaploid wheat check (Varella et al., 2018). No QTL were reported from the study, due a low phenotypic variation within the population (n = 193), however the potential for success of

introgression of alleles from barley into bread wheat is not possible without the use of genetic transformation (Sherman et al., 2001).

Host-Plant Preference

Recent research has shifted focus to developing varieties based on WSS host-plant preference (Talbert et al., 2014). Antixenosis forms of resistance may be more durable than antibiosis or tolerance mechanisms (Berzonsky et al., 2002). Sherman et al. (2010) identified a novel QTL on chromosome 2D (*Qwss.msub*-2D) for WSS infestation and cutting, while two other tightly linked QTL on 4A (*Qwss.msub*-4A.1 and *Qwss.msub*-4A.2) had different effects on WSS cutting and infestation. Near-isogenic lines (NILs) from Conan, Reeder, and 'Scholar' (PI 607557) were evaluated for WSS preference associated with the QTLs *Qwss.msub*-2D and *Qwss.msub*-4A.1 (Varella et al., 2017). Both *Qwss.msub*-4A.1 and *Qwss.msub*-2D affected female oviposition behavior and ability to find a suitable host (Varella et al., 2017). An evaluation of spring wheat germplasm from regions with endemic WSS populations found that the *Qwss.msub*-4A preference QTL was widely present in current elite spring wheat cultivars from North America (Varella et al., 2015).

The preference for different host plants can be affected by emissions of plant volatiles (Piesik et al., 2008). Wheat has been previously characterized as having a 'green odor' due to the presence of eight different 6-carbon aldehydes and alcohol volatile compounds (Piesik et al., 2008). The release of three different green-leaf volatiles, namely (Z)-3-hexenyl acetate, (Z)-3-hexenol, and 6-methyl-5-hepten-2-one, is associated with increased female WSS activity (Piesik et al., 2008). Another study using Conan and Reeder spring wheats showed increased infestation and oviposition from the presence of (z)-3-hexenyl acetate volatiles (Weaver et al., 2009). Because a higher amount of (z)-3-hexehyl acetate is present in Reeder during the stem elongation stage (Weaver et al., 2009), Y-tube olfactometer studies have shown that female WSS have a selection preference for Reeder over Conan (Varella et al., 2017). This result may be due to the difference in alleles present at the *Qwss.msub*-4A.1 locus between Conan and Reeder (Sherman et al., 2010), however no differences were observed between individual carrying the Conan

allele and a pure air control treatments (Varella et al., 2017). Wheat varieties with expression of specific plant volatiles may be useful as a trap crop (Piesik et al., 2008; Varella et al., 2017; Weaver et al., 2009), with a study by Buteler et al. (2010) identifying five different winter wheat cultivars for potential trap crop use in Montana. However, there are likely still additional genes affecting WSS preference that have not yet been identified (Varella et al., 2017).

Genotyping for WSS Resistance Traits in Winter Wheat

All the previously mentioned QTL studies have been done in spring wheat, with a lack of literature on QTL mapping for WSS resistance in winter wheat. Stem solidness traits have been introgressed into elite winter wheat cultivars, but the identification of novel WSS resistant traits associated with host-plant preference in spring wheat may also be beneficial for use in winter wheat. All the above identified QTLs are potential sources of resistance that can be used to complement already implemented stem solidness traits. To identify novel or current antixenosis alleles in elite winter wheat breeding germplasm, individuals need to be quickly screened for polymorphic molecular markers that are also closely linked to a QTL of interest (Collard et al., 2005). The use of next-generation-sequencing (NGS) along with QTL mapping will provide molecular markers that are useful for use in marker-assisted selection breeding efforts for WSS.

The use of NGS has drastically reduced the cost of genotyping and allows for large numbers of individuals to be sequenced for genetic studies (Poland et al., 2012). One exciting application of NGS has been the development of genotyping-by-sequencing (GBS) in commodity crops for generating large quantities of single nucleotide polymorphism (SNP) markers (Poland et al., 2012). Key features of GBS are low cost, no ascertainment bias, shortened laboratory protocol, and no size selection of DNA fragments (He et al., 2014; Poland et al., 2012). Application of GBS can be useful for genotyping individuals with or without a reference genome (Elshire et al., 2011).

Genotyping-by-Sequencing

GBS was originally developed at Cornell University by Edward Buckler's lab group (Elshire et al., 2011) and was modified for use in wheat and barley shortly thereafter (Poland et al., 2012). Briefly, two different methylation-sensitive enzymes (*PstI* and *MspI*) are used to produce uniform cut sites across the genome - reducing genome complexity by targeting only gene rich regions (Poland et al., 2012). Both restriction enzymes differ in their prevalence within the genome, with *PstI* (6 base pairs) being a rare cutting enzyme in comparison to the more common cutter *MspI* (4 base pairs) (Poland et al., 2012). After enzyme digestion, unique barcode adapters are ligated to each DNA fragment for pooling of individuals into a single library (Poland et al., 2012). Barcodes range in length from 4 to 9 bp and must meet three specific criteria for use: 1) barcodes must be two or more bp different from other barcodes; 2) barcodes cannot contain sequential bp of the same nucleotides; and 3) barcodes cannot contain or recreate (when ligated) the *PstI* or *MspI* restriction sites (Poland et al., 2012). After pooling of individuals, libraries are sequenced on individual flow lanes – generating short sequence reads between 50 to 300 bp (He et al., 2014). Reads are trimmed to 64 bp (barcode plus sequence) to reduce computational restraints (Poland et al., 2012). By using a cheap and robust barcoding system, samples can be multiplexed together for further cost reductions (He et al., 2014). Up to 384 individuals can be pooled together for sequencing on one Illumina flow lane (Glaubitz et al., 2014), resulting in a cost of $8 per sample (E. Hudson-Arns, personal communication).

Low cost per sample and reduced complexity in preparation of sequencing libraries has allowed for wide application of GBS, however difficulties persist with application of generated data. Pitfalls of GBS include a higher percentage of missing data (especially at higher levels of multiplexing), reduction in read depth at higher multiplexing levels, misalignment of reads to a reference genome, and complex bioinformatic tools for analyzing sequencing data (He et al., 2014; Poland et al., 2012). Sequencing on newer platforms, such as the Illumina HiSeq 4000, can generate between 280-330 million reads per flow lane (Illumina, Inc., San Diego, CA). The high number of sequencing reads causes analysis of GBS data to be more complex than other types of data (Liu et al., 2014). This challenge is even greater when

considering the size and complexity of the hexaploid wheat genome – totaling 17 Gb (five times the size of the human genome) and being comprised of 80% repetitive sequences (Brenchley et al., 2012). The large size and amount of repetitive sequences is a result of interspecific hybridization events that occurred with the diploid *Aegilops tauschii* (DD) and the tetraploid *Triticum turgidum* (AABB) – resulting in common wheat being an allohexaploid (AABBDD) (Brenchley et al., 2012). Each of the three sub genomes (A, B, and D) retained high genetic similarity, which can cause a SNP to map to a homoeologous chromosome (e.g., 1A, 1B, and 1D) or even to non-homoeologous chromosomes (e.g., 1A and 2B) (Arruda et al., 2016). This can result in incorrect SNP calls (Spindel et al., 2013). However, these issues can be overcome by higher read coverage on newer sequencing platforms and using programs like TASSEL-GBS to streamline bioinformatics (Glaubitz et al., 2014).

QTL Mapping

One major application of molecular markers is in the mapping of QTL. The goal of QTL mapping is to characterize the underlying genetic basis, either qualitative or quantitative, for observed phenotypic trait variation (Mackay et al., 2009; Mauricio, 2001). The idea of QTL mapping is not new, as Karl Sax (1923) discovered a major QTL for seed size in common bean (*Phaseolus vulgaris*) linked to seed coat color. However, the advent of SNP markers and computational tools has allowed for more complex traits to be mapped. A common analogy to describe QTL mapping is referring to the genome as the highway with SNPs acting as the mile markers (Collard et al., 2005). Once a roadmap of genetic markers has been created, statistical techniques can then locate regions within the genome that might contain genes controlling to the trait of interest (Mauricio, 2001). Quantitative trait loci mapping has been used in numerous crops to identify traits related to disease and insect resistance, end-use quality, and abiotic stresses - to name a few. Effective QTL mapping consists of three steps: development of a mapping population, creation of a genetic map, and QTL analysis (Collard et al., 2005).

Creation of a Mapping Population

For any QTL mapping study to be successful, an appropriate population, with parents differing for the trait of interest, needs to be created first. Quantitative trait loci can be identified by evaluating segregating progeny from a bi-parental cross or through association mapping, which uses a large panel of genetically different individuals (Mackay et al., 2009). With inbreeding species such as wheat, bi-parental populations are created by crossing two highly homozygous individuals which differ for a trait of interest (Collard et al., 2005). Examples of populations useful for bi-parental QTL mapping include recombinant inbred lines, F_2 individuals, doubled haploids, and backcrosses. Doubled haploid populations are advantageous over other types, because completely homozygous individuals can be obtained in a short timeframe (1-2 years) (Santra et al., 2017) and the populations are considered to be "immortal" as they do not change genetically with successive generations of seed production. Androgenesis (using anther culture methods) and wheat-maize wide hybridization are the two methods most commonly used to create doubled haploid populations in hexaploid wheat (Santra et al., 2017).

The number of individuals needed for a mapping population will vary depending on mapping resolution, trait heritability, and type of study being conducted (Mackay et al., 2009). Generally, bi-parental studies require fewer individuals than association studies, with Vales et al. (2005) suggesting that 150 individuals being sufficient for a bi-parental cross. However, smaller populations decrease the probability of identifying loci with small effects, overestimating a QTL's effect, and an inability to detect significant QTL x QTL interaction (Vales et al., 2005). These issues decrease the accuracy of measuring the recombination frequency and determining final marker order (Collard et al., 2005).

Genetic Mapping

The next step in QTL mapping is the creation of a genetic linkage map from the genotypic data. A genetic linkage map consists of ordered markers derived from their percentages of recombination (converted to centiMorgan, cM) (Collard et al., 2005). A cM value of less than 10% is equal to the recombination frequency, however genetic distance is not equal to physical distance (Mackay et al.,

2009). Markers closer together do not segregate independently and are said to be in linkage disequilibrium (LD) (Mackay et al., 2009). When recombination is 50% between two markers they are no longer in linkage disequilibrium, suggesting they are far apart on the same chromosome, located on different chromosome arms or different chromosomes (Mackay et al., 2009). Linkage between markers is calculated by setting a logarithm of odds (LOD) value of greater than 3.0 for linkage map construction (Collard et al., 2005). A LOD score of 3.0 means a linkage is 1000 times more likely than no linkage between two markers (Zuo et al., 2019). Markers are then arranged by LOD values for determining linkage groups (LG) before reordering markers in each LG (Verma et al., 2015).

Exclusion of markers based on percentage of missing data across the population and segregation distortion have been used to ensure that only high quality markers are used in the final genetic map. Missing values reduces the number of correctly ordered markers (more pronounced at smaller intervals) (Hackett & Broadfoot, 2003) – decreasing QTL detection and increasing the false-discovery rate (Zhang et al., 2010). Excluding all the highly distorted markers decreases the total genetic distances but increases the marker interval (Zuo et al., 2019). Instead, retaining distorted markers allows for improved grouping (within the same chromosome) and increased marker coverage (Zuo et al., 2019). Segregation distortion will affect the QTL position, if the marker is far away, but their effects can be ignored in larger populations (Zhang et al., 2010). Every genetic map is unique and a product of the mapping population used (Collard et al., 2005).

QTL Analysis

The three most common methods for detecting QTLs are single-marker analysis, simple interval mapping, and composite interval mapping (Collard et al., 2005). Single-marker analysis, the simplest of the three methods by which detection of QTLs occurs, is done one marker at a time by conducting paired *t*-tests of all molecular markers with the trait of interest (Bernardo, 2020; Collard et al., 2005). Limitations of single-marker analysis are 1) the inability to detect a QTL if it's not near a marker, and 2) adjacent markers can unknowingly detect the same or different QTLs (Bernardo, 2020). Simple interval mapping

analyzes the interval between pairs of markers by using maximum likelihood to estimate the location of a QTL (Collard et al., 2005). A logarithm of odds (LOD) value is used to declare the presence of a QTL – with a LOD value of 3.0 being equal to a probability level of 0.001 (Bernardo, 2020). Problems with simple interval mapping include inaccurate estimation of the QTL position (especially if two QTLs are located close together) and can result in the detection of ghost (nonexistent) QTLs between flanking markers (Bernardo, 2020). Composite-interval mapping (CIM) overcomes the limitations of single-marker analysis and interval mapping by combining interval mapping with multiple regression (Bernardo, 2020; Collard et al., 2005). Location of a QTL between two markers is estimated by simple interval mapping and the effect of each QTL is estimated by multiple regression (Bernardo, 2020). This is done for each trait by using a subset of markers as cofactors - chosen by stepwise regression (Verma et al., 2015). Composite interval mapping is preferred over interval mapping because it reduces the background variation from other QTL (Bernardo, 2020).

Because many SNP markers are used in QTL analysis, the control of the family-wise error rate is important to limit the detection of spurious QTL. Two methods to reduce the rate of false-discovery are the use of a Bonferroni correction and permutation testing (Bernardo, 2020). A Bonferroni correction may be problematic since the estimate is strongly dependent on the marker density and size of the chromosomes (Cheverud, 2001). Modern approaches have instead used permutation testing because of their advantage in calculating the significance threshold from the population used for analysis. The LOD value produced from permutation testing also takes into account non-normality of phenotypes, missing data, and biased allele frequencies (Cheverud, 2001). Current software programs allow for thousands of permutation tests to be run simultaneously for multiple phenotypes in a short amount of time.

Many different software packages are available for QTL analysis. Current packages and programs available include R/qtl (Broman et al., 2003), QTL IciMapping (Meng et al., 2015), and QTL Cartographer (North Carolina State University, Raleigh, NC). R/qtl (package for the statistical software R (R Core Team, 2018)) and QTL IciMapping (standalone software) are both free programs that allow for creating genetic maps, permutation testing, and performing QTL scans. In comparison, QTL Cartographer

performs similar functions as above but requires a license fee for each user. Regardless of software used, results from QTL analysis can estimate additive effects, dominance effects, epistatic effects, and percent R^2 (contribution to phenotypic variance). Results from QTL analysis allow for better understanding of genetic factors controlling a phenotype of interest and if a given QTL may be useful for marker-assisted selection.

REFERENCES

Ainslie, C. N. (1920). The western grass-stem sawfly – a pest of small grains . USDA. Bulletin No. 157.

Arruda, M. P., Lipka, A. E., Brown, P. J., Krill, A. M., Thurber, C., Brown-Guedira, G., … Kolb, F. L. (2016). Comparing genomic selection and marker-assisted selection for Fusarium head blight resistance in wheat (Triticum aestivum L.). *Molecular Breeding*, *84*(36). https://doi.org/10.1007/s11032-016-0508-5

Bekkerman, A., & Weaver, D. K. (2018). Modeling joint dependence of managed ecosystems pests: The case of the wheat stem sawfly. *Journal of Agricultural and Resource Economics*, *43*(2), 172–194. https://doi.org/10.22004/ag.econ.273445

Beres, B. L., Carcamo, H. A., & Bremer, E. (2009). Evaluation of alternative planting strategies to reduce wheat stem sawfly (Hymenoptera: Cephidae) damage to spring wheat in the northern great plains. *Journal of Economic Entomology*, *102*(6), 2137–2145. https://doi.org/10.1603/029.102.0617

Beres, B. L., Cárcamo, H. A., & Byers, J. R. (2007). Effect of wheat stem sawfly damage on yield and quality of selected canadian spring wheat. *Journal of Economic Entomology*, *100*(1), 79–87. https://doi.org/10.1093/jee/100.1.79

Beres, B. L., Carcamo, H. A., Weaver, D. K., Dosdall, L. M., Evenden, M. L., Hill, B. D., … Spaner, D. M. (2011b). Integrating the building blocks of agronomy and biocontrol into an IPM strategy for wheat stem sawfly. *Praire Soils and Crops*, *4*, 54–65.

Beres, B. L., Dosdall, L. M., Weaver, D. K., Cárcamo, H. A., & Spaner, D. M. (2011a). Biology and integrated management of wheat stem sawfly and the need for continuing research. *Canadian Entomologist*, *143*(2), 105–125. https://doi.org/10.4039/n10-056

Beres, B. L., Carcamo, H. A., Byers, J. R., Clarke, F. R., Pozniak, C. J., Basu, S. K., & DePauw, R. M. (2013). Host plant interactions between wheat germplasm source and wheat stem sawfly Cephus cinctus Norton (Hymenoptera : Cephidae) I . Commercial cultivars. *Canadian Journal of Plant Science*, 93, 607–617. https://doi.org/10.4141/CJPS2012-088

Beres, B. L., Hill, B. D., Cárcamo, H. A., Knodel, J. J., Weaver, D. K., & Cuthbert, R. D. (2017). An artificial neural network model to predict wheat stem sawfly cutting in solid-stemmed wheat cultivars. *Canadian Journal of Plant Sciecne*, *336*, 329–336.

Bernardo, R. (2020). *Breeding for Quantitative Traits in Plants*. Woodbury, MN: Stemma Press

Berzonsky, W. A., Ding, H., Haley, S. D., Harris, M. O., Lamb, R. J., McKenzie, R. I. H., … Ohm H. W., (2002). Breeding Wheat for Resistance to Insects. In J. Janick (Ed) *Plant Breeding Reviews, Volume 22* (pp. 221–296). Hoboken, NJ: Wiley & Sons, Inc.

Biyiklioglu, S., Alptekin, B., Akpinar, B. A., Varella, A. C., Hofland, M. L., Weaver, D. K., … Budak, H. (2018). A large-scale multiomics analysis of wheat stem solidness and the wheat stem sawfly feeding response , and syntenic associations in barley , brachypodium , and rice. *Functional and Integrative Genomics 18*, 241–259. https://doi.org/10.1007/s10142-017-0585-5

Boswell, E. (2015). Insecticide now available to fight wheat stem sawfly in Montana. Retrieved from https://www.montana.edu/news/15597/insecticide-now-available-to-fight-wheat-stem-

Brenchley, R., Spannagl, M., Pfeifer, M., Baker, G. L. A., D'Amore, R., Allen, A. M. … Hall, N. (2012). Analysis of the bread wheat genome using whole-genome shotgun sequencing. *Nature*, *491*, 705–710. https://doi.org/10.1038/nature11650

Broman, K. W., Wu, H., Sen, S., & Churchill, G. A. (2003). R/qtl: QTL mapping in experimental crosses. *Bioinformatics*, *19*(7), 889-890. https://doi.org/10.1093/bioinformatics/btg11

Bruckner, P., & Berg, J. (2016). Recommended Solid-Stemmed Winter Wheat Varieties. Retrieved from http://plantsciences.montana.edu/foundationseed/quickfacts/Solid Winter Wheat Varieties 2016.pdf

Buteler, M., Weaver, D. K. Bruckner, P. L., Carlson, G. R., Berg, J. E., & Lamb, P. F. (2010). Using agronomic traits and semiochemicl production in winter wheat cultivars to identify suitable trap crops for the wheat stem sawfly. *The Canadian Entomologist, 142*(3), 222-233. https://doi.org/10.4039/n09-072

Cheverud, J. M. (2001). A simple correction for multiple comparisons in interval mapping genome scans. *Heredity*, *87*(1), 52–58. https://doi.org/10.1046/j.1365-2540.2001.00901.x

Cockrell, D. M., Griffin-Nolan, R. J., Rand, T. A., Altilmisani, N., Ode, P. J., & Peairs, F. (2017). Plant-insect interactions host plants of the wheat stem sawfly (Hymenoptera: Cephidae), *Environmental Entomology*, 46(4), 847–854. https://doi.org/10.1093/ee/nvx104

Collard, B. C., Jahufer, M. Z. Z., Brouwer, J. B., & Pang, E. C. K. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica*, *142*, 169–196. https://doi.org/10.1007/s10681-005-1681-5

Cook, J. P., Weaver, D. K., Varella, A. C., Sherman, J. D., Hofland, M. L., Heo, H.-Y. … Talbert, L. E. (2019). Compersion of three alleles at a major solid stem QTL for wheat stem sawfly resistance and agronomic performance in hexaploid wheat. *Crop Breeding and Genetics, 59*(4), 1639-1647. https://doi.org/10.2135/cropsci2019.01.0009

Cook, J. P., Wichman, D. M., Martin, J. M., Bruckner, P. L., & Talbert, L. E. (2004). Identification of microsatellite markers associated with a stem solidness locus in wheat. *Crop Science*, *44*, 1397–1402.

Criddel, N. (1922). The western wheat stem sawfly in Canada. *Journal of Economic Entomology*, *15*(3), 221. https://doi.org/10.1093/jee/15.3.221

Delaney, K. J., Weaver, D. K., & Peterson, R. K. D. (2010). Photosynthesis and yield reductions from wheat stem sawfly (Hymenoptera : Cephidae): Interactions with wheat solidness , water stress , and phosphorus deficiency. *Journal of Economic Entomology*, *103*(2), 516–524. https://doi.org/10.1603/EC09229

Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., & Mitchell, S. E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS ONE*, *6*(5). https://doi.org/10.1371/journal.pone.0019379

Fisher, M. (2017). Combating wheat stem sawfly in Colorado. *Crops and Soils Magazine*, (November-December), 4–8. Retrieved from dl.sciencesocieties.or/publications/crops-and-soils

Fulbright, J., Wanner, K., Bekkerman, A., & Weaver, D. (2017). Wheat stem sawfly biology. *Montana State University Extension*, *MontGuide* (MT201107AG). Retrived from https://agresearch.montana.edu/wtarc/producerinfo/entomology-insect-ecology/WheatStemSawfly/WSS-MontGuide.pdf

Glaubitz, J. C., Casstevens, T. M., Lu, F., Harriman, J., Elshire, R. J., & Sun, Q. (2014). TASSEL-GBS: A high capacity genotyping by sequencing analysis pipeline. *PLoS ONE*, *9*(2). https://doi.org/10.1371/journal.pone.0090346

Hackett, C. A., & Broadfoot, L. B. (2003). Effects of genotyping errors, missing values and segregation distortion in molecular marker data on the construction of linkage maps. *Heredity*, *90*(1), 33–38. https://doi.org/10.1038/sj.hdy.6800173

Hayat, M. A., Martin, J. M., Lanning, S. P., McGuire, C. F., & Talbert, L. E. (1995). Variation for stem solidness and its association with agronomic traits in spring wheat. *Canadian Journal of Plant Science*, *75*, 775–780. https://doi.org/10.4141/cjps95-131

He, J., Zhao, X., Laroche, A., Lu, Z.-X., Liu, H., & Li, Z. (2014). Genotyping-by-sequencing (GBS), an ultimate marker-assisted selection (MAS) tool to accelerate plant breeding. *Frontiers in Plant Science*, *484*(5). https://doi.org/10.3389/fpls.2014.00484

Holmes, N. D. (1975). Effects of moisture, gravity, and light on the behavior of larvae of the wheat stem sawfly, Cephus cinctus (Hymenoptera: Cephidae). *Canadian Entomologist*, *107*, 391–401.

Houshmand, S., Knox, R. E., Clarke, F. R., & Clarke, J. M. (2007). Microsatellite markers flanking a stem solidness gene on chromosome 3BL in durum wheat. *Molecular Breeding*, *20*(3), 261–270. https://doi.org/10.1007/s11032-007-9088-8

Hudons-Arns, E. (2019, September 23). Personal interview.

Irell, B., & Peairs, F. B. (2010). Wheat stem aawfly : A new pest of Colorado wheat. *Colorado State University Extension*, (Fact Sheet No. 5.612). Retrieved from www.ext.colostate.edu

Ivie, M. A. (2001). On the geographic origin of the wheat stem sawfly (Hymenoptera : Cephidae). *American Entomologist*, *47*(2), 84–97.

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

Lanning, S. P., Fox, P., Elser, J., Martin, J. M., Blake, N. K., & Talbert, L. E. (2006). Microsatellite markers sssociated with a secondary stem solidness locus in wheat. *Crop Science*, *46*, 1701–1703. https://doi.org/10.2135/cropsci2005.10-0379

Lanning, S. P., Blake, N. K., Sherman J., Talbert, L. E. (2008). Variable production of tetraploid and hexaploid progney lines from spring wheat by durum wheat crosses. *Crop Science*, 48(1). https://doi.org/ 10.2135/cropsci2007.06.0334

Large, E. C. (1945). Growth stages in cereals. Illustration of the feeks scale. *Plant Pathology, 3*, 128-129

Lesieur, V., Martin, J.-F., Weaver, D. K., Hoelmer, K. A., Smith, D. R., Morrill, W. L., … Bon, M.-C. (2016). Phylogeography of the wheat stem sawfly, Cephus cinctus Norton (Hymenoptera: Cephidae): Implications for pest management. *PLOS ONE*, *11*(12), 1–19. https://doi.org/10.1371/journal.pone.0168370

Liu, H., Bayer, M., Druka, A., Russell, J. R., Hackett, C. A., Poland, J. A., … Waugh, R. (2014). An evaluation of genotyping by sequencing (GBS) to map the breviaristatum-e (ari-e) locus in cultivated barley. *BMC Genomics*, *104*(15). https://doi.org/10.1186/1471-2164-15-104

Mackay, T. F., Stone, E. A., & Ayroles, J. F. (2009). The genetics of quantitative traits: Challenges and prospects. *Annual Review of Genetics*, *10*(8), 303–339. https://doi.org/10.1038/nrg2612

Mauricio, R. (2001). Mapping quantitative trait loci in plants: Uses and caveats for evolutionary biology. *Nature Reviews Genetics*, *2*(5), 370–381. https://doi.org/10.1038/35072085

Meng, L., Li, H., Zhang, L., & Wang, J. (2015). QTL IciMapping : Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. *The Crop Journal*, *3*, 269–283. https://doi.org/10.1016/j.cj.2015.01.001

Merten, C. (2019, May 20). Personal interview. Mertens Diversified Ag, LLC, New Raymer, CO.

Morrill, W. L., & Kushnak, G. D. (1996). Wheat stem sawfly (Hymenoptera: Cephidae) adaptation to winter wheat. *Environmental Entomology*, *25*(5), 1128–1132. https://doi.org/10.1093/ee/25.5.1128

Morrill, W. L., Kushnak, G. D., Bruckner, P. L., & Gabor, J. W. (1994). Wheat stem sawfly (Hymenoptera: Cephidae) damage, rates of prasitism, and overwinter survival in resistant wheat lines. *Journal of Economic Entomology*, *87*(5), 1373–1376. https://doi.org/10.1093/jee/87.5.1373

Morrill, W. L., & Weaver, D. K. (2000). Host plant quality and male wheat stem sawfly (Hymenoptera: Cephidae) fitness. *Journal of Entomological Science*, *35*(4), 478–842. https://doi.org/10.18474/0749-8004-35.4.478%0A

Nilsen, K. T., Clarke, J. M., Beres, B. L., & Pozniak, C. J. (2016). Sowing density and cultivar effects on pith expression in solid-stemmed durum wheat. *Agronomy Journal,* 108(1), 219–228. https://doi.org/10.2134/agronj2015.0298

Peairs, F. (2018, October 18). Personal interview.

Piesik, D., Weaver, D. K., Runyon, J. B., Buteler, M., Peck, G. E., & Morrill, W. L. (2008). Behavioural responses of wheat stem sawflies to wheat volatiles. *Agricultural and Forest Entomology*, *10*(3). https://doi.org/10.1111/j.1461-9563.2008.00380.x

Platt, A. W., & Farstad, C. W. (1946). The reaction of wheat varieties to wheat stem sawfly attack. *Scientific Agriculture*, *26*, 231–247.

Poland, J., Brown, P. J., Sorrells, M. E., & Jannink, J.-L. (2012). Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. *PLoS ONE*, *7*(2). https://doi.org/10.1371/journal.pone.0032253

Portman, S. L., Jaronski, S. T., Weaver, D. K., & Reddy, G. P. (2018). Advancing biological control of the wheat stem sawfly: New strategies in a 100-yr struggle to manage a costly pest in the northern great plains. *Annals of the Entomological Society of America*, *111*(3), 1–7. https://doi.org/10.1093/aesa/say002

R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from http://www.R-project.org/.

Robertson, H. M., Waterhouse, R. M., Walden, K. K. O., Ruzzante, L., Reijnders, M. J. M. F., Coates, B. S., … Budak, H. (2018). Genome sequence of the wheat stem sawfly, Cephus cinctus, representing an early-branching lineage of the hymenoptera, illuminates evolution of Hymenopteran chemoreceptors. *Genome Biology and Evolution*, *10*(11), 2997–3011. https://doi.org/10.1093/gbe/evy232

Sax, K. (1923). The association of size differences with seed-coad pattern and pigmentation in Phaseolus vulgaris. *GENETICS, 8*(6), 552-560.

Santra, M., Wang, H., Seifert, S., & Haley, S. (2017). Doubled haploid laboratory protocol for wheat using wheat--maize wide hybridization. In P. L. Bhalla & M. B. Singh (Eds.), *Wheat Biotechnology: Methods and Protocols* (pp. 235–249). https://doi.org/10.1007/978-1-4939-7337-8_14

Shanower, T. G., & Hoelmer, K. A. (2004). Biological control of wheat stem sawflies: Past and future. *Journal of Agricultural and Urban Entomology*, *21*(4), 197–221.

Sherman, J. D., Blake, N. K., Martin, J. M., Kephart, K. D., Smith, J., Clark, D. R., … Talbert, L. E. (2015). Agronomic impact of a stem solidness gene in near-isogenic lines of wheat. *Crop Science*, *55*, 514–520. https://doi.org/10.2135/cropsci2014.05.0403

Sherman, J. D., Smith, L. Y., Blake, T. K., & Talbert, L. E. (2001). Identification of barley genome segments introgressed into wheat using PCR markers. *Genome*, *44*(1), 38–44. https://doi.org/10.1139/g00-092

Sherman, J. D., Weaver, D. K., Hofl, M. L., Sing, S. E., Buteler, M., Lanning, S. P., … Talbert, L. E. (2010). Identification of novel QTL for sawfly resistance in wheat. *Crop Science*, *50*, 73–86. https://doi.org/10.2135/cropsci2009.03.0145

Spindel, J., Wright, M., Chen, C., Cobb, J., Gage, J., Harrington, S., … McCouch, S. (2013). Bridging the genotyping gap: Using genotyping by sequencing (GBS) to add high-density SNP markers and new value to traditional bi-parental mapping and breeding populations. *Theoretical and Applied Genetics*, *126*(11), 2699–2716. https://doi.org/10.1007/s00122-013-2166-x

Talbert, L. E., Sherman, J. D., Hofland, M. L., Lanning, S. P., Blake, N. K., Grabble, R., … Weaver, D. K. (2014). Resistance to Cephus cinctus Norton, the wheat stem sawfly, in a recombinant inbred line population of wheat derived from two resistance sources. *Plant Breeding*, *133*(4). https://doi.org/10.1111/pbr.12184\

Vales, M. I., Schon, C. C., Capettini, F., Chen, X. M., Corey, A. E., Mather, D. E., … Hayes, P. M. (2005). Effect of population size on the estimation of QTL : A test using resistance to barley stripe rust. *Theoretical and Applied Genetics*, *111*, 1260–1270. https://doi.org/10.1007/s00122-005-0043-y

Varella, A. C., Talbert, L. E., Achhami, B. B., Blake, N. K., Hofland, M. L., Sherman, J. D., … Weaver, D. K. (2018). Characterization of resistance to Cephus cinctus (Hymenoptera : Cephidae) in barley germplasm. *Functional & Integrative Genomics, 111*(2), 923–930. https://doi.org/10.1093/jee/toy025

Varella, A. C., Weaver, D. K., Blake, N. K., Hofland, M. L., Heo, H.-Y., Cook, J. P., … Talbert, L. E. (2019). Analysis of recombinant inbred line populations derived from wheat landraces to identify new genes for wheat stem sawfly resistance. *Theoretical and Applied Genetics*, *132*, 2195–2207. https://doi.org/10.1007/s00122-019-03347-8

Varella, A. C., Weaver, D. K., Peterson, R. K. D., Sherman, J. D., Hofland, M. L., Blake, N. K., … Talbert, L. E. (2017). Host plant quantitative trait loci affect specific behavioral sequences in oviposition by a stem - mining insect. *Theoretical and Applied Genetics*, *130*(1), 187–197. https://doi.org/10.1007/s00122-016-2805-0

Varella, A. C., Weaver, D. K., Sherman, J. D., Blake, N. K., Heo, H. Y., Kalous, J. R., … Talbert, L. E. (2015). Association analysis of stem solidness and wheat stem sawfly resistance in a panel of North American spring wheat germplasm. *Crop Science*, *55*(5). 2046-2055. https://doi.org/10.2135/cropsci2014.12.0852

Verma, S., Gupta, S., Bandhiwal, N., Kumar, T., Bharadwaj, C., & Bhatia, S. (2015). High-density linkage map construction and mapping of seed trait QTLs in chickpea (Cicer arietinum L.) using genotyping-by-sequencing (GBS). *Scientific Reports*, *5*, 1–14. https://doi.org/10.1038/srep17512

Weaver, D. K., Buteler, M., Hofland, M. L., Runyon, J. B., Nansen, C., Talbert, L. E., … Carlson, G. R. (2009). Cultivar preferences of ovipositing wheat stem sawflies as influenced by the amount of volatile attractant. *Journal of Economic Entomology*, *102*(3), 1009–1017. https://doi.org/10.1603/029.102.0320

Weaver, D. K., Sing, S. E., Runyon, J. B., & Morrill, W. L. (2004). Potential impact of cultural practices on wheat stem sawfly (Hymenoptera : Cephidae) and associated parasitoids. *Journal of Agricultural and Urban Entomology*, *21*(4), 271–287.

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

Zhang, L., Wang, S., Li, H., Deng, Q., Zheng, A., Li, S., … Wang, J. (2010). Effects of missing marker and segregation distortion on QTL mapping in F2 populations. *Theoretical and Applied Genetics*, *121*(6), 1071–1082. https://doi.org/10.1007/s00122-010-1372-z

Zuo, J. F., Niu, Y., Cheng, P., Feng, J. Y., Han, S. F., Zhang, Y. H., … Zhang, Y. M. (2019). Effect of marker segregation distortion on high density linkage map construction and QTL mapping in soybean (Glycine max L.). *Heredity*, *123*(5), 579–592. https://doi.org/10.1038/s41437-019-0238-7

CHAPTER II – CHARACTERIZATION AND QUANTITATIVE TRAIT LOCI (QTL) ANALYSIS FOR WHEAT STEM SAWFLY (HYMENOPTERA: CEPHIDAE) RESISTANCE IN WINTER WHEAT

INTRODUCTION

Edward Norton first documented the wheat stem sawfly (*Cephus cinctus*) infesting native grasses of Colorado in 1872 (Fulbright, et al., 2017; Ivie, 2001; Lesieur et al., 2016). However, it was not until 2010 that damage in winter wheat (*Triticum aestivum*) first appeared along Highway 14 in Weld County, Colorado (Irell & Peairs, 2010). Wheat stem sawfly (WSS) has become the number one threat to winter wheat in the western Great Plains over the past ten years. Colorado wheat producers planted 870,000 hectares of wheat in 2019, worth an estimated value of 387 million dollars (USDA NASS, 2019), but severe yield losses caused by WSS threaten to disrupt the livelihoods of rural communities. As the spread of WSS continues in Colorado, identification of new resistance sources will be needed for improving winter wheat varieties.

Direct damage from WSS occurs in two main ways: 1) reduced kernel weight (affecting grain yield) and 2) lodging of wheat at harvest. Larval feeding disrupts translocation of carbohydrates, protein, and other minerals to developing wheat kernels (Morrill et al., 994), and can cause yield losses of up to 20% (Weaver et al., 2004). Additional damage occurs at plant senescence, when larvae cut a notch in the stem for overwintering preparation (Beres et al., 2011a). Girdled stems are susceptible to lodging from wind and rain events – resulting in a 5-10% yield loss due to a lower efficiency in harvesting lodged plants by having to combine slower, in only one direction, and the inability to pick cut stems off the ground (Fisher, 2017). Damage from lodging and reduced kernel weight can cause yield losses greater than 25% (Beres et al., 2011a), however growers have reported damage of more than 50% in extremely susceptible varieties (C. Mertens, personal communication). Additional economic losses occur in wheat-

based cropping systems, common in eastern Colorado, due to a reduction in crop residue persistence and soil moisture availability (C. Mertens, personal communication).

Controlling WSS has proven difficult due to the protection provided by the wheat stem during larval development. Approximately 97% of the WSS lifecycle occurs within the wheat stem (Beres et al., 2011b), inhibiting the use of chemical controls (Weiss & Morrill, 1992). Current control options have instead relied on a mixture of cultural and physical management tools including burning of stubble, tillage of soil, swathing, and use of trap crops (Beres et al., 2011a). With limited options available, the primary control option has instead centered around use of a resistant cultivars to reduce the damage from the WSS.

For over 70 years, deposition of pith within the stem has been the main method of plant resistance against WSS (Beres et al., 2011a). The main source of stem solidness used by breeders is conferred by the presence of a major quantitative trait locus (QTL) located on the long arm of chromosome 3B (Cook et al., 2004) that was originally introgressed from the Portuguese landrace 'S-615' (CI12157) into the spring wheat variety 'Rescue' (Cltr 12435) (Beres et al., 2011a). Rescue helped to reduce damages by 95% in comparison to susceptible varieties (Weiss & Morrill, 1992), however the success of Rescue was short lived, when high rates of WSS sawfly cutting was observed in Regina, SK, Canada due to inconsistent expression of pith (Beres et al., 2007). It was discovered that stem solidness was greatly affected by multiple environmental factors; causing varieties with the S-615 allele to have varying pith expression when exposed to varying light intensities (Beres et al., 2011b), sowing density (Nilsen et al., 2016), and precipitation amounts (Beres et al., 2017). The limitations of solid-stemmed varieties have resulted in the recent identification of novel QTL for reducing WSS infestation and cutting by host-plant preference (Sherman et al., 2010; Talbert et al., 2014; Varella et al., 2017).

Recent research has shifted focus to developing varieties based on WSS host-plant preference. Sherman et al. (2010) identified a novel QTL on chromosome 2D (*Qwss.msub*-2D) for WSS infestation and cutting, while two other tightly linked QTL on 4A (*Qwss.msub*-4A.1 and *Qwss.msub*-4A.2) reduced WSS cutting and infestation. Near-isogenic lines (NILs) from Conan, Reeder, and 'Scholar' (PI 607557)

were evaluated for WSS preference associated with the QTLs *Qwss.msub*-2D and *Qwss.msub*-4A.1 (Varella et al., 2017). Both *Qwss.msub*-4A.1 and *Qwss.msub*-2D affected female oviposition behavior and ability to find a suitable host (Varella et al., 2017). An evaluation of spring wheat germplasm from regions with endemic WSS populations found that the *Qwss.msub*-4A preference QTL was widely present in current elite spring wheat cultivars from North America (Varella et al., 2015).

All the above-mentioned QTL studies have been conducted in spring wheat, and a lack of literature on QTL mapping for WSS resistance in winter wheat is apparent. Stem-solidness traits have already been introgressed into elite winter wheat cultivars, but the identification of novel WSS resistant traits associated with host-plant preference, as previously done in spring wheat, may also prove useful in winter wheat. In this light, four parental lines showing resistance to WSS cutting were selected from the Colorado State University (CSU) Wheat Breeding Program to create two doubled haploid (DH) populations for use in genetic mapping of WSS resistance. Each DH population was planted at two location in eastern Colorado during the 2018-2019 and 2019-2020 growing seasons. The main objectives of this study were to 1) phenotype individuals from two DH populations for wheat stem sawfly damage under field conditions, 2) identify QTL associated with reduced WSS damage, and 3) characterize the resistance mechanisms for each identified QTL.

MATERIALS AND METHODS

Doubled Haploid Population Development

Selection of parents used in development of each mapping population was based on observations of WSS cutting scores from CSU Elite Trials conducted in northern Colorado during the 2014-2016 field seasons. From these preliminary evaluations, four hollow-stemmed hard red winter wheat genotypes were selected for DH population development: 'Denali' (PI 664256; Haley et al., 2012), 'Hatcher' (PI 638512; Haley et al., 2005), 'Avery' (PI 676977; Haley et al., 2018), and 'CO11D1397' [CO050337-2/Byrd (PI 664257; Haley et al., 2012)]. Hatcher and CO11D1397 both have both shown reduced stem cutting under WSS pressure, while Denali and Avery were selected as the susceptible parents due to their higher degree of stem cutting under WSS infestation.

Crosses between Denali/Hatcher and Avery/CO11D1397 were completed in the greenhouse during Fall 2014 and Spring 2015 for the development of each DH population. Completely homozygous individuals were obtained by the wheat-maize wide hybridization protocol as described by Santra et al. (2017). The Avery/CO11D1397 DH population consisted of 142 individuals, while the Denali/Hatcher DH population was larger with 208 individuals

Field Experiments

Site Locations

Two sites in Colorado, New Raymer and Orchard, were selected for evaluation of the two DH populations. Both the New Raymer site (40.57˚N, 103.90˚W) and the Orchard site (40.48˚N, 104.11˚W), were chosen for their prior history of severe WSS infestation and damage (Figure 1). The New Raymer and Orchard sites are both managed as no-till production systems and have a similar clay loam soil profile with an average 38 cm of annual precipitation (Web Soil Survey, 2018). Experiments were conducted for two consecutive years, 2018-19 (hereafter designated as 2019) and 2019-20 (hereafter designated as 2020), adjacent to wheat fields that had severe WSS infestations in the previous year. Trials in 2019 were

planted on 25 September 2018 into maize (*Zea mays*) stubble at New Raymer and millet (*Panicum miliaceum*) stubble at Orchard. Because of time requirements to evaluate two populations, only the Avery/CO11D1397 population was selected for planting in the 2020 field season. The Avery/CO11D1397 population was chosen over the Denali/Hatcher population based on preliminary QTL results showing more major-effect QTL being detected in the Avery/CO11D1397 population. At both locations, the planting of the Avery/CO11D137 population was done on 24 September 2019 in fields adjacent to the prior year experiments.

Experimental Design of Field Trials

Field trial experiments were randomized as an augmented latinized row-column design in R (R Core Team, 2018) using the *DiGGer* package (Coombes, 2009). Five control varieties were included in both experiments: 'Bearpaw' (PI 665228; Carlson et al., 2013; solid stem), Byrd (hollow stem), 'Fortify SF' (semi-solid stem), 'Monarch' (PI 691606, hollow stem), 'Snowmass' (PI 658597; Haley et al., 2011; hollow stem), and Snowmass 2.0 (PI 691605, hollow stem). These varieties were selected based on prior observations of cutting under WSS infestation. Both DH populations, and their respective parents, were replicated three times in each trial whereas the control varieties were replicated 18 times in Avery/CO71D1396 and 15 times in Denali/Hatcher.

Trials were planted with a 6-row Hege head-row planter (WINTERSTEIGER AG, Austria). Each entry was planted at a rate of 5 g per plot into paired rows, with 23 cm spacing between rows and 33 cm spacing between each plot. Each plot was 1 m long, with a total trial dimension at each site of 70 rows by 18 columns (107 m x 9 m) in 2018 and 60 rows by 9 columns in 2019 (90 m x 4.5 m).

Phenotypic Evaluation

Phenotypic data were collected for the following traits: plant height, heading date, physiological maturity, cutting score, kernel weight, and stem solidness. Plant height was measured from the soil surface to the tip of the spikes (excluding awns). Heading date and physiological maturity were recorded

as the number of days from January 1 until 50% of the spikes had emerged from the leaf sheath (boot) and 50% of the plants within the plot had completely lost color in the peduncles. At harvest maturity (about 7 days after the last physiological maturity assessment), WSS cutting scores were determined by using a visual rating scale of 1 to 9; with 1 representing 10% or less cutting from WSS infestation and 9 representing greater than 90% WSS cutting. To evaluate kernel weight, 10 wheat spikes from each plot were collected at random at harvest. Heads from each plot were threshed and cleaned before conducting seed counts using the phone app CountThings (Dynamic Ventures, Inc., Cupertino, CA). Final kernel weight was calculated by dividing the sample weight by the number of counted seeds. During the 2019 field season stems from parents and control varieties were collected prior to harvest to assess stem solidness. Collected stems were dissected at each internode and scored for pith expression on a scale of 1 (hollow) to 5 (solid), and the values for all five internodes were summed for a final score range of 5 (hollow) to 25 (solid) (Cook et al., 2004). Results from the stem solidness assessment are presented in the Appendix.

Statistical Analysis

Statistical analysis of phenotypic data in individual environments was done in R (R Core Team, 2018). For each trait in each environment, the normality of the phenotypic distribution was tested using the Shapiro–Wilks test in base R. Model testing for spatial adjustment using different covariate structures was done in each single environment using ASReml-R (Butler et al., 2017) , and the best model for each trait was selected based on the lowest log likelihood value and visual inspection of plots of the residuals. The general form of the linear-mixed model used for single environments was:

$$Y_{ijk} = \mu + G_i + R_j + C_k + \varepsilon_{ijk}$$

where the phenotypic value of an individual (Y_{ijk}) is a function of the overall mean (μ), the random effect of the *i*th genotype (G_i), the random effect of *j*th row (R_j), the random effect of *k*th column (C_k), and the residual error (ε_{ijk}). Heritability estimates were calculated on a line-mean bases as described by Fehr (1991). After selecting the best model for each trait for each individual environment, best linear unbiased

predictior (BLUP) values were estimated using the *predict.asreml* function from ASReml-R. Phenotypic correlations using the Spearman rank method were conducted between all measured traits using the estimated BLUP values as input.

Genotyping

Both DH populations were grown in the greenhouse for DNA extraction from leaf samples. Seeds of each genotype (3 per individual for Denali/Hatcher and 10 per individual for Avery/CO11D1397) were planted into 1 cell of a 96-well horticultural flat (The Blackmore Company, Belleville, MI). Approximately 5 cm of plant tissue was collected from each plant for each individual at the 1st leaf stage and stored at -80 °C for a minimum of 48 hours. Samples were freeze-dried before DNA extraction in a 96-well format using a King Fisher 96 magnetic bead extraction kit (ThermoFisher Scientific Inc., Waltham, MA). DNA was quantified and the concentration was normalized before construction of the genotyping-by-sequencing (GBS) library.

Libraries for sequencing (384-plex) were prepared by using a modified protocol as described by Poland et al. (2012) with *PstI* and *MspI* as restriction enzymes. Sequencing was performed at the University of Illinois in Urbana, IL on an Ilumina HiSeq4000 platform. Received FASTQ files were processed for SNPs using the TASSEL 5.0-GBSv2 pipeline (Glaubitz et al., 2014) with IWGSC RefSeq v2.0 as the reference genome for alignment. Briefly, tag counts were generated and merged into a database using the GBSSeqToTagDBPlugin; with default parameters – except the minimum kmer length was increased to 30 for better read alignment to the reference genome when using the BWA alignment tool (Li & Durbin, 2009). Only reads with a MAPQ score greater than 20 were kept when converting the output alignment file back to database format with the SAMToGBSdbPlugin. SNPs were called using the DiscoverySNPCallerPluginV2, and only minimum locus coverage (0.2) and minimum minor allele frequency (0.02) were changed from default settings (0.1 and 0.01). SNPs with an inbreeding coefficient less than 0.8 and an average sample read depth below 1 were removed before creating the final Hapmap

file using the ProductionSNPCallerPluginV2. Missing SNP data in the Hapmap file were imputed in TASSEL 5.2 (Glaubitz et al., 2014) using the LD-KNNI method (Money et al., 2015).

QTL Analysis

Linkage Map Construction

Linkage map construction was completed by using the *mstmap* function (Wu et al., 2008) from the R/ASMap v1.0-4 package (Taylor & Butler, 2017) in R. Prior to linkage mapping, polymorphic SNP markers were filtered in TASSEL 5.2 and excluded from the analysis if 1) the marker missing data level was > 30% (Avery/CO11D1397) or > 20% (Denali/Hatcher) and 2) the percentage of heterozygous allele calls was > 10%. Remaining SNPs were changed from a nucleotide-based to a parent-based (A, B, H) configuration in TASSEL 5.2 before importing into R. Additional filtering was completed within R/ASMap by removing markers that co-located (retaining only one) or formed linkage groups with five or fewer markers. Seven individuals in the Avery/CO11D1397 population and nine individuals in the Denali/Hatcher population were discarded due to higher than expected recombination rates caused by single and doubled crossovers events. Map distances (cM) were calculated using the Kosambi function with a significance threshold p-value = $1.0e^{-12}$ for linkage group formation. Final marker order was checked by plotting physical vs. genetic distances. Two packages in R, ggplot2 (Wickham, 2016) and LinkageMapView (Ouellette et al., 2017) were used for graphics creation.

Detection of QTLs

Standard interval mapping was conducted by the Haley-Knott regression method (Haley & Knott, 1992) using the *scanone* function in R/qtl v1.44-9 (Broman et al., 2003). Genotypic data and phenotypic data for the following traits were analyzed: kernel weight, cutting score, heading date, and physiological maturity. Logarithm of odds significance thresholds for detection of QTLs were determined by 2000 permutation test analysis (Hussain et al., 2017; Verma et al., 2015). Confidence intervals for each QTL were calculated by finding the region on both sides of the peak LOD value corresponding to a decreased

LOD score of one (Collard et al., 2005). For each QTL, the additive effect and the percent of phenotypic variation explained (PVE) by the detected QTL was calculated using the *fitqtl* function in R/qtl. Results from the Denali/Hatcher QTL analysis are presented in the Appendix. Best linear unbiased prediction values were used for QTL analysis in each individual environment for each trait.

RESULTS AND DISCUSSION

Phenotypic Analysis

Phenotypic Variability of Mapping Population

Before model selection and BLUP estimation, each trait within each environment was evaluated for assumptions of normality. Wheat stem sawfly infestation was severe in both years, with extensive cutting damage occurring at each trial location. Mean values for cutting scores had a distribution skewed to the left in all environments, except for Orchard 2020 ($p = 0.14$) (Figure 2). A large proportion of DH individuals showed greater stem cutting from WSS infestation, with the mean cutting score being above 5.0 in each environment (Table 1). Results from the Shapiro-Wilks test confirmed that the cutting scores were significant for non-normality in New Raymer 2019, Orchard 2019, and New Raymer 2020 (each $p < 0.001$) (Table 1). Additionally, heading date ($p = 0.002$) and physiological maturity ($p = 0.002$) were also significant for non-normality at New Raymer 2019 (Table 1). Heading dates for Orchard and New Raymer were similar in the 2019 season, however in 2020 both locations were more than a week earlier for heading date compared with 2019 - with Orchard being six days ahead of New Raymer (Table 1). Physiological maturity was also earlier in the 2020 field season than the previous year (Table 1). Data were not collected for physiological maturity at Orchard in the 2020 season due to unusually high temperatures in May and June and the trial maturing earlier than expected. Kernel weight was normally distributed in all three environments (data were not obtained at the Orchard site in 2019 because the cooperator harvested the field prior to head collection). No significant Shapiro-Wilks test values for plant height were observed, with measurements only conducted in the first year. Statistical analysis was still completed despite the non-normality of cutting scores, heading date, and physiological maturity in the above mentioned environments. Transformations were not done due to the residual errors being normally distributed.

Variation between Avery and CO11D1397 for all the measured traits was minimal. Initial field testing of Avery and CO11D1397 showed that Avery had a greater degree of stem cutting compared with

CO11D1397, however little variation in cutting scores between these two was observed in this study. Avery and CO11D1397 are half-sibs (with Byrd as the common parent), and the DH population was initially developed for use within a breeding program, thus Avery and CO11D1397 are highly structured according to geographic origins and market class restrictions. Despite the lack of diversity between the two parents, transgressive segregants were still identified for each trait (Table 1).

BLUP and Heritability Estimates

Best linear unbiased predictions and line-mean heritability estimates for traits evaluated in each environment are presented in Table 1. Several spatial models were used for correcting spatial trends present in each trait x environment combination. Best linear unbiased predictor values for Avery and CO11D1397 differed very little for each of the measured traits. The population mean was consistently between the two parental values, however DH individuals with low cutting scores were identified in each environment; except for Orchard 2019 where the lowest DH values were right in the middle of the 1-9 rating scale. The highest observed line-mean heritability was for plant height (0.89-0.91) in 2019. Cutting score heritability ranged from moderate to high across the four environments (0.44-0.77). Kernel weight and heading date were also moderately heritable, with line-mean heritability for kernel weight being slightly higher than that for heading date; ranging from 0.40-0.64 and 0.40-0.59. The lowest line-mean heritability estimates were for physiological maturity (0.28-0.41).

Correlation Analysis

Spearman rank correlation coefficients (r_S) between traits were calculated by using estimated BLUP values from single environments (Figure 3). Cutting score was negatively correlated with heading date and physiological maturity in 20 of 21 individual-environment analyses, ranging from r_S = -0.20 to -0.56 for heading date and r_S = -0.23 to -0.54 for physiological maturity. In 2019 kernel weight and cutting score were not correlated, however kernel weight in 2020 was negatively correlated with cutting score at both New Raymer (r_S = -0.27, p = 0.008) and Orchard (r_S = -0.15, p = 0.033) locations. A positive

correlation for plant height was observed between kernel weight (r_S = 0.31-0.32) and physiological maturity (r_S = 0.19-0.22) at the New Raymer site, and all individual-environment analyses for heading date (r_S = 0.29-0.40). In addition, the correlation coefficients were positive for heading date and physiological maturity in each environment, with the highest values observed at Orchard in 2019 (r_S = 0.69, $p < 0.001$) and 2020 (r_S = 0.65, $p < 0.001$).

Results from the correlation analysis are in general agreement with prior work on WSS infestation and damage in common wheat. Sherman et al. (2010) observed a negative correlation between infestation and heading date and suggested that selecting individuals that showed less WSS cutting may also indirectly select for later heading date. Lines that are early heading give a greater opportunity for WSS oviposition to occur due to elongating stems being available for a longer period of time during the WSS flight period (Sherman et al., 2010) - an observation also shared by Morrill and Kushnak (1996). Reduction in kernel weight from stem WSS infestation has been well documented in wheat (Delaney et al., 2010; Morrill et al., 1994; Weaver et al., 2004), however these observations can be confounded with other environmental variables (Delaney et al., 2010). Cutting score was negatively correlated with kernel weight in two of three environments and correlations between kernel weight and both heading date and physiological maturity were negative as well (Figure 3). These results suggest that kernel weight was negatively affected not only by WSS infestation but also by heading date and maturity. Temperatures were higher in the 2020 field season, resulting in the flowering period occurring during hotter conditions than 2019 – negatively affecting grain filling.

Genetic Linkage Map Construction

Initial genetic map construction resulted in a highly inflated map with a total length of 18,584 cM (Table 2). Only the chromosomes 3D, 4A, 4D, and 5D had values within the expected range of 200 cM, while the remaining chromosomes often had genetic distances values greater than 1,000 cM (Table 2). The initial map was created from SNP discovery done in the TASSEL-GBSv2 pipeline using the default parameters. To reduce the total genetic distance of the linkage map, a new set of parameters was then

implemented in TASSLE-GBSv2 pipeline. By increasing the minimum kmer length to 30, aligning reads to the updated IWGSC RefSeq v2.0 reference, removing markers with a MAPQ score of 20, and setting an average read depth of greater than one, a genetic map for QTL analysis with similar chromosomal distances to previous QTL studies in wheat was created (Cook et al., 2018; Varella et al., 2019). Changes in the filtering parameters resulted in the revised genetic map used for QTL analysis to have 18 out of the 21 chromosomes within expected centiMorgan distances (Table 2). Evaluation of the revised map showed a greater agreement between the genetic and physical positions - compared to the initial genetic map (Figure 4). All SNP markers were assigned to a chromosome in the revised linkage map created and genetic distances were reduced by 86.8% across the whole genome compared to the genetic map created using the initial TASSEL parameters (Table 2).

Discrepancies between maps likely resulted from a combination of sequencing errors and tag misalignments to the reference genome, and these problems were addressed by implementing altered filtering option for the TASSEL-GBSv2 pipeline. High read coverage is needed to increase the confidence in the base call, however high levels of multiplexing results in lower read coverage (Elshire et al., 2011) In the current study, 384 samples were multiplexed into a single sequencing library, resulting in 47% of SNPs having an average read depth of less than one. In addition, alignment of unique sequence reads onto the common wheat genome can be difficult because of large proportion of long sequence repeats and high genetic similarity between the three sub-genomes (Li et al., 2004). Most of the QTL analysis literature neglect to discuss the parameters used for GBS discovery, resulting in uncertainty of the quality for the marker data used.

A total of 776 polymorphic SNP markers were assigned to 21 linkage groups, creating a final genetic map with a total size of 2,461 cM (Table 3). Marker distribution across the sub-genomes was not uniform, with the B genome having 492 (63%) SNP markers, the A genome 195 (25%) SNP markers, and the D genome having the fewest SNP markers at 89 (11%) (Table 5). A goodness of fit test showed there was a significant deviation of the observed allele frequency from the expected 1:1 ratio (x^2= 343.4, df = 1, $p < 0.001$), with a greater proportion of CO11D1397 alleles present. Average SNP marker spacing was

3.3 cM, however large gaps (> 9 cM) were observed on all chromosomes except chromosomes 3D, 5D, 6B, and 7B (Figure 5).

The low number and uneven distribution of SNP markers can be attributed to the high genetic similarity between Avery and CO11D1397 and low polymorphism on the D genome. Figure 6 shows the distribution of SNP markers across each chromosome after KNNI imputation and before any filter parameters were applied in TASSEL 5.2. Large sections of each chromosome consist of monomorphic markers, with chromosomes from the B genome harboring most of the polymorphic markers. Because Avery and CO11D1397 have a common parent (Byrd), large portions of the genome are conserved through identity by descent. Low marker coverage on the D genome is common, and a study of SNP polymorphisms in US wheat showed that the D genome had the lowest level of polymorphisms compared to the A and B genomes (Chao et al., 2009). Most areas with polymorphic markers were located on the distal ends of each chromosome, thus our genetic map consists of large gaps in genomic coverage.

QTL Analysis

A total of 11 QTLs were identified for cutting score on chromosomes 1B, 2B, 2D, 3A, 4B, 4D, 5B, 6A, and 7D (Table 4 and Figure 7). Avery contributed the resistance allele for eight of the QTLs (located on chromosomes 1B, 2B, 2D, 3A, 4B, 5B, 6A, and 7D), whereas CO11D1397 contributed the resistance allele for the remaining four QTLs (located on chromosomes 4D and 5B). Ten of the 11 QTL were considered as major-effect (R^2 > 10%), while the remaining QTL on chromosome 3AS only had a minor-effect (R^2 < 10%). Major QTLs on chromosomes 1BL, 2BS, 2DS, and 5BS were significant in three of the four environments, with the QTL on chromosome 2DS had the largest effect. The phenotypic variation explained by this QTL ranged from 10% in Orchard 2020 to 18.7% at New Raymer 2020. The Avery allele at this QTL position was associated with a 0.7–1.2 point reduction in WSS cutting scores. Quantitative trait loci on chromosomes 1BL and 2BS explained 12.6-23.6% and 11.9–18.5% of the phenotypic variation and reduced cutting scores by 0.7–1.1 and 0.7–1.0, respectively. Avery contributed the resistance allele for both QTLs. An additional two QTLs on chromosomes 3AS and 7DS were

significant in two of the four environments and resulted in reduced cutting scores of 0.5 and 0.8 when the Avery allele was present. The QTL on chromosome 5BS was the only QTL significant in more than one environment with the resistance allele coming from CO11D1397. The presence of the CO11D1397 allele accounted for 7.9–20.8% of the phenotypic variation and reduced the cutting score by 0.2 in New Raymer 2020 and 0.4 in New Raymer 2019.

Three QTLs were detected for heading date on chromosomes 2B and 5A (Table 4 and Figure 7). All three of the QTLs were major-effect, however none were significant in more than a single environment. Two different QTLs were identified on chromosome 2B - located on opposite ends of the chromosome. Phenotypic variation explained by these two QTLs range from 12.8% for chromosome 2BS in Orchard 2020 and 10.2% for chromosome 2BL in New Raymer 2019. The allelic effect from the chromosome 2BS QTL accounted for a decrease in 0.4 days to heading while the 2BL QTL increased heading date by 0.1 days, with the CO11D1397 allele present. The QTL on 5A explained 10.4% of the phenotypic variation in heading date and a reduction of 0.3 days when the Avery allele was present.

Four QTLs were identified for physiological maturity on chromosomes 2B, 3A, 4D and 7B (Table 4 and Figure 7). All four QTLs were major-effect, accounting for 11.8% (2B), 11.3% (3AL), 10.1% (4DS), and 10.0% (7BL) of the phenotypic variation. As with heading date, none of the QTLs were significant in more than one environment. QTLs on chromosomes 3AL, 4DS, and 7BL all reduced the physiological maturity date by 0.7, 0.3, and 0.3 days, while the 2B QTL increased the physiological maturity date by 0.2 days. The reduction in days to physiological maturity was associated with the Avery allele.

Two different QTLs were identified for kernel weight on chromosomes 1B and 7D (Table 4 and Figure 7). The 1BS QTL was significant in all 3 of the environments and had an allelic effect of 0.2-0.4 mg increase in kernel weight when the CO11D1397 allele was present. Phenotypic variation explained by the chromosome 1BS QTL was also high at 10.4% in New Raymer 2019 to 12.6% in New Raymer 2020. The chromosome 7DS QTL was significant in two of the three environments and was associated with an

increase of 0.3–0.4 mg kernel weight when the Avery allele was present. Phenotypic variation attributed to this QTL was low at 7.7% in New Raymer 2020 and 10.0% in New Raymer 2019.

Characterization of Putative WSS QTLs

The QTL on chromosome 2DS (hereafter referred to as *Qwss.csu*-2DS) showed the largest effect in reducing WSS cutting scores. This QTL is located between the flanking markers S2D_ 37287852 - S2D_ 37745314 (37.3-37.7 Mb) using the IWGSC RefSeq v2.0 reference (Table 4). There was no co-localization observed with other identified QTLs. Sherman et al. (2010) and Varella et al. (2017) both reported the presence of a QTL on chromosome 2D for reducing WSS infestation and cutting. Female WSS use their ovipositor to assess the suitability of a host for multiple cues before oviposition (Varella et al., 2017), with one of those cues thought to be related to emissions of different volatile organic compounds (Piesik et al., 2008). However, the strong negative relationship between cutting score and heading date suggests that there may be an association between the *Qwss.csu*-2DS QTL and genes that affect flowering time. The photoperiod gene *Ppd-D1* is also located on the short arm of chromosome 2D, and the dominant allele form shows insensitivity to daylength (Chen et al., 2018). The absence of this allele would be associated with later flowering and possibly allowing for the plant to escape WSS oviposition during the flight period. Further validation may help to determine if *Qwss.csu*-2Ds is in tight linkage or pleiotropic with *Ppd-D1*.

The QTL on chromosomes 1BL (*Qwss.csu*-1BL) and 5BL (*Qwss.csu*-5BL) have been previously reported to be associated with WSS infestation by altering the flowering time of genotypes considered to be resistant. Qw*ss.csu*-1BL and *Qwss.csu*-5BL are located on the long arms of chromosomes 1B and 5B – between the flanking markers S1B_ 556746763 - S1B_ 570574571 and S5B_ 598891452 - S5B_ 697055707 (Table 4). Prior work by Sherman et al. (2010) identified similar QTLs on chromosomes 1B and 5B that explained 8.0% and 26.0% of the phenotypic variation for reduced WSS infestation. It was concluded that both QTLs affected heading date in the study and the QTL on 5B was associated with vernalization gene *Vrn-B1* (Sherman et al., 2010) - making it possible that *Qwss.csu*-5BL is either in tight

linkage or is pleiotropic with the same gene. It has been shown that varieties carrying the *Vrn-B1* allele can have a heading date 2.2 days later than varieties without it (Grogan et al., 2016). However, *Qwss.csu*-5BL was only detected in Orchard 2020 and accounted for a much smaller proportion of the phenotypic variation (12%) than in the study by Sherman et al. (2015). While Sherman et al. (2010) did not suggest any genes associated with the QTL on chromosome 1B, more recent studies of earliness *per se* (*Eps*) gene suggest there may be an association between *Qwss.csu*-1BL and *Eps-B1*. Fine adjustments to flowering-time are made by *Eps* genes independently of vernalization or photoperiod requirements, and it has been suggested that an Eps gene may be present on the long arm of group 1 chromosomes (Zikhali & Griffiths, 2015). The presence of the *Eps-B1* gene would explain the delay in heading date for individuals with reduced cutting scores in the Avery/CO11D1397 population.

Additionally, the QTL on chromosome 2BS (*Qwss.csu*-2BS) was found to be associated with altered flowering time. The QTL is located on the short arm of chromosome 2B between the SNP markers S2B_ 111454309 and S2B_ 244490927 (Table 6). In our current study, the confidence interval for physiological maturity 2B QTL did not have an overlap with *Qwss.csu*-2BS QTL (Figure 7). However, the photoperiod gene *Ppd-B1* has been mapped to the short arm of chromosome 2B, and increased copy number for the gene is associated with earlier flowering (Kiseleva et al., 2017). Reduced copy number for *Ppd-B1* would cause a delay in heading date and thus maturity for individuals within the Avery/CO11D1397 population.

Two QTLs for cutting score (*Qwss.csu*-3AS and *Qwss.csu*-5BS) were present in both years at the New Raymer location (Table 4). Each QTL is located on the short arm of their respective chromosome and are flanked by the SNP markers S3A_ 10222683 - S3A_ 573145273 for *Qwss.csu*-3AS and S5B_ 71904280 - S5B_ 330567893 for *Qwss.csu*-5BS (Table 4). Only *Qwss.csu*-3AS co-localize with another detected QTLs for physiological maturity (Table 4 and Figure 7). Two novel QTLs on chromosomes 3A and 5B were found to reduce larval mortality in a genome-wide association study (GWAS) of North American spring wheat germplasm, however the physical positions of the reported QTL were not given (Varella et al., 2015). Results from Varella et al. (2015) concluded that the mechanism of resistance for

both QTLs was antibiosis related (due to GWAS entries varying in temporal expression of stem solidness), however none of the parents in the current study varied for stem solidness. Because Avery and CO11D1397 are both hollow-stemmed varieties, *Qwss.csu*-3AS and *Qwss.csu*-5BS instead appear to be antixenosis related but further evaluation is needed.

A QTL on the short arm of chromosome 7D (*Qwss.csu*-7DS) for stem cutting was identified at the New Raymer site in 2019 and again 2020. There is no mention in the current WSS literature to indicate the presence of a QTL on chromosome 7D for reduced cutting resulting from wheat stem sawfly infestation. Coinciding on the chromosome was the presence of a QTL for kernel weight at a physical position of 14.4–453.5 Mb (Table 4) Based on the map (Figure 7) the kernel weight QTL appears to completely co-localize with *Qwss.csu*-7DS – with *Qwss.csu*-7DS also having a 1 LOD interval of 14.4-453.6 Mb (Table 4). Other QTL mapping studies for kernel traits have shown that chromosome 7D is important for influencing kernel morphology (kernel width and length) and kernel weight (Groos et al., 2003; Xin et al., 2020). The relationship between the two QTL identified in this study for kernel weight and cutting score is unknown and warrants further evaluation.

Additionally, another kernel weight QTL on chromosome 1B was present in each the three environments where harvest of wheat heads occurred. The physical position for the confidence interval of this QTL was quite large (14.4-453.5 Mb), however it did not overlap with the *Qwss.csu*-1BL QTL for WSS cutting (Table 4 and Figure 7). This QTL could be linked to other QTLs previously described for kernel traits such as kernel length, kernel width, and kernel weight (Li et al., 2015). Reduced kernel weight from stem feeding by wheat stem sawfly can be confounded with other environmental variables (Delaney et al., 2010), and a strong genotype by environment effect was present for all kernel traits in a QTL study conducted by Zhang et al. (2015). For this study, it is unclear if the identified QTL was directly related to a reduction in WSS larval feeding damage or an unrelated QTL controlling a kernel trait. Quantitative trait loci analysis for kernel traits often results in identification of a large number of QTL that are directly influenced by environmental conditions (Li et al., 2015; Xin et al., 2020), however

further work is necessary to clarify if the 1B QTL for kernel weight is a mechanism for reduced damage caused by WSS larval feeding.

CONCLUSION

In this study, 11 QTLs, seven major-effect and four minor-effect, were identified in the Avery/CO11D1397 DH population for reduced WSS cutting. Phenotypic variation between Avery and CO11D1397 was minimal due to the shared maternal parent Byrd, however the presence of transgressive segregants for all traits allowed for the mapping of QTL for resistance to the wheat stem sawfly in Colorado winter wheat. The initial hypothesis of this study was that the resistance observed for the CO11D1397 parent was caused by WSS host-plant preference, but correlation analyses and QTL mapping results suggest that several identified QTL may be tightly linked to heading date and physiological maturity. Known functional genes exist that affect flowering time, including photoperiod (*Ppd*), vernalization (*Vrn*), and earliness *per se* (*Esp*). Of the six QTL identified for cutting score in multiple environments, three QTLs were associated with flowering time: *Qwss.csu*-2DS (potentially associated with *Ppd-D1*), *Qwss.csu*-2BS (potentially associated with P*pd-B1*), and *Qwss.csu*-1BL (potentially associated with *Eps-B1*). Results from this study suggest that a relationship between lower cutting score and a later flowering date exists for genotypes within the Avery/CO11D1397 DH population. As wheat breeders in many environments do not want to develop later heading and maturing varieties, these QTL are likely not useful for improving resistance to WSS through reduced stem cutting. The only major-effect QTL associated with reduced stem cutting score but not associated with heading date or physiological maturity were the previously unreported QTL *Qwss.csu*-7DS and *Qwss.csu*-5BS. These QTL might be a form of antixenosis resistance, since both parents are hollow-stemmed varieties. Further fine mapping and validation studies are needed to confirm the presence of these QTL in different populations and possible association with a functional gene. Introgression of antixenosis alleles into cultivars with alleles for stem solidness may help in developing new wheat varieties with more durable WSS resistance.

TABLES AND FIGURES

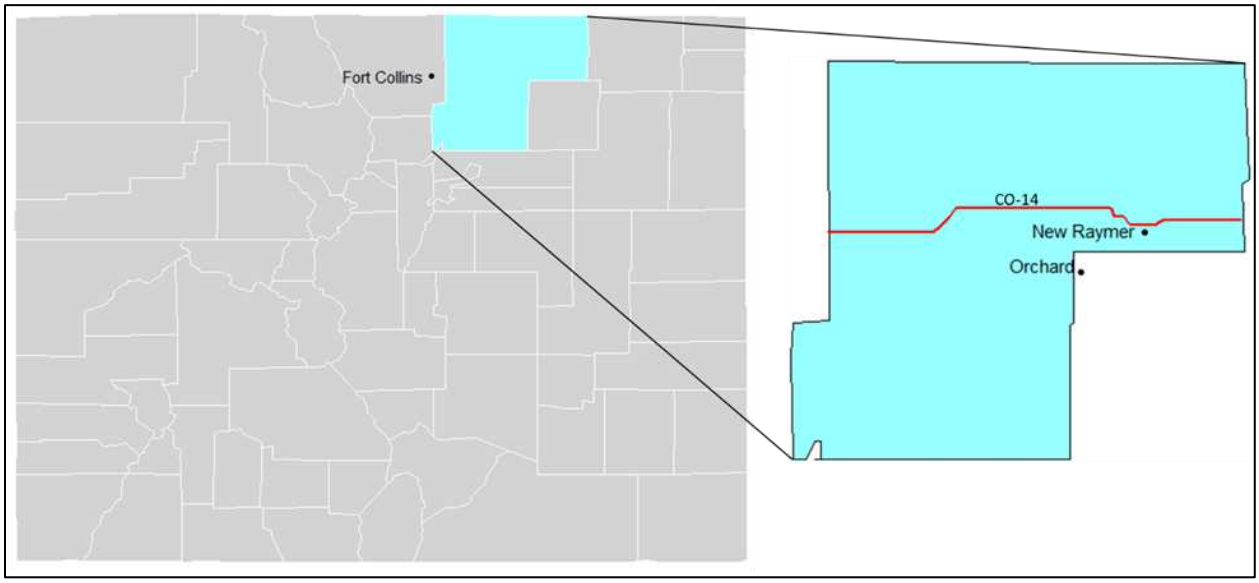

Figure 1. Location of field trials in and near Weld Country, Colorado.

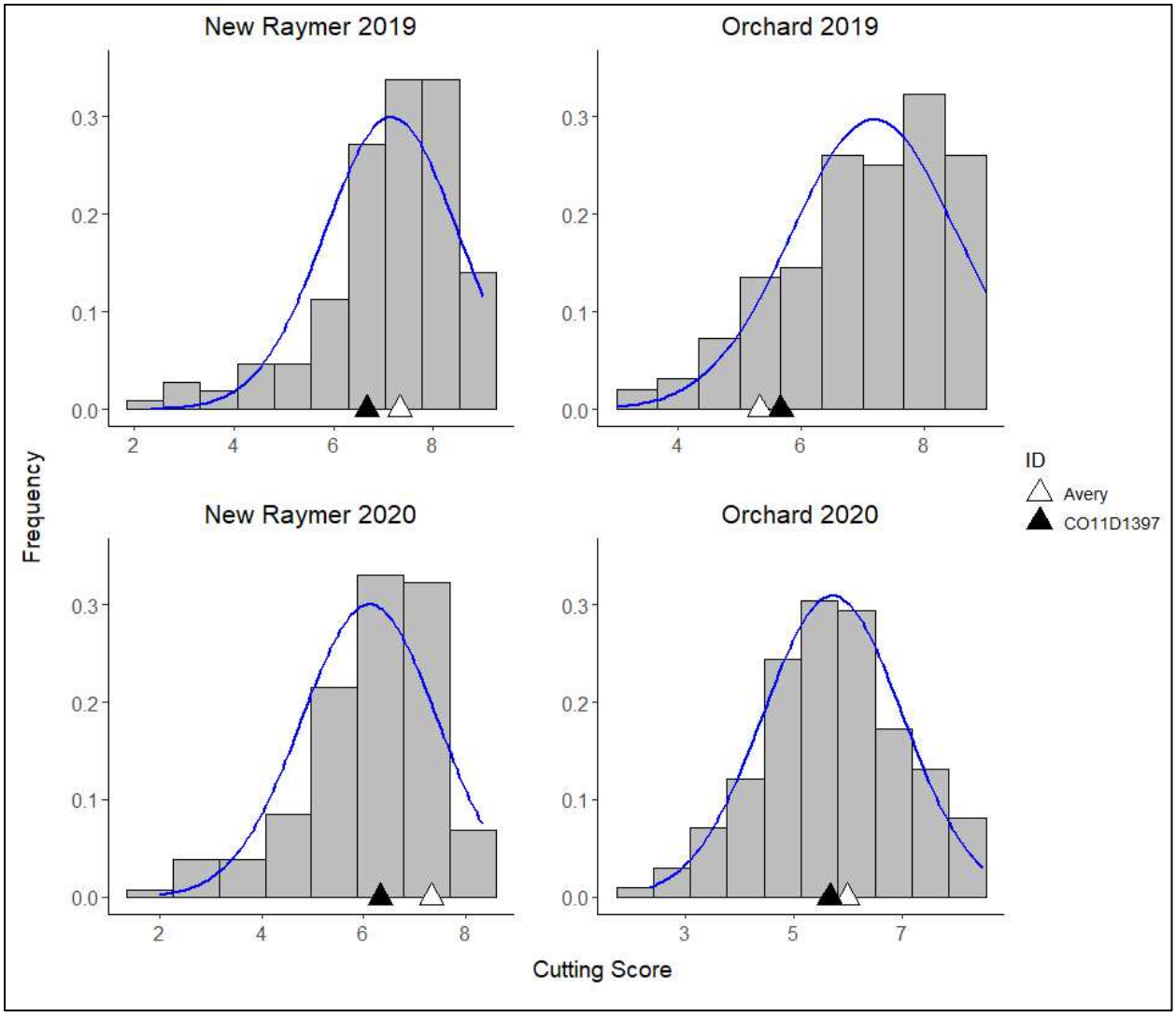

Figure 2. Frequency distribution of wheat stem sawfly cutting scores measured in the Avery /CO11D1397 doubled haploid (DH) population. Values represent the mean of each DH for a single environment. Parental values for each trait are indicated by triangular markers on the x-axis (black = CO11D1397, white = Avery).

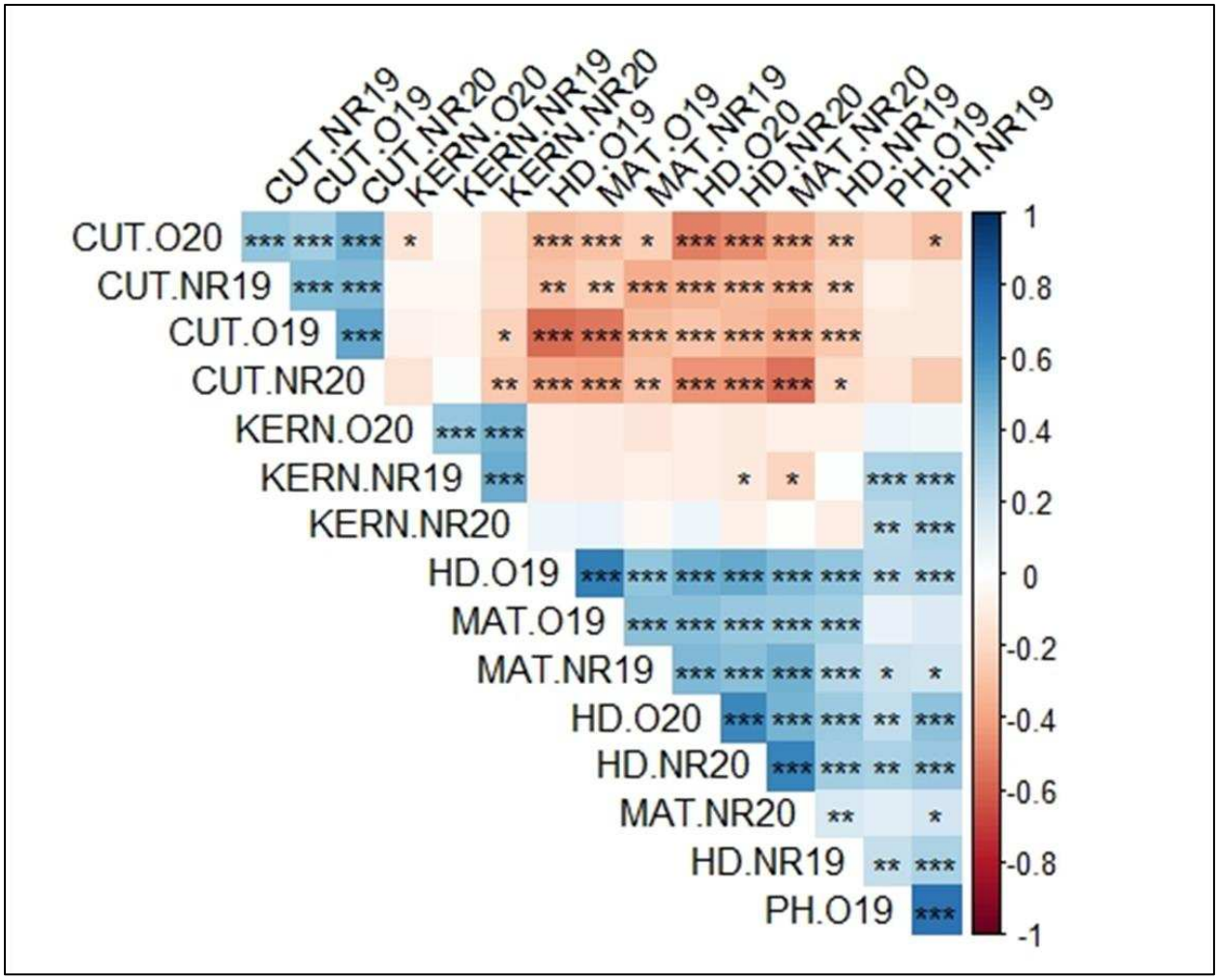

Figure 3. Heatmap of Spearman rank correlation coefficients among traits based on best linear unbiased predictor (BLUP) estimates within a single environment.

Names include trait, location, and year; CUT, cutting score; KERN, kernel weight; PH, plant height; MAT, physiological maturity; HD, heading date; NR19, New Raymer 2019; O19, Orchard 2019; NR20, New Raymer 2020; O20, Orchard 2020.

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

Table 1. Best linear unbiased predator (BLUP) estimates for parental values, population mean, and population range; line-mean heritability; and Shapiro-Wilks test value in the Avery/CO11D1397 doubled haploid population for all measured traits in each environment.

Trait	Environment	Avery	CO11D1397	Population Mean	Population Range	H^2†	SW‡
Cutting score (1-9 scale)	New Raymer 2019	7.5	6.4	7.1	3.8 - 8.6	0.77	0.000
	Orchard 2019	6.3	6.1	6.9	5.1 - 8.1	0.44	0.000
	New Raymer 2020	6.3	6.2	5.9	3.2 - 7.2	0.59	0.000
	Orchard 2020	5.4	6.0	5.8	3.8 - 7.2	0.70	0.141
Kernel weight (mg kernel^{-1})	New Raymer 2019	30.0	28.3	29.5	26.7 - 32.3	0.64	0.336
	New Raymer 2020	26.0	26.3	26.4	24.2 - 28.8	0.52	0.521
	Orchard 2020	24.1	23.3	24.0	21.8 - 25.6	0.40	0.971
Heading date (days from Jan. 1)	New Raymer 2019	165.6	166.2	165.9	164.8 - 67.3	0.59	0.002
	Orchard 2019	165.3	168.0	165.8	163.1 - 169.3	0.40	0.131
	New Raymer 2020	158.4	159.2	159.2	156.7 - 162.4	0.54	0.200
	Orchard 2020	152.1	152.8	152.7	150.7 - 155.4	0.40	0.539
Physiological maturity (days from Jan. 1)	New Raymer 2019	197.8	198.2	197.9	195.9 - 199.3	0.41	0.002
	Orchard 2019	199.2	201.5	199.5	197.2 - 202.1	0.28	0.496
	New Raymer 2020	188.7	189.9	189.7	187.8 - 192.3	0.31	0.133
Plant height (cm)	New Raymer 2019	79.5	77.7	80.3	74.7 - 85.6	0.89	0.062
	Orchard 2019	82.3	78.7	81.5	75.7 - 88.9	0.91	0.229

† Line-mean heritability.
‡ Shapiro-Wilks p-value.

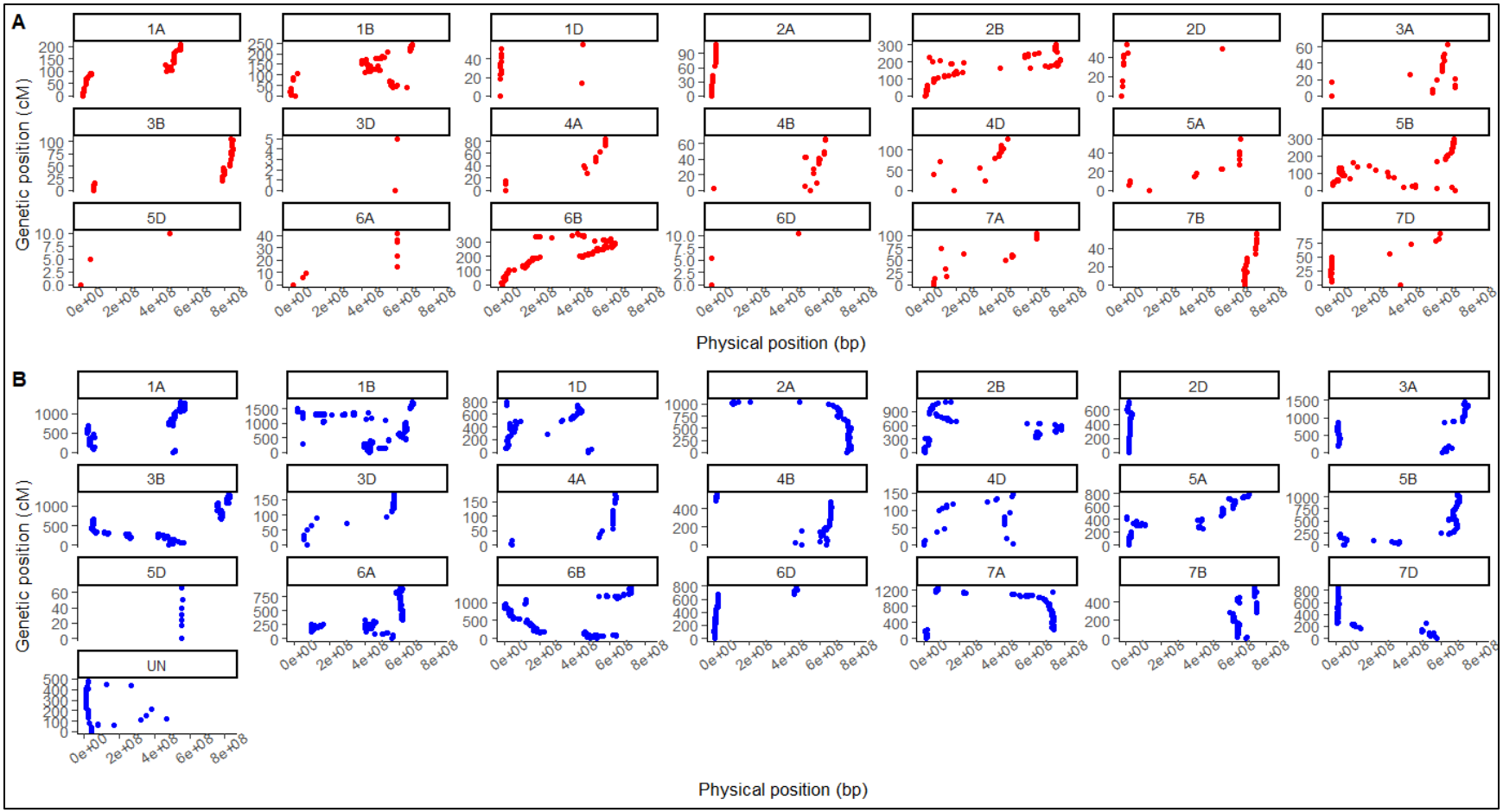

Figure 4. Comparison between the genetic and physical positions for the revised **(A)** and the initial **(B)** linkage maps. The y-axis indicates the genetic position (cM), while the x-axis shows the physical position (bp) for each chromosome. Only the initial linkage map had SNP markers that were unable to be assigned (UN) to the reference genome.

Table 2. Comparison of genetic distance (cM) after the implementation of altered filtering parameters. Using the updated IWGSC RefSeq v2.0 reference genome resulted in no unassigned single-nucleotide polymorphisms; thus, no unknown linkage group bin was created with the revised linkage map.

Chromosome	Genetic Distances (cM)		Difference (%)
	Before	After	
1A	1307	209	84.0
1B	1732	245	85.9
1D	798	56	93.0
2A	1050	109	89.6
2B	1118	302	73.0
2D	718	53	92.6
3A	1460	63	95.7
3B	1277	106	91.7
3D	166	5	97.0
4A	173	84	51.4
4B	547	66	87.9
4D	145	129	11.0
5A	782	55	93.0
5B	1039	300	71.1
5D	66	10	84.8
6A	912	42	95.4
6B	1401	363	74.1
6D	770	11	98.6
7A	1251	104	91.7
7B	554	57	89.7
7D	839	92	89.0
Unknown	481	-	-
Total	18584	2459	86.8

Table 3. Summary of single-nucleotide polymorphisms (SNP) markers in the Avery/CO11D1397 double haploid population for each chromosome in the revised linkage map.

Chromosome	Total SNPs	Unique Loci	Length (cM)
1A	74	70	209
1B	81	70	245
1D	20	15	56
2A	29	25	109
2B	96	84	302
2D	11	10	53
3A	21	18	63
3B	42	39	106
3D	2	2	5
4A	20	16	84
4B	16	15	66
4D	22	18	129
5A	20	14	55
5B	94	78	300
5D	3	3	10
6A	8	8	42
6B	138	128	363
6D	3	3	11
7A	23	19	104
7B	26	23	57
7D	28	26	92
Total	776	684	2461

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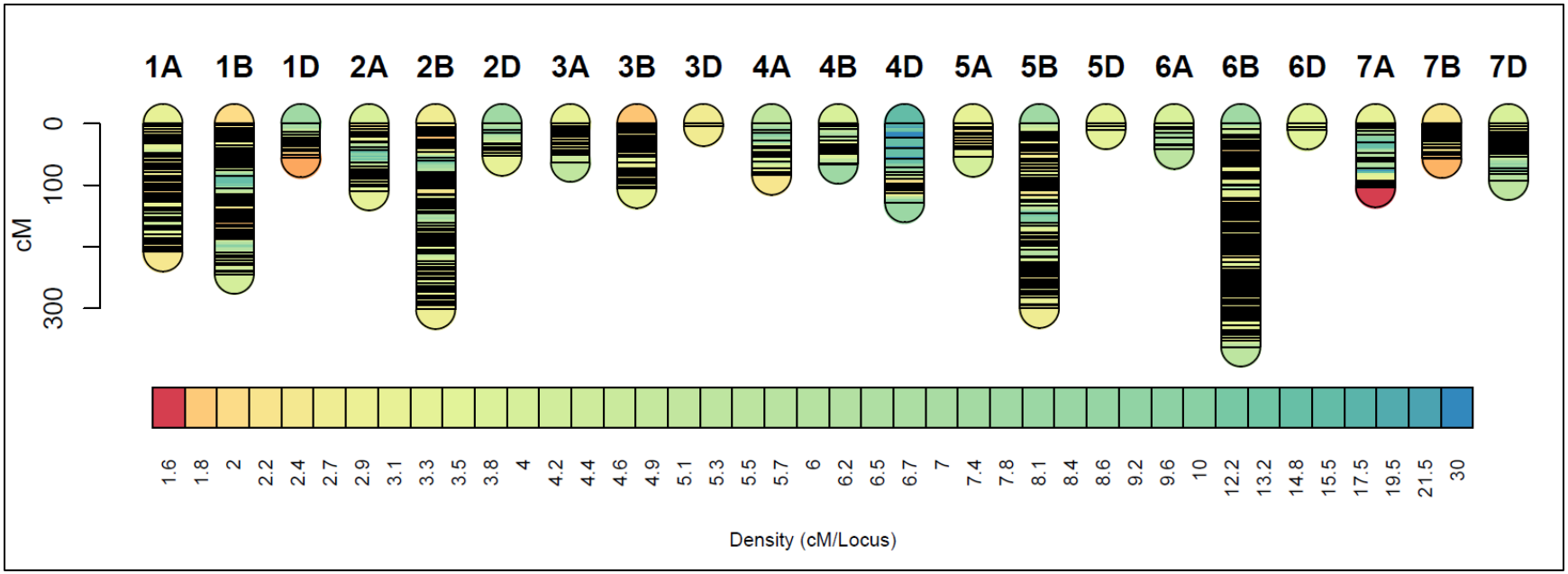

Figure 5. Distribution and density of single-nucleotide polymorphisms markers on the linkage map for the Avery/CO11D1397 doubled haploid population.

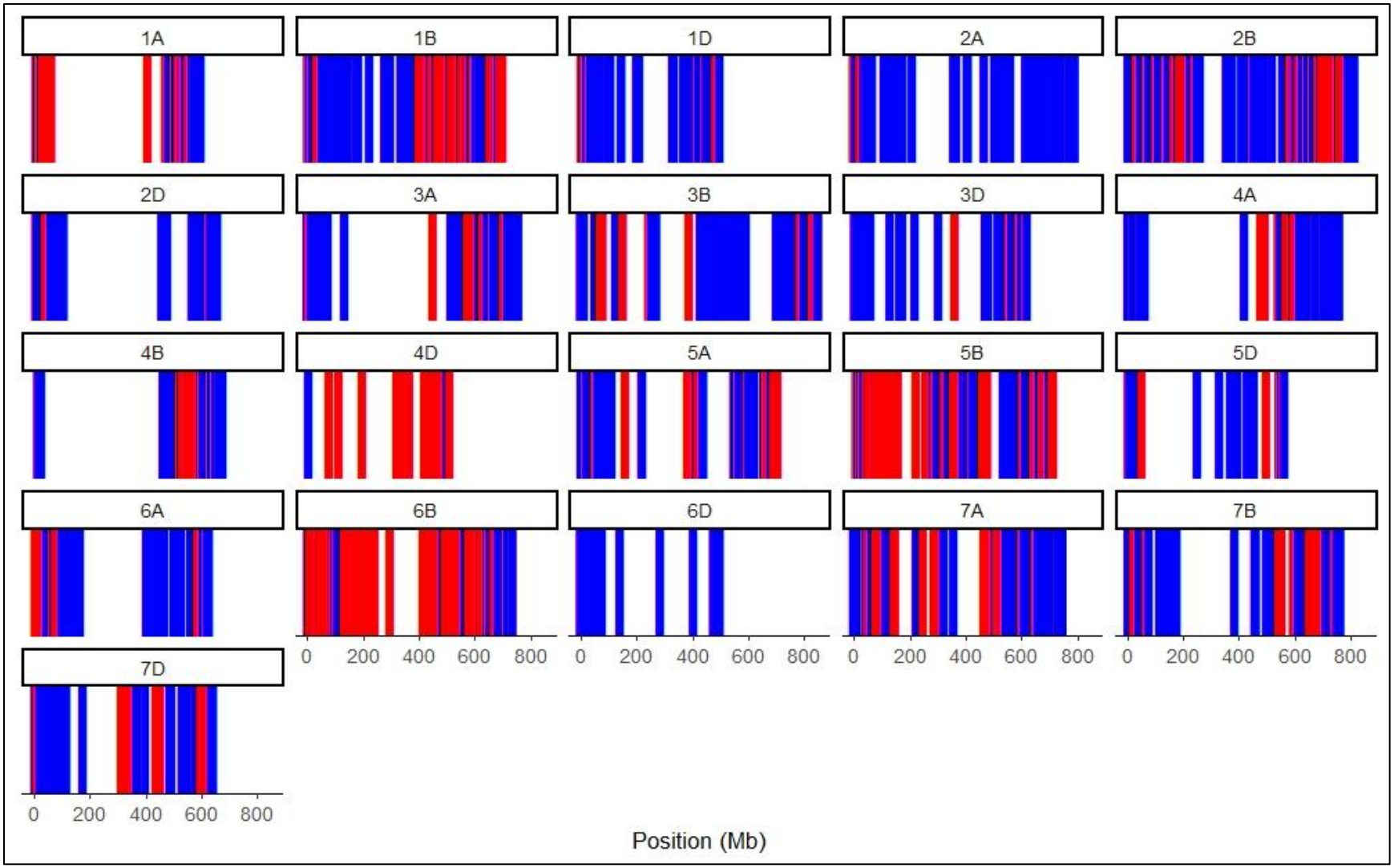

Figure 6. Distribution of markers per chromosome for the Avery/CO11D1397 double haploid population after completing the single-nucleotide polymorphisms discovery using TASSEL-GBSv2. Monomorphic markers are represented in blue while polymorphic markers are displayed in red.

Table 4. Quantitative trait loci (QTL) associated with measured phenotypic traits in the Avery/CO11D1397 doubled haploid population.

Trait†	Chr	Position‡	CI¶	New Raymer 2019			Orchard 2019			New Raymer 2020			Orchard 2020		
		(cM)	(Mb)	LOD	R^2§ (%)	Effect#	LOD	R^2§ (%)	Effect#	LOD	R^2§ (%)	Effect#	LOD	R^2§ (%)	Effect#
CUT	1BL	67.2	556.7-570.6	8.0	23.6	-1.1	-	-	-	7.4	22.1	-1.0	4.0	12.6	-0.7
	2BS	198.6	111.5-244.5	6.1	18.5	-1.0	-	-	-	3.8	11.9	-0.8	3.9	12.2	-0.7
	2DS	48.1	37.3-37.7	6.0	18.1	-1.2	-	-	-	6.1	18.7	-1.1	3.1	10.0	-0.7
	3AS	8.0	10.2-573.1	2.9	9.2	-0.5	-	-	-	2.9	9.4	-0.5	-	-	-
	4BL	2.2	460.1-544.1	4.1	12.7	-0.6	-	-	-	-	-	-	-	-	-
	4DS	56.6	79.3-113.5	-	-	-	3.5	11.2	0.2	-	-	-	-	-	-
	4DS	23.0	194.0-455.5	-	-	-	-	-	-	3.5	11.2	0.3	-	-	-
	5BL	0.0	598.9-697.1	-	-	-	-	-	-	-	-	-	3.9	12.2	-0.4
	5BS	83.5	71.9-330.6	6.9	20.8	0.4	6.9	20.7	0.3	2.4	7.9	0.2	-	-	-
	6AS	5.9	71.9-330.6	3.6	11.3	-0.5	-	-	-	-	-	-	-	-	-
	7DS	54.1	14.4-453.5	6.1	18.5	-0.8	-	-	-	6.4	19.3	-0.8	-	-	-

Table 4. (Continued)

Trait†	Chr	Position‡ (cM)	CI¶ (Mb)	New Raymer 2019			Orchard 2019			New Raymer 2020			Orchard 2020		
				LOD	R^2§ (%)	Effect#	LOD	R^2§ (%)	Effect#	LOD	R^2§ (%)	Effect#	LOD	R^2§ (%)	Effect#
HD	2BL	278.6	663.2-756.3	3.2	10.2	-0.1	-	-	-	-	-	-	-	-	-
	2BS	60.9	34.7-75.8	-	-	-	-	-	-	-	-	-	4.1	12.8	0.4
	5A	9.6	160.1-563.5	-	-	-	-	-	-	-	-	-	3.3	10.4	-0.3
MAT	2B	38.4	36.5-75.8	3.7	11.8	0.2	-	-	-	-	-	-	-	-	-
	3AL	34.3	10.2-660.2	3.6	11.3	-0.7	-	-	-	-	-	-	-	-	-
	4DS	128.6	79.3-487.4	-	-	-	3.1	10.1	-0.3	-	-	-	-	-	-
	7BL	8.7	691.0-700.7	-	-	-	3.1	10.0	-0.3	-	-	-	-	-	-
KERN	1B	127.9	46.0-507.4	3.2	10.4	-0.4	-	-	-	4.0	12.6	-0.3	3.8	12.1	-0.2
	7DS	54.1	14.4-453.5	3.1	10.0	0.4	-	-	-	2.4	7.7	0.3	-	-	-

† Phenotypic trait; CUT, cutting score; HD, heading date; MAT, physiological maturity; KERN, kernel weight.
‡Peak QTL position.
¶ One LOD confidence interval on either side of the QTL reported in megabase pair position from the International Wheat Genome Sequencing Consortium (IWGSC) RefSeq v2.0.
§ Percent of phenotypic variation explained by the QTL.
# Additive effect of the Avery allele.

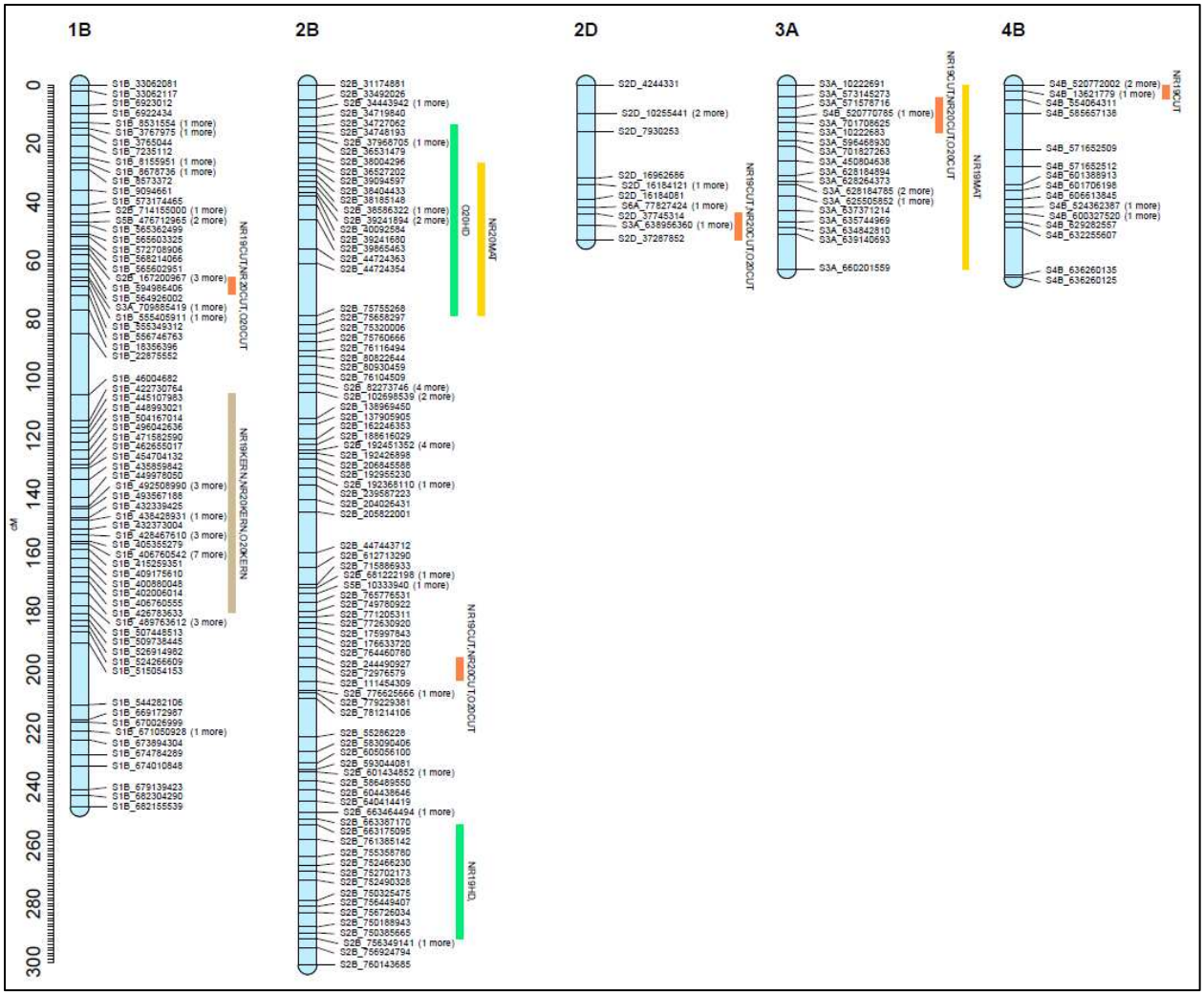

Figure 7. Chromosomal locations of quantitative trait loci (QTL) detected for cutting score (*orange*), heading date (*green*), physiological maturity (*gold*), and kernel weight (*brown*) in the Avery/CO11D1397 doubled haploid (DH) population in four Colorado environments in 2019 and 2020. Quantitative trait loci intervals represent a one LOD score drop. Quantitative trait loci names are denoted by trait (CUT, cutting score; HD, heading date; MAT, physiological maturity; KERN, kernel weight), site (O, Orchard; NR, New Raymer), and year (19, 2019; 20, 2020). The number of single-nucleotide polymorphisms (SNP) markers with the same genetic position but different physical positions is indicated in parenthesis next to the SNP marker.

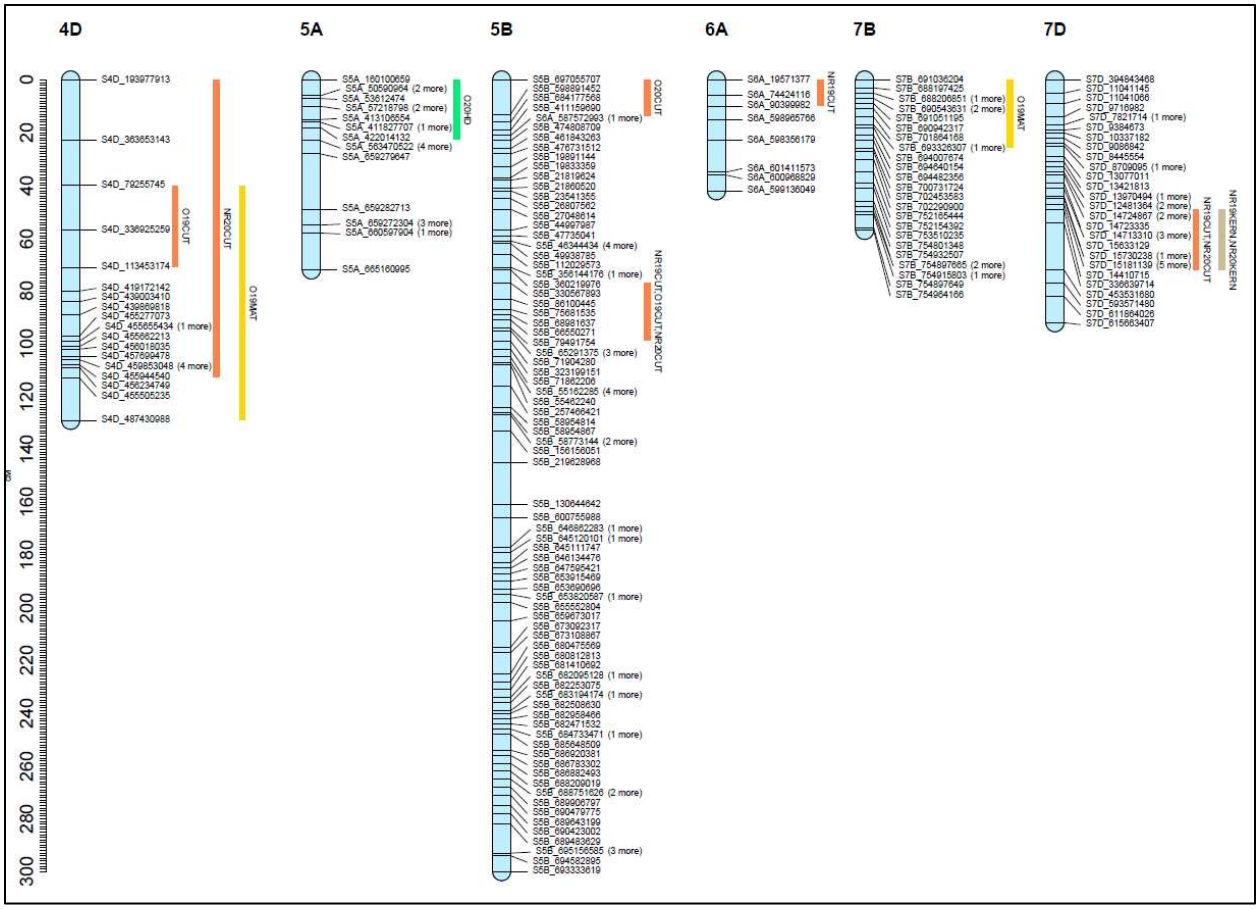

Figure 7. (Continued)

REFERENCES

Beres, B. L., Carcamo, H. A., & Byers, J. R. (2007). Effect of wheat stem sawfly damage on yield and quality of selected Canadian spring wheat. *Journal of Economic Entomology*, *100*(1), 79–87. https://doi.org/10.1093/jee/100.1.79

Beres, B. L., Carcamo, H. A., Weaver, D. K., Dosdall, L. M., Evenden, M. L., Hill, B. D., … Spaner, D. M. (2011b). Integrating the building blocks of agronomy and biocontrol into an IPM strategy for wheat stem sawfly. *Praire Soils and Crops*, *4*, 54–65.

Beres, B. L., Dosdall, L. M., Weaver, D. K., Cárcamo, H. A., & Spaner, D. M. (2011a). Biology and integrated management of wheat stem sawfly and the need for continuing research. *Canadian Entomologist*, *143*(2), 105–125. https://doi.org/10.4039/n10-05

Beres, B. L., Hill, B. D., Cárcamo, H. A., Knodel, J. J., Weaver, D. K., & Cuthbert, R. D. (2017). An artificial neural network model to predict wheat stem sawfly cutting in solid-stemmed wheat cultivars. *Canadian Journal of Plant Sciecne*, *336*, 329–336.

Broman, K. W., Wu, H., Sen, S., & Churchill, G. A. (2003). R/qtl: QTL mapping in experimental crosses. *Bioinformatics*, *19*(7). https://doi.org/10.1093/bioinformatics/btg11

Butler, D. G., Cullis, B. R., Gilmour, A. R., Gogel, B. G., & Thompson, R. (2017). *ASReml-R Reference Manual Version 4*. VSN International Ltd.

Carlson, G. R., Berg, J. E., Stougaard, R. N., Eckhoff, J. L., Lamb, P. F., Kephart, K. D., .. Bruckner, P. (2013), Registration of 'Bearpaw' wheat. *Journal of Plant Registrations*, 7, 180-183. https://doi:10.3198/jpr2012.07.0009crc

Chao, S., Zhang, W., Akhunov, E., Sherman, J., Ma, Y., Lou, M.-C., & Dubcovsky, J. (2009). Analysis of gene-derived SNP marker polymorphism in US wheat (Triticum aestivum L.) cultivars. *Molecular Breeding*, 23, 23–33. https://doi.org/10.1007/s11032-008-9210-6

Chen, L., Du, Y., Lu, Q., Chen, H., Meng, R., Cui, C., … Hu, Y.-G. (2018). The photoperiod-insensitive allele Ppd-D1a promotes earlier flowering in Rht12 dwarf plants of bread wheat. *Frontiers in Plant Science*, *9*(1312). https://doi.org/10.3389/fpls.2018.01312

Collard, B. C., Jahufer, M. Z. Z., Brouwer, J. B., & Pang, E. C. K. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica*, *142*, 169–196. https://doi.org/10.1007/s10681-005-1681-5

Cook, J. P., Heo, H.-Y., Varella, A. C., Lanning, S. P., Blake, N. K., Sherman, J. D., … Talbert, L. E. (2018). Evaluation of a QTL mapping population composed of hard red spring and winter wheat alleles using various marker platforms. *Crop Science*, *58*(2), 701-712 . https://doi.org/10.2135/cropsci2017.08.0488

Cook, J. P., Wichman, D. M., Martin, J. M., Bruckner, P. L., & Talbert, L. E. (2004). Identification of microsatellite markers associated with a stem solidness locus in wheat. *Crop Science*, *44*, 1397–1402.

Coombes, N. E. (2009). DiGGer design search tool in R [R package]. Retrieved from http://www.austatgen.org/software/.

Delaney, K. J., Weaver, D. K., & Peterson, R. K. D. (2010). Photosynthesis and yield reductions from wheat stem sawfly (Hymenoptera : Cephidae): Interactions with wheat solidness , water stress , and phosphorus deficiency. *Journal of Economic Entomology*, *103*(2), 516–524. https://doi.org/10.1603/EC09229

Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., & Mitchell, S. E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS ONE*, *6*(5). https://doi.org/10.1371/journal.pone.0019379

Fehr, W. (1991*).* Heritability. In E. L. Fehr & J. Jession (Eds.) *Principles of cultivar development: Theory and technique* (pp. 94-104). New York, NY: Macmillian Publishing Company.

Fisher, M. (2017). Combating wheat stem sawfly in Colorado. *Crops and Soils Magazine*, (November-December), 4–8. Retrieved from dl.sciencesocieties.or/publications/crops-and-soils

Fulbright, J., Wanner, K., Bekkerman, A., & Weaver, D. (2017). Wheat stem sawfly biology. *Montana State University Extension*, *MontGuide* (MT201107AG).Retrived from https://agresearch.montana.edu/wtarc/producerinfo/entomology-insect-ecology/WheatStemSawfly/WSS-MontGuide.pdf

Glaubitz, J. C., Casstevens, T. M., Lu, F., Harriman, J., Elshire, R. J., & Sun, Q. (2014). TASSEL-GBS: A high capacity genotyping by sequencing analysis pipeline. *PLoS ONE*, *9*(2). https://doi.org/10.1371/journal.pone.0090346

Grogan, S. M., Anderson, J., Baenziger, P. S., Frels, K., Guttieri, M. J., Haley, S. D., … Byrne, P. F. (2016). Phenotypic plasticity of winter wheat heading date and grain yield across the US great plains. *Crop Science*, 56, 2223-2236. https://doi:10.2135/cropsci2015.06.0357

Groos, C., Robert, N., Bervas, E., & Charmet, G. (2003). Genetic analysis of grain protein-content, grain yield and thousand-kernel weight in bread wheat. *Theoretical and Applied Genetics*, *106*, 1032–1040. https://doi.org/10.1007/s00122-002-1111-1

Haley, S. D., Johnson, J. J., Peairs, F. B., Stromberger, J. A., Hudson-Arns, E. E., Seifert, S. A., … Anderson, V. A. (2018). Registration of 'Avery' hard red winter wheat. *Journal of Plant Registrations*, 12, 362-366. https://doi:10.3198/jpr2017.11.0080crc

Haley, S. D., Johnson, J. J., Peairs, F. B., Stromberger, J. A., Heaton, E. E., Seifert, S. A., … Kottke, R. A. (2011), Registration of 'Snowmass' wheat. *Journal of Plant Registrations*, 5, 87-90. https://doi:10.3198/jpr2010.03.0175crc

Haley, S. D., Johnson, J. J., Peairs, F. B., Stromberger, J. A., Hudson, E. E., Seifert, S. A., … Kottke, R. A., Valdez (2012). Registration of 'Byrd' wheat. *Journal of Plant Registrations*, 6, 302-305. https://doi:10.3198/jpr2011.12.0672crc

Haley, S .D., Johnson, J. J., Peairs, F. B., Stromberger, J. A., Hudson, E. E., Seifert, S. A., … Kottke, R. A. (2012). Registration of 'Denali' wheat. *Journal of Plant Registrations*, 6, 311-314. https://doi:10.3198/jpr2011.12.0675crc

Haley, C. S., & Knott, S. A. (1992). A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity*, *69*, 315–324.

Haley, S., Quick, J., Johnson, J., Peairs, F., Stromberger, J., Clayshulte, S., … Kolmer, J. (2005). Registration of 'Hatcher' wheat. *Crop Science*, 45, 2654-2656. https://doi:10.2135/cropsci2005.0030

Hussain, W., Baenziger, S. P., Belamkar, V., Guttieri, M. J., Venegas, J. P., Easterly, A., … Poland, J. (2017). Genotyping-by-sequencing derived high-density linkage map and its application to QTL mapping of flag leaf traits in bread wheat. *Scientific Reports*, *7*(1), 1–15. https://doi.org/10.1038/s41598-017-16006-z

Irell, B., & Peairs, F. B. (2010). Wheat stem sawfly: A new pest of Colorado wheat. *Colorado State University Extension*, (Fact Sheet No. 5.612). Retrieved from www.ext.colostate.edu

Ivie, M. A. (2001). On the geographic origin of the wheat stem sawfly (Hymenoptera : Cephidae). *American Entomologist*, *47*(2), 84–97.

Kiseleva, A., Potokina, E., & Salina, E. (2017). Features of Ppd-B1 expression regulation and their impact on the flowering time of wheat near-isogenic lines. *BMC Plant Biology*, *17*(172). https://doi.org/10.1186/s12870-017-1126-z

Lesieur, V., Martin, J.-F., Weaver, D. K., Hoelmer, K. A., Smith, D. R., Morrill, W. L., … Bon, M.-C. (2016). Phylogeography of the wheat stem sawfly, Cephus cinctus Norton (Hymenoptera: Cephidae): Implications for pest management. *PLOS ONE*, *11*(12), 1–19. https://doi.org/10.1371/journal.pone.0168370

Li, H., & Durbin, R. (2009). Transform, fast and accurate short read alignment with Burrows–Wheeler. *Bioinformatics*, *25*(14), 1754–1760.

Li, Q., Zhang, Y., Liu, T., Wang, F., Liu, K., Chen, J., & Tian, J. (2015). Genetic analysis of kernel weight and kernel size in wheat (Triticum aestivum L.) using unconditional and conditional QTL mapping. *Molecular Breeding*, *34*(194). https://doi.org/10.1007/s11032-015-0384-4

Li, W., Zhang, P., Fellers, J. P., Friebe, B., & Gill, B. S. (2004). Sequence composition, organization, and evolution of the core Triticeae genome. *The Plant Journal*, *40*(4), 500-511.

Merten, C. (2019, May 20). Personal interview. Mertens Diversified Ag, LLC, New Raymer, CO.

Money, D., Gardner, K., Migicovsky, Z., Schwaninger, H., Zhong, G.-Y., & Myles, S. (2015). LinkImpute: Fast and accurate genotype imputation for nonmodel organisms. *G3: Genes, Genomes, Genetics*, *5*(5), 2383–2390. https://doi.org/10.1534/g3.115.021667

Morrill, W. L., & Kushnak, G. D. (1996). Wheat stem sawfly (Hymenoptera: Cephidae) adaptation to winter wheat. *Environmental Entomology*, *25*(5), 1128–1132. https://doi.org/10.1093/ee/25.5.1128

Morrill, W. L., Kushnak, G. D., Bruckner, P. L., & Gabor, J. W. (1994). Wheat stem sawfly (Hymenoptera: Cephidae) damage, rates of parasitism, and overwinter survival in resistant wheat lines. *Journal of Economic Entomology*, *87*(5), 1373–1376. https://doi.org/10.1093/jee/87.5.1373

Nilsen, K. T., Clarke, J. M., Beres, B. L., & Pozniak, C. J. (2016). Sowing density and cultivar effects on pith expression in solid-stemmed durum wheat. *Agronomy Journal*, *108*(1), 219–228. https://doi.org/10.2134/agronj2015.0298

Ouellette, L. A., Reid R. W., Blanchard, S. G., & Brouwer, C. R. (2017). LinkageMapView - rendering high resolution linkage and QTL maps. *Bioinformatics*, 34(2), 306-307. https://doi.org/10.1093/bioinformatics/btx576

Piesik, D., Weaver, D. K., Runyon, J. B., Buteler, M., Peck, G. E., & Morrill, W. L. (2008). Behavioural responses of wheat stem sawflies to wheat volatiles. *Agricultural and Forest Entomology*, *10*(3). https://doi.org/10.1111/j.1461-9563.2008.00380.x

Santra, M., Wang, H., Seifert, S., & Haley, S. (2017). Doubled haploid laboratory protocol for wheat using wheat--maize wide hybridization. In P. L. Bhalla & M. B. Singh (Eds.), *Wheat Biotechnology: Methods and Protocols* (pp. 235–249). https://doi.org/10.1007/978-1-4939-7337-8_14

Sherman, J. D., Weaver, D. K., Hofl, M. L., Sing, S. E., Buteler, M., Lanning, S. P., … Talbert, L. E. (2010). Identification of novel QTL for sawfly resistance in wheat. *Crop Science*, *50*, 73–86. https://doi.org/10.2135/cropsci2009.03.0145

Soil Survey Staff (2018). Web soil survey: Soil data mart. Retrieved from https://websoilsurvey.sc.egov.usda.gov/.

Talbert, L. E., Sherman, J. D., Hofland, M. L., Lanning, S. P., Blake, N. K., Grabble, R., … Weaver, D. K. (2014). Resistance to Cephus cinctus Norton, the wheat stem sawfly, in a recombinant inbred line population of wheat derived from two resistance sources. *Plant Breeding*, *133*(4). https://doi.org/10.1111/pbr.12184

Taylor, J., & Butler, D. (2017). R package ASMap: Efficient genetic linkage map construction and diagnosis. *Journal of Statistical Software, 79*(6). https://doi.org/10.18637/jss.v079.i06

R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from http://www.R-project.org/.

Sherman, J. D., Blake, N. K., Martin, J. M., Kephart, K. D., Smith, J., Clark, D. R., … Talbert, L. E. (2015). Agronomic impact of a stem solidness gene in near-isogenic lines of wheat. *Crop Science*, *55*, 514–520. https://doi.org/10.2135/cropsci2014.05.0403

USDA National Agricultural Statistics Service. (2019). NASS - Quick Stats. Retrieved from https://data.nal.usda.gov/dataset/nass-quick-stats

Varella, A. C., Weaver, D. K., Peterson, R. K. D., Sherman, J. D., Hofland, M. L., Blake, N. K., … Talbert, L. E. (2017). Host plant quantitative trait loci affect specific behavioral sequences in oviposition by a stem - mining insect. *Theoretical and Applied Genetics*, *130*(1), 187–197. https://doi.org/10.1007/s00122-016-2805-0

Varella, A. C., Weaver, D. K., Sherman, J. D., Blake, N. K., Heo, H. Y., Kalous, J. R., … Talbert, L. E. (2015). Association analysis of stem solidness and wheat stem sawfly resistance in a panel of North American spring wheat germplasm. *Crop Science*, *55*(5). https://doi.org/10.2135/cropsci2014.12.0852

Varella, A. C., Zhang, H., Weaver, D. K., Cook, J. P., Hofland, M. L., Lamb, P., … Talbert, L. E. (2019). A novel QTL in durum wheat for resistance to the wheat stem sawfly associated with early expression of stem solidness. *G3: Genes, Genomes, Genetics*, 9(6), 1–36. https://doi.org/10.1534/g3.119.400240

Verma, S., Gupta, S., Bandhiwal, N., Kumar, T., Bharadwaj, C., & Bhatia, S. (2015). High-density linkage map construction and mapping of seed trait QTLs in chickpea (Cicer arietinum L.) using genotyping-by-sequencing (GBS). *Scientific Reports*, *5*, 1–14. https://doi.org/10.1038/srep17512

Weaver, D. K., Sing, S. E., Runyon, J. B., & Morrill, W. L. (2004). Potential impact of cultural practices on wheat stem sawfly (Hymenoptera : Cephidae) and associated parasitoids. *Journal of Agricultural and Urban Entomology*, *21*(4), 271–287.

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

Wickham, H. (2016). ggplot2: Elegant graphics for data analysis [R package]. Retrieved from https://ggplot2.tidyverse.org

Wu, Y., Bhat, P. R., Close, T. J., & Lonardi, S. (2008). Efficient and accurate construction of genetic linkage maps from the minimum spanning tree of a graph. *PLoS Genetics*, *4*(10). https://doi.org/10.1371/journal.pgen.1000212

Xin, F., Zhu, T., Wei, S., Han, Y., Zhao, Y., Zhang, D., … Ding, Q. (2020). QTL mapping of kernel traits and validation of a major QTL for kernel length-width ratio using SNP and bulked segregant analysis in wheat. *Scientific Reports*, *10*(25). https://doi.org/10.1038/s41598-019-56979-7

Zhang, R., Hou, F., Feng, Y., Zhang, W., Zhang, M., & Chen, P. (2015). Characterization of a Triticum aestivum–Dasypyrum villosum T2VS·2DL translocation line expressing a longer spike and more kernels traits. *Theoreticla and Applied Genetics,* 128**,** 2415–2425. https://doi.org/10.1007/s00122-015-2596-8

Zikhali, M., & Griffiths, S. (2015). The effect of earliness per se (Eps) genes on flowering time in bread wheat. In Y. Ogihara, S. Takumi, & H. Handa (Eds). *Advances in Wheat Genetics: From Genome to Field.* Tokyo: Springer. https://doi.org/10.1007/978-4-431-55675-6_39

APPENDIX

Stem Solidness Analysis

Figure A shows results from the stem solidness evaluation of parents from each double haploid population along with control varieties during the 2018-2019 field season. Only two of the ten varieties had a stem solidness value greater than 10. Avery and CO11D137 had the lowest solidness ratings of 6.7 and 6.9, while Denali and Hatcher were slightly higher at 9.5 and 8.2. Solidness ratings below 10 classify each of these varieties as being hollow-stemmed. Fortify SF (12.7) and Bearpaw (18.4) had stem solidness ratings greater than 10, with Fortify SF being classified as a semi-solid and Bearpaw being the only solid-stemmed variety in the trial.

Denali/Hatcher QTL Analysis

A total of 1,758 polymorphic SNP markers were assigned to 21 linkage groups, creating a final genetic map with a total size of 5,493 cM (Figure B). Marker distribution across the genome was unequal; with the B genome having 870 (49%) SNP markers, the A genome consisting of 670 (38%) SNP markers, and the D genome having the least SNP markers at 219 (12%) (Figure B). The final genetic linkage map had an average marker spacing of 4.5 cM, however large gaps (> 10 cM) were observed on all chromosomes. The genetic map is inflated due to higher than expected genetic recombination frequencies, and ten chromosomes (2A, 2B, 3A, 3B, 4B, 5A, 5B, 6B, 7A, 7D) have a genetic distance greater than 300 cM (Figure B).

Six different QTLs were identified for cutting score on chromosomes 2A, 2D, 3A, 4B, 4D, and 5B (Table A and Figure C). Hatcher contributed the resistance allele for only one of the QTL (located on chromosome 2D), whereas Denali contributed the resistance allele for the remaining five QTLs. Major-effect QTL (R^2 > 10%) were identified for *Qwss.csu*-2DS (R^2 = 10.4-13.6%) and *Qwss.csu*-5BS (R^2 = 12.4-13.9%) and were significant in both environments. The *Qwss.csu*-5BS QTL had the largest effect

with the Denali allele contributing a 0.2–0.3 point reduction in WSS cutting scores, however the *Qwss.csu*-2DS QTL reduced cutting by a similar amount (0.2-0.3) when the Hatcher allele was present. Two minor-effect QTLs on chromosome 3A and 4D QTL explained 9.4-105% and 8.0–9.5% of the phenotypic variation while reducing the cutting score by 0.2– 0.3. An additional two QTLs on 2A and 4B were identified by the analysis, however they were only present in a single environment.

Four QTL were detected for heading date on chromosomes 2D, 4D, 5A, and 7B (Table A and Figure C). Two of four QTL (2D and 4D) were considered major-effect and both were significant in each of the environments. The QTL on chromosome 2D explained 25.1% and 26.0% of the phenotypic variation in New Raymer 2019 and Orchard 2019. Allelic effect from the 2D QTL decreased the heading date by an average of 1 day when the Denali allele was present. The QTL identified on 4D had a phenotypic variation of 12.2-14.4% and reduced heading date by 0.7 days with the Hatcher allele. The remaining two QTL on 5A and 7B were not considered to be major-effect and were only present in a single environment.

Quantitative trait loci were identified for physiological maturity date on chromosomes 2D and 4D (Table A and Figure C). One QTL was major-effect, accounting for 16.3% (New Raymer 2019) and 24.0% (Orchard 2019) of the phenotypic variation. The effect of the QTL was considerably different between the two environments, with a -0.3 day reduction in New Raymer 2019 and a -0.7 day reduction in Orchard 2019 – in the Denali allelic state. The other QTL on 4D contributed a much smaller proportion of the phenotypic variation at only 7.5% in Orchard 2019. The Hatcher allele reduced the physiological maturity for the 2D QTL by 0.4 days.

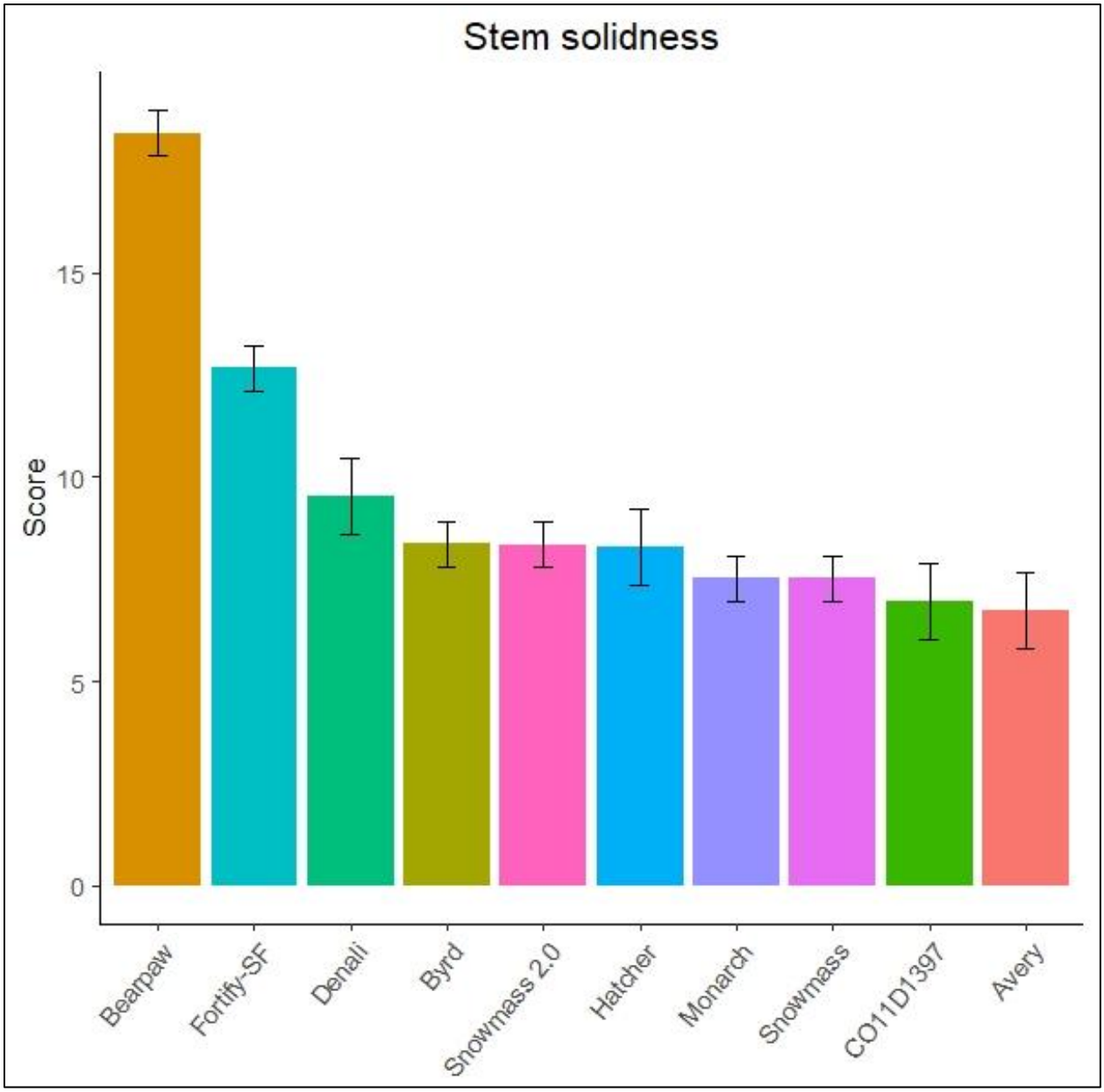

Figure A. Best linear unbiased predictor values for stem solidness of parents from each double haploid population and control varieties. Stems were collected from each trial location during the 2018-2019 field season. Stems were dissected at each internode and scored for pith expression on a scale of 1 (hollow) to 5 (solid), and the values for all five internodes were summed for a final score range of 5 (hollow) to 25 (solid). Standard error bars for stem solidness are represented above each variety.

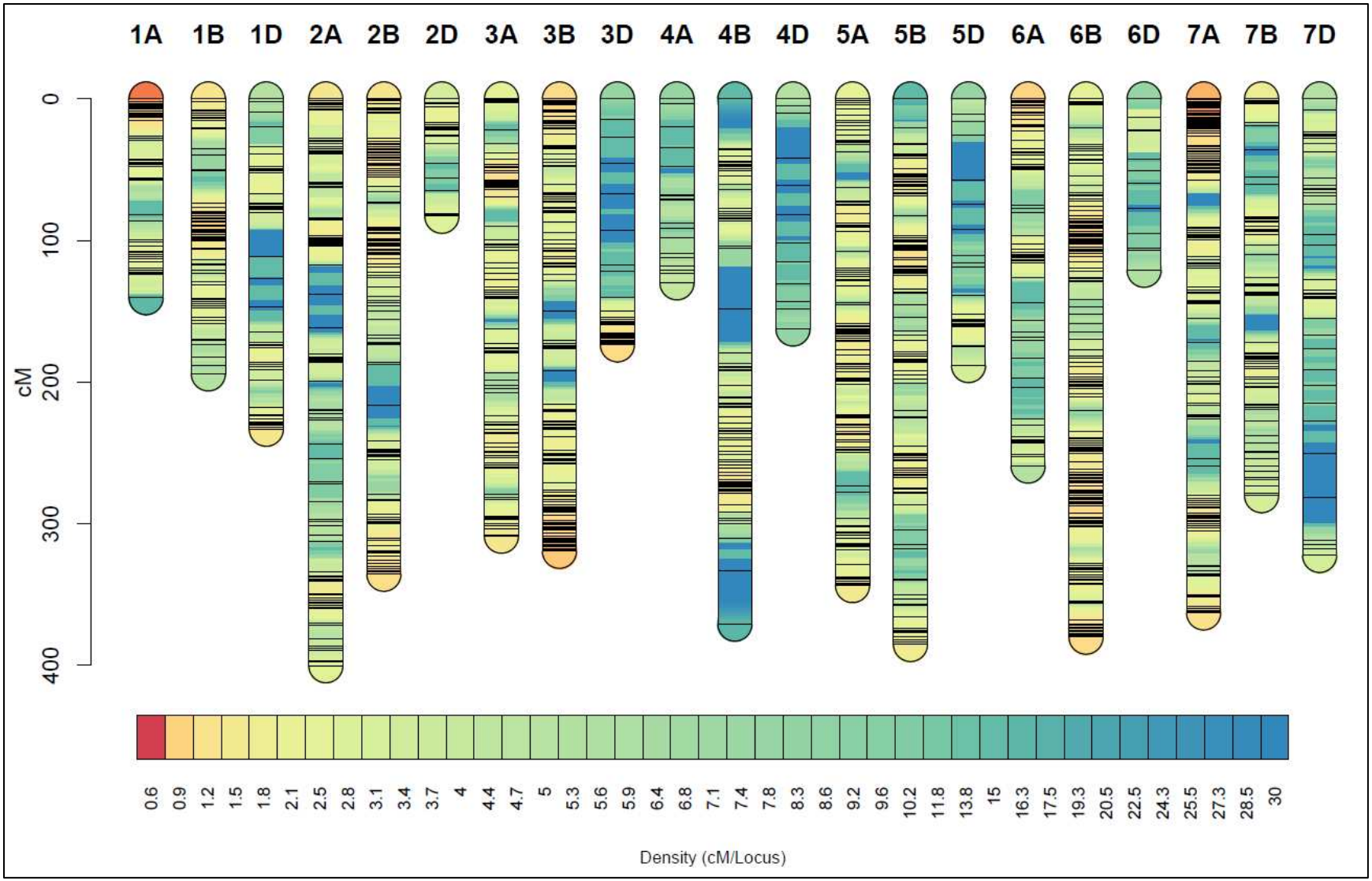

Figure B. Distribution and density of single-nucleotide polymorphisms markers on the final linkage map for the Denali/Hatcher doubled haploid population.

Table A. Quantitative trait loci (QTL) associated with measured phenotypic traits in the Denali/Hatcher double haploid population.

Trait†	Chr	Position‡	CI¶	New Raymer 2019			Orchard 2019		
		(cM)	(Mb)	LOD	R^2§ (%)	Effect#	LOD	R^2§ (%)	Effect#
CUT	2A	83.9	53.8-745.9	3.8	8.4	-0.3	-	-	-
	2DS	82.7	62.0-476.2	4.8	10.4	0.3	6.3	13.6	0.2
	3AL	308.9	743.3-753.0	4.8	10.5	-0.3	4.3	9.4	-0.2
	4B	148.3	7.5-672.6	-	-	-	3.3	7.4	-0.2
	4DS	60.7	194.0-483.2	3.6	8.0	-0.3	4.3	9.5	-0.2
	5BS	185.3	35.4-112.0	6.5	13.9	-0.3	5.7	12.4	-0.2
HD	2DS	81.7	62.0-112.9	13.0	26.0	-0.9	12.5	25.1	-1.1
	4DL	143.3	497.9-511.9	6.7	14.4	0.7	5.6	12.2	0.8
	5AL	190.1	503.9-549.7	-	-	-	4.0	8.7	0.6
	7BL	310.0	490.4-738.5	3.9	8.7	0.8	-	-	-
MAT	2DS	82.8	62.0-112.9	7.7	16.3	-0.23	11.8	24.0	-0.7
	4DS	143.3	113.5-511.9	-	-	-	3.4	7.5	0.4

† Phenotypic trait; CUT, cutting score; HD, heading date; MAT, physiological maturity.
‡Peak QTL position.
¶ One LOD confidence interval on either side of the QTL reported in megabase pair position from the International Wheat Genome Sequencing Consortium (IWGSC) RefSeq v2.0.
§ Percent of phenotypic variation explained by the QTL.
# Additive effect of the Denali allele.

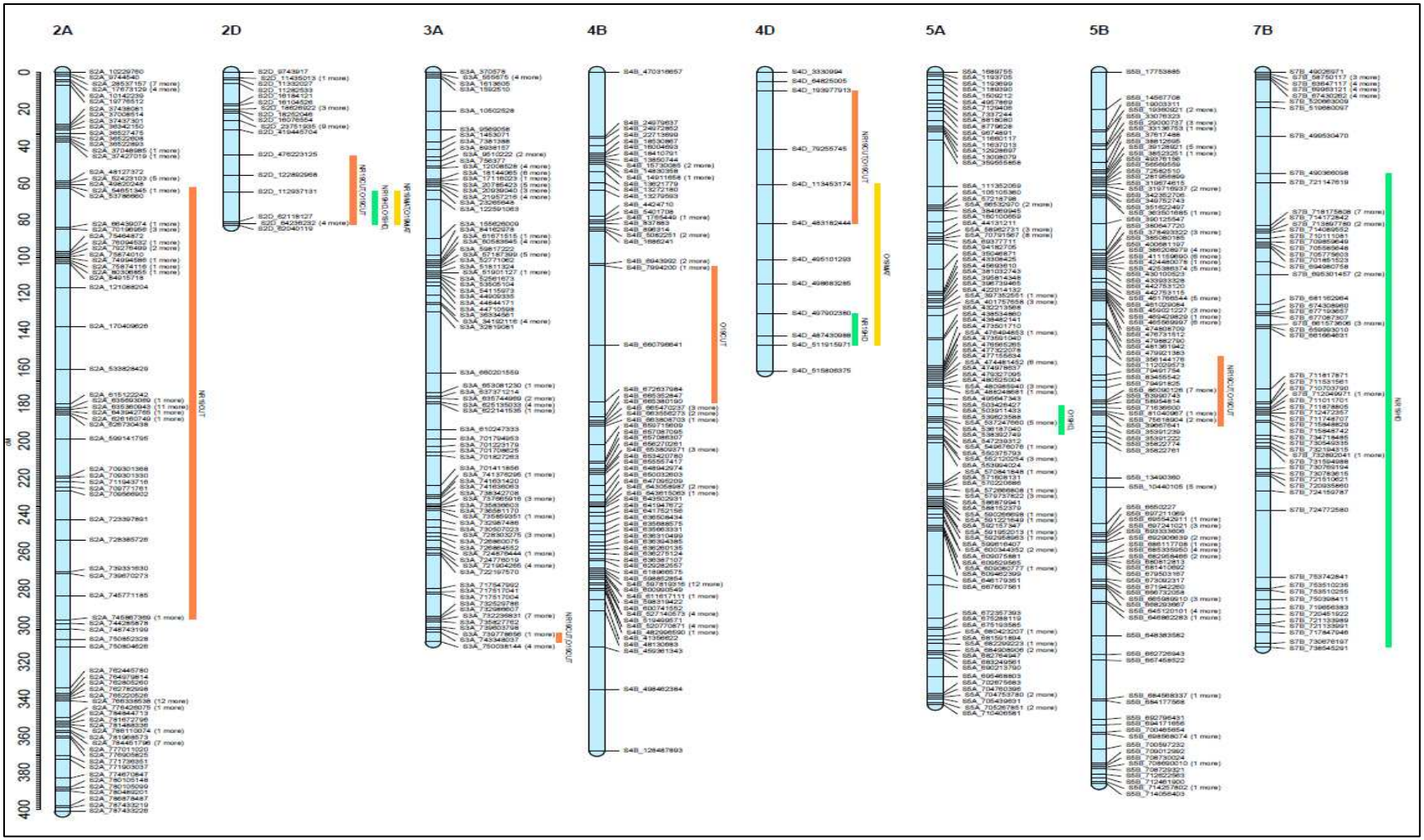

Figure C. Chromosomal locations of quantitative trait loci (QTL) detected for cutting score (*orange*), heading date (*green*), and physiological maturity (*gold*) in the Denali/Hatcher double haploid population in two Colorado environments in 2019. Quantitative trait loci intervals represent a one LOD score drop. Quantitative trait loci names are denoted by trait (CUT, cutting score; HD, heading date; MAT, physiological maturity), site (O, Orchard; NR, New Raymer), and year (19, 2019). The number of single-nucleotide polymorphisms (SNP) markers with the same genetic position but different physical positions is indicated in parenthesis next to the SNP marker.