Identificaton of microsatellite markers associated to a solid stem QTL in wheat
by Jason Patrick Cook

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Plant Sciences
Montana State University

Abstract:
The research that was conducted involved phenotyping and genotyping a doubled haploid (DH) winter wheat population derived from a 'Rampart' (solid stems) X 'Jerry' (hollow stem) cross to identify molecular markers linked to solid stem genes, which provide wheat stem sawfly resistance. Additionally, the DH population was used to determine if a relationship exists between stem solidness and other important traits, such as yield. The DH population was genotyped using GWM and BARC microsatellite primers that spanned the whole-wheat genome. To efficiently genotype the population, bulked segregent analysis was used to identify polymorphism between groups of solid stem and hollow stem individuals. Four microsatellite markers (GWM247, GWM340, GWM547, and BARC77) were found linked to a single solid stem QTL (designate Qss.msub-3BL) on chromosome 3BL. Linear regression analysis showed Qss.msub-3BL contributes at least 76% of the total variation for stem solidness. GWM247, GWM340, GWM547 are more closely linked to Qss.msub-3BL then BARC77. Additionally, linear regression analysis showed no relationship between Qss.msub-3BL and other traits. Also, trait to trait correlation analysis revealed no correlation to stem solidness and other traits except for plant height. It is hoped that GWM247, GWM340, and GWM547 will be useful for selecting solid stem varieties without deleterious affects on yield.

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TO A SOLID STEM QTL IN WHEAT

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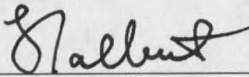MONTANA STATE UNIVERSITY
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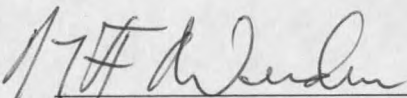
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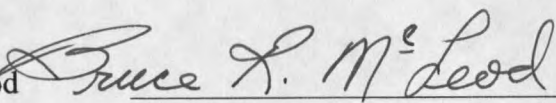(Signature)   Date

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iv

ACKNOWLEDGMENTS

TABLE OF CONTENTS

vi

TABLE OF CONTENTS - CONTINUED

LIST OF TABLES

LIST OF FIGURES

ix

ABSTRACT

The research that was conducted involved phenotyping and genotyping a doubled haploid (DH) winter wheat population derived from a 'Rampart' (solid stems) X 'Jerry' (hollow stem) cross to identify molecular markers linked to solid stem genes, which provide wheat stem sawfly resistance. Additionally, the DH population was used to determine if a relationship exists between stem solidness and other important traits, such as yield. The DH population was genotyped using GWM and BARC microsatellite primers that spanned the whole-wheat genome. To efficiently genotype the population, bulked segregent analysis was used to identify polymorphism between groups of solid stem and hollow stem individuals. Four microsatellite markers (GWM247, GWM340, GWM547, and BARC77) were found linked to a single solid stem QTL (designate *Qss.msub-3BL*) on chromosome 3BL. Linear regression analysis showed *Qss.msub-3BL* contributes at least 76% of the total variation for stem solidness. GWM247, GWM340, GWM547 are more closely linked to *Qss.msub-3BL* then BARC77. Additionally, linear regression analysis showed no relationship between *Qss.msub-3BL* and other traits. Also, trait to trait correlation analysis revealed no correlation to stem solidness and other traits except for plant height. It is hoped that GWM247, GWM340, and GWM547 will be useful for selecting solid stem varieties without deleterious affects on yield.

CHAPTER 1

INTRODUCTION

Wheat stem sawfly (WSS), *Cephus cinctus* Norton, is a pest that inflicts severe economic damage to the winter and spring wheat, *Triticum aestivum* L., production areas of the Northern Great Plains of North America. The pest is a native species of North America, originally preferring wild grasses, primarily *Agropyron* spp (Criddle 1923). In the early 1900's, wheat stem sawfly switched to wheat as its primary host (Wallace and McNeal 1966). Damage caused by WSS is two-fold and only inflicted by the larva. Larva will first tunnel inside the stem, feeding on vascular tissue and parenchyma cells (Holmes 1954). The larval tunneling and feeding disrupts water and nutrient translocation to the developing kernels, causing up to 22% decrease in test weight and more then 1% loss in protein content (Holmes 1977). Secondly, when the larva is mature it migrates towards the base of the stem and cuts a ring or girdle around the stem wall. The girdling weakens the stem, substantially increasing lodging with consequent yield loss (Morrill et al.1992).

Despite considerable effort to control WSS proliferation and migration with cultural, chemical, and biological methods, only plant host resistance has proven to be effective. Plant host resistance is found in wheat accessions that have stems filled with pith, referred to as solid stems (Kemp 1934). The pith impedes larval growth and migration, greatly reducing stem cutting and population abundance (Wallace and McNeal 1966). The first publicly released WSS resistant cultivar was 'Rescue' (Stoa 1947).

Cytogenetic and inheritance analyses have determined that several genes may control solid stem expression. Larson and MacDonald (1959) identified the presence of potential genes for stem solidness on chromosomes 3B, 3D, 5A, 5B, and 5D. Inheritance studies have shown that 3 or 4 genes cause stems to be solid, but one gene in particular appears to account for more then twice the genetic variation compared to the other two or three genes (McNeal 1956, McKenzie 1965).

Acceptance of WSS resistant varieties has been minimal in areas where WSS population levels are low or non-existent. Solid stem varieties yield significantly less then hollow stem varieties in areas where little WSS pressure is present (Weiss and Morrill 1992). Early research showed a significant negative correlation between stem solidness and yield (McNeal et al.1965). However, more recent studies have indicated that the negative correlation between stem solidness and yield was not significant (Hayat et al. 1995). Hayat's data attributes low yield in solid stem varieties to the poor genetic background of the solid stem source rather then pleiotropy or deleterious linkage.

Breeding high yielding WSS resistant cultivars is problematic because of the subjectivity of solid stem scoring and variation of expression due to environmental effects. Marker Assisted Selection (MAS) could enhance the accurate identification of breeding lines with solid stem genes. By using molecular markers to ensure the presence of solid stem genes, backcrossing would become a viable option for developing WSS resistant wheat varieties in high yielding genetic backgrounds.

Microsatellite markers have become a popular DNA marker system in wheat (Plashke et al. 1995, Roder et al. 1995, Bryan et al. 1997). A microsatellite map

developed by Roder et al. (1998) demonstrates that microsatellite loci are evenly

distributed across the wheat genome providing excellent coverage for marker analysis.

Recently, several microsatellites have been identified linked to both pest and disease

resistance in wheat (Chantret et al. 2000, Huang et al. 2000, Liu et al. 2001, Ghislain et

al. 2001, Liu et al. 2002). This report details the identification of microsatellite markers

closely linked to a stem solidness gene in wheat. The markers may be suitable for MAS

of WWS resistant wheat varieties.

4

CHAPTER 2

LITERATURE REVIEW

History of Wheat Stem Sawfly

Wheat stem sawfly (WSS), *Cephus cinctus* Norton, is a native insect of North America, preferentially living in areas where annual precipitation ranges from 250-500 mm (Weiss and Morrill 1992). Originally, the insect inhabited large-stemmed native grasses (Ainsle 1920, Criddle 1923). In 1895, sawfly larva was observed in several native grass species in the Northwest Territories of Canada (Ainslie 1920). Observations in 1905 and 1906 indicated sawfly preferentially inhabited *Agropyron* spp grasses in Wyoming and the Dakotas (Ainslie 1920). By 1908, sawfly was found to inhabit native grasses as far west as Oregon and as far south as California and Nevada (Ainslie 1929).

In the late 1800's, farmers began to cultivate the native grasslands of the Northern Plains for wheat production. As the abundance of native grasses dwindled and the abundance of wheat increased the insect was forced to adapt to spring wheat as its primary host (Criddle 1922, Ainslie 1929). The first report of sawfly damage in spring wheat occurred in 1895 at Moose Jaw, Saskatchewan (Ainslie 1920). Subsequent reports in 1900, from Bozeman, Montana; in 1907, from Minot, North Dakota; and in 1908, from Manitoba and Saskatchewan, indicated wheat stem sawfly was becoming a potential pest of spring wheat production (Ainslie 1920). By 1908 and 1910, severe economic damage was reported in Minot, ND and Bainville, MT respectively (Ainslie 1929, Montana Agricultural Experiment Station and Montana Extension Service 1946). Severe losses

were also reported in the Southern Prairie Provinces of Canada in 1926 and 1931 (Atkinson 1931, King 1929). From 1943 –1955, the economic impact of sawfly increased as its area of infestation expanded in Montana, North Dakota, and Canada (Mills 1945, Montana Agricultural Experiment Station and Montana Extension Service, 1946, Bird 1955). By 1954, annual losses to spring wheat production had reached $17 million in Montana and North Dakota (Davis 1955).

Initially, winter wheat escaped sawfly damage due to early maturation (Wallace and McNeal 1966). Unfortunately, sawfly adapted to the growth pattern of winter wheat between 1970 and 1985 (Morrill and Kushnak 1996). By 1985, consistent reports of sawfly infestation in winter wheat were documented (Morrill and Kushnak 1996). Presently, wheat stem sawfly is the primary economic pest for winter and spring wheat production in Montana, North Dakota, and the Southern Prairie Provinces of Canada.

<u>Insect Morphology</u>

<u>Adult</u>

Adult sawflies are slender and approximately 1cm long (Morrill 1995). The insect has a black body with yellow markings on the abdomen (Wallace and McNeal 1966). Sawfly have two pairs of clear wings that appear golden in the sunlight (Morrill 1995).

In late May to early June, adult sawfly begin to emerge from wheat stubble. The male sawfly generally emerges before the female sawfly (Holmes 1982). The duration of

emergence can last 3 to 4 weeks (Morrill et al. 1992). Environment dictates the timing of emergence with ideal conditions combining a warm moist May, a hot June, adequate moisture for vigorous plant growth, and sporadic dry periods to allow sawfly to emerge (Seamans 1945). Emergence also coincides with the host plant growth stage suitable for ovipositing. Once the sawfly emerges, it typically lives 5 to 8 days (Wallace and McNeal 1966).

After emergence, the female sawfly will seek suitable stems, one that is young, succulent, elongating, and has a diameter between 2.8 and 3.4 mm, for depositing its eggs (Holmes and Peterson 1960). Using Zadoks et al. (1974) growth stage code, Morrill and Kushnak (1996) indicated that the plant growth stages susceptible to ovipositing started at growth stage 31 (first detectable internode) and ended at stage 40 (boot). Sawfly are relatively weak flyers capable of traveling no further then 2 km (Morrill 1995). The female will typically oviposit its eggs into stems that are in close proximity to the site of emergence (Criddle 1911, Ainslie 1920, Holmes 1975). Adults are most active during the day when the temperature ranges from 17°-32° C and wind speed is minimal (Seamans 1945). Once the female finds a suitable stem, it will insert its saw-like ovipositor through the stem tissue and oviposit the egg (Wallace and McNeal 1966). A female sawfly will deposit one egg per stem and is capable of laying eggs in approximately 30 stems, depending on lifespan and vigor of the female sawfly (Ainslie 1920). Although a female sawfly will deposit only one egg per stem, subsequent female sawfly may also deposit eggs into the stem (Wallace and McNeal 1966). The developing

larvae will compete with one another until only one remains (Holmes 1954, Weiss and Morrill 1992).

Wheat stem sawfly is haplodiploid; the genome of the female has 18 chromosomes and the male has nine chromosomes (Mackay 1956). Sex of the sawfly is determined by selective egg fertilization at the time of oviposition (Flanders 1946). Typically, equal numbers of male and female sawfly persist in the environment (McGinnis 1950). However, male dominated populations can occur if late emerging female sawflies are not able to find mates (Jacobson and Farstad 1952).

The Egg and Larva

Wheat stem sawfly eggs are crescent-shaped, glossy, and milky-white in color (Ainslie 1920). The size of the egg depends on the size of the female sawfly. Eggs are typically 1.00 – 1.25mm long and 0.33-0.42mm wide (Ainslie 1920). The egg will incubate inside the stem for approximately seven days before the larva hatches (Ainslie 1920).

Newly emerged larvae are colorless and transparent until they begin feeding on plant tissue giving them a yellow green coloration (Wallace and McNeal 1966). The larva head is easily identified by its pale brown coloration, eyespots, and dark brown four-pointed mandibles (Wallace and McNeal 1966). Average length of the larva is 2.24 mm and an average width is 0.28 mm (Wallace and McNeal 1966). As the larva develops, it progresses through four to five instars (Ainslie 1920, Farstad 1940).

Sawfly larvae obtain nutrition by migrating up and down the stem feeding on plant tissue. Holmes (1954) found that parenchyma tissue makes up the majority of the ingested plant material, however as the larva matures, it might ingest vascular tissue as well. Sawfly larvae are cannibalistic when they encounter either sawfly eggs or another feeding larva. The larva that is the lowest in the stem usually destroys all other larvae and eggs above it. Because eggs are usually laid first in the lower portion of the stem, the first larva to develop is most likely to survive (Wallace and McNeal 1966). Only one larva will survive within a stem.

Completion of larval development usually coincides with plant senescence. As the plant begins to senesce, visible and infrared light transmitted though the stem wall changes, triggering the larva to migrate towards the stem base (Holmes 1975). Once the larva reaches the stem base, it will cut or girdle a V-shaped notch near the soil surface. After girdling, a frass plug approximately 4 mm in length is compactly inserted directly below the V-shaped notch (Wallace and McNeal 1966). The plug adds rigidity to the stem, forcing the stem to break cleanly where the V-shaped notch is cut, creating a stub (Wallace and McNeal 1966). If the stem collapsed upon itself, adult sawfly would not be able to emerge the following spring (Ainslie 1920). The remaining stub and frass plug provides an overwintering site for the sawfly larva, protecting it from extreme environmental conditions (Salt 1946a, Holmes and Farstad 1956). Inside the stub, the larva will form a transparent cocoon and enter obligatory diapause (Wall 1952, Villacorta et al. 1971). In the spring, larvae will pupate after spending a minimum of 90 days at 10°

C in diapause (Salt 1947). Pupation lasts 7 to 14 days and then the adult will emerge (Criddle 1922, Holmes 1954).

Plant Hosts

Wheat stem sawfly larvae have been found in several cultivated and native plant species. The preferred cultivated host is *Triticum aestivum* (common wheat), however sawfly will also infest other *Triticum* spp. such as *T. compactum, T. spelta, T. sphaerococcum, T. carthlicum, T. dicoccum, T. durum,* and *T. monococcum* but with limited success primarily due to narrow stem diameters (Wallace and McNeal 1966). Sawfly will also infest *Hordeum vulgare* L. (barley), *Secale cereale* L. (rye), *Avena sativa* (oats), and *Linum usitatissimum* L., Linaceae (flax), but larva mortality is usually high and in the case of oats mortality is nearly 100% (Farstad 1944, Farstad and Platt 1946, Wallace and McNeal 1966).

Along with cultivated plant species, sawfly will also infest many native plant species as well. It is well documented that *Elymus* spp. are preferred by sawfly (Criddle 1923). *Agropyron* species that have been infested include *E. caninum, E. cristatum, E. dasystachyum, E. elongatum, E. intermedium, E. repens,* and *E. smithii* (Wallace and McNeal 1966). Other native species such as *Beckmannia syzigachne, Bromus inermis* and *Bromus secalinus,* to name a few, have had larvae detected in their stems (Wallace and McNeal 1966). Female sawfly will typically shun grasses with narrow stems (Wallace and McNeal 1966). Variation of grass phenology at the time of sawfly

emergence will dictate which grass species will be most likely infested with sawfly larvae (Wallace and McNeal 1966).

Crop Damage

Damage inflicted by wheat stem sawfly is two-fold and only caused by the larva inside the stem. First, larval feeding will damage vascular tissue disrupting carbohydrate and water translocation to the developing kernels (Holmes 1954). Evidence of carbohydrate translocation disruption can be observed by the presence of darkened spots, caused by the accumulation of carbohydrates, on the sub-nodal regions of the stem (Morrill et al. 1992). Reduction of carbohydrate and water translocation reduces kernel weight and numbers. Kernel weight reduction ranges from 2.8 – 10%, depending on the wheat variety (Morrill et al. 1992). Other studies have shown kernel weight reductions to be 10.8 – 22.3% (Holmes 1977), 5 – 20% (McNeal et al. 1955), and 3% (Munro et al. 1947). Holmes (1977) also observed a reduction in grain protein content that ranged between 0.6 – 1.2%.

The sawfly larva causes additional damage when it reaches maturity and ceases to feed. At the end of the growing season the larva will migrate to the base of the stem and cut a V-shaped notch or girdle nearly completely through the stem wall (Holmes 1975). Wind will induce the cut stem to break away causing extensive lodging (Weiss and Morrill 1992). Lodging increases the difficulty of harvesting the grain and also reduces grain quality (Holmes 1977).

Methods of Control

Since the inception of sawfly as a pest in wheat, substantial effort has been put forth to control the pest. Cultural, chemical, and biological strategies have been studied for their effectiveness. The single most effective means of control is solid stem resistant wheat cultivars. Cultural, chemical, and biological strategies alone have not been found to be economically effective because of the biology of sawfly. Emergence of adult sawfly is sporadic over a 3 to 4 week period, making it very difficult to eradicate all of the adults at one time. Also, the stem and soil protects the larva from desiccation while it feeds during the growing season and freezing during its winter dormancy. If sawfly infestation is not reduced below 7 to 9%, an infestation of 70 to 80% will likely occur the following year (Holmes 1982).

Cultural Control

Initial efforts for managing sawfly were focused on the use of cultural methods of control. Norman Criddle, a farmer hired by the Manitoba provincial government, initiated the first studies for controlling sawfly. From extensive research, Criddle (1911, 1913, 1915, 1922) proposed several strategies including: tillage, early mowing of rye grasses, refraining from disturbing grasses that are hosts to wheat stem sawfly parasites, planting trap crops in which larvae will not survive, planting non-host crops, early harvesting, and swathing. Since Criddle's research, further studies have produced mixed results for the effectiveness of cultural management techniques in controlling sawfly.

Shallow tillage, alternative seeding dates, swathing, and crop rotations have constituted the majority of the strategies chosen for cultural control research.

Shallow tillage, at depths less then 0.3 meters, is a common technique for weed control, but also has been extensively studied for sawfly management (Callenbach and Hansmeir 1944, Mills 1945, Holmes and Farstad 1956, Morrill et al. 1993). The purpose of shallow tillage is to disturb the soil surrounding stems cut by sawfly larva, exposing the overwintering larvae to the harsh environment (Holmes and Farstad 1956). Salt (1946, 1961a, 1961b) found that freezing and desiccation of larvae in exposed wheat stems significantly increased mortality. Both fall and spring tillage were studied for their effectiveness, however spring tillage appeared to be less effective because larva would sometimes re-enter diapause and emerge the following year (Church 1955, Holmes and Farstad 1956). Morrill et al. (1993) conducted a study, using shallow tillage in the fall, which showed larval survival rate in exposed stems to be 7.3% and 8.0% in 1990-1991 and 1991-1992, respectively. The drawback of using shallow tillage is the difficulty in freeing an adequate number of stems from the soil to sufficiently reduce sawfly populations below an economic threshold (Morrill et al. 1993). Large-scale tillage can also be disadvantageous because it reduces the amount of snow captured to increase soil moisture, and soil erosion may occur (Morrill et al. 1993).

Altering seeding dates has been shown to reduce sawfly infestation. The objective of altered seeding dates is to de-synchronize wheat development and sawfly emergence (Weiss et al. 1987), and is accomplished by seeding winter wheat early or delaying the seeding of spring wheat. By planting winter wheat early, the plants will be to

advanced (boot stage), and by delaying spring wheat seeding, the plants should be too immature (prior to stem elongation), at the time of adult emergence making it difficult for the female sawfly to find a suitable host. Callenbach and Hansmeier (1944) recommended seeding spring wheat after May 20 in highly infested sawfly areas. There are, unfortunately, risks associated with altering seeding dates. Late planting subjects spring wheat to higher possibility of moisture stress. Low levels of moisture will result in significant crop losses due to low germination. Losses may also occur if the plants are actively growing during July, which is one of the hottest and driest months of the year.

The use of swathing has long been considered a potential method for reducing sawfly-inflicted damage (Criddle 1922, Callenbach and Hansmieier 1944, Mills 1945). The primary purpose of swathing is to cut and windrow grain before lodging occurs to increase yields. Swathing was also studied for its potential in reducing sawfly population levels. Holmes and Peterson (1965) found no significant reduction of sawfly populations after swathing at the recommended grain moisture level of 35%. The larva had successfully migrated to the base of the stem before the grain was swathed. They determined that swathing would have to occur when the grain moisture levels were between 55 to 61% to adequately reduce sawfly numbers.

Dodds (1957) and Molberg (1963) observed that swathing grain before moisture levels dropped below 35% and 38% respectively, would reduce yield and test weight. Molberg (1963) reported losses as high as 14 bushels per acre from grain that was swathed at 55% moisture. Dodd (1957), however, found no significant yield differences in grain that was swathed at moisture levels ranging from 35.4% and 40.9%. The

potential risk for yield and test weight loss when grain is swathed at high moisture levels has prevented farmers from this method for reducing sawfly populations. However, swathing at the recommended grain moisture level of 35% is widely used in areas that are highly infested with sawfly to help reduce losses associated with lodging.

Crop rotations are a proven method for reducing sawfly populations and the damage that they inflict on wheat (Munro 1944, Callenbach and Hansmeier 1945, Butcher 1946). Using crop rotations with non-susceptible hosts limits the opportunity for female sawfly to oviposit and produce progeny, thereby reducing sawfly populations. There are several hosts, including flax, oats, and mustards that are not susceptible to sawfly infestation (Platt and Farstad 1946). Additionally, hosts, such as fall rye that are minimally affected by sawfly infestation, can also be used (Wallace and McNeal 1966). Unfortunately, economics associated with continuous planting of wheat makes it undesirable for producers to rotate a large amount of acreage into a non-host crop (Weiss and Morrill 1992).

Chemical Control

Insecticides have been thoroughly investigated for controlling wheat stem sawfly, including both foliar and systemic seed treatments. Foliar treatments are applied by spraying the insecticide onto the foliage of a growing crop. Systemic seed treatments are applied to seeds prior to planting and are translocated through the plant as it develops. Neither foliar nor systemic insecticides have provided acceptable control of wheat stem sawfly (Holmes and Hurtig 1952, Skoog and Wallace 1964, Wallace and McNeal 1966).

Wallace (1962) evaluated the systemic insecticide heptachlor. He reported sawfly larval mortalities ranging from 61.2% to 96.3% in 'Thatcher' spring wheat with most mortality occurring in the early instar larvae. Holmes and Peterson (1963a) also evaluated heptachlor on 'Thatcher' and reported inconsistent larval control. They concluded that heptachlor was only effective in the lower two internodes on early instar larvae. Mature larvae in higher internodes could tolerate heptachlor and successfully lodge the host plant.

In a more recent study, three foliar insecticides were evaluated for sawfly control. Blodgett et al. (1996) evaluated Lorsban 4E-SG (chlorpyrifos), Furadan 4F (carbofuran), and Warrior 1E (lambdacyhalothrin) in winter wheat at various rates. The insecticides were sprayed directly on 2 to 3 node winter wheat during peak sawfly emergence. Fifty stems were randomly chosen from each plot to determine the level of plot infestation. No significant differences were recorded in larvae per stem between control and treated plots.

Adult and larval biology of wheat stem sawfly makes control with conventional insecticides difficult and uneconomical. Sawfly larvae are protected from insecticides inside the stem, which make foliar insecticides impractical for larval control. It is also difficult to control adult sawflies with foliar insecticides because they emerge sporadically over a 3 to 4 week period, so a single insecticide application has little effect on reducing ovipositing females. While possible to kill sawfly adults with foliar insecticides, targeting the adults would require applications at three to five-day intervals

over the entire adult emergence cycle. This is prohibitively expensive in a wheat production system.

Biological Control

Use of biological controls, primarily parasitic insects, has been unsuccessful. In native grasses, wheat stem sawfly is attacked by nine species of hymenopterous parasites (Holmes et al. 1963). Two species, *Bracon cephi* (Gahan) and *Bracon lissogaster* (Muesebeck), have been found to parasitize sawfly in wheat (Somsen and Luginbill 1956, Holmes et al. 1963). The female parasite will seek sawfly larva by tapping on the stem with its antennae to determine the location of the larva (Somsen and Luginbill 1956). Once detected, the parasitoid will insert its ovipositor through the stem to paralyze the larva and place an egg on top of the larva. The egg will hatch, producing a larval parasite that feeds on the sawfly larva (Nelson and Farstad 1953). *Bracon cephi* and *Bracon lissogaster* have two generations per year in native grasses, but in wheat, the second generation is often not completed, possibly due to grain harvesting (Criddle 1923, Somson and Luginbill 1956, Holmes et al. 1963). Loss of the second generation limits the population size of the sawfly parasites, which therefore decreases the ability of the parasite to control sawfly.

Attempts with biological control agents from abroad have also occurred. In 1930, approximately 6,000 adult *Collyria calcitrator* (Gravenhorst), an egg parasite from Europe, was released in Saskatchewan (Smith 1931). Unfortunately, the released parasites never became established. Further releases of *Collyria calcitrator* over a nine-

year period were also unsuccessful (Weiss and Morrill 1992). *Bracon terebella* (Wesnsen), a European hymenopterous larval parasite, was released in the 1950's, and it also failed to be established (Davis et al. 1955). The reasons for the establishment failures have never been fully understood. The most likely explanation may be European parasitoids are not adapted to the North American climate. Overall, biological agents may hold promise for controlling wheat stem sawfly, however, current parasitoid population levels are insufficient to effectively reduce sawfly numbers.

Host Plant Resistance

Host plant resistance is the single most effective strategy for controlling sawfly in wheat (Roberts 1954, Holmes and Peterson 1962, Weiss and Morrill 1992). Resistance enables the plant to repel or tolerate pest infestation without causing a significant negative impact on productivity. Sawfly resistance in wheat was identified when a positive correlation between stem solidness and reduced sawfly damage was observed (Shchegolev 1926, Kemp 1934, Farstad 1940, Eckroth and McNeal 1953, Holmes and Peterson 1962). The first observation of sawfly resistance in solid stem wheat was reported in the 1920's. Shchegolev (1926) tested rye, barley, wheat, and oats and found solid stem wheat to be resistant to sawfly. A further investigation by Kemp (1934) concluded solid stem wheat could reduce sawfly damage to inconsequential levels. The potential for developing wheat stem sawfly resistant wheat compelled the Canadian government to collect solid stem accessions for the development of an agronomically suitable sawfly resistant cultivar for the Northern Plains. A solid stem spring wheat

cultivar from Portugal, S-615, was crossed with a hollow stem spring wheat cultivar 'Apex' to generate a solid stem cultivar, 'Rescue' (Stoa 1947). 'Rescue' was initially released in Canada in 1946 and then in the United States in 1947 (Wallace and McNeal 1966). It was reported that the first year 'Rescue' was used in a highly infested sawfly area, damage was reduced to 5% while hollow stem varieties sustained nearly 95% losses (Platt et al. 1948). The success of 'Rescue' has prompted further development of solid stem cultivars, including winter wheat, with 'Rescue' being the solid stem source.

Even though 'Rescue' was successful in reducing sawfly damage, it possessed poor agronomic characteristics. Yields were generally 8 to 15% less then hollow stem varieties in areas with low sawfly infestation, and it lacked good milling and baking qualities (Stoa 1947). The low yield potential has caused reduced grower acceptance of solid stem cultivars. However, when sawfly infestations are high, solid stem varieties will yield equal to or greater than their hollow stem counterparts (Weiss and Morill 1992). By developing higher yielding solid stem cultivars, grower acceptance would likely increase.

Solid Stem Wheat

Stem solidness in wheat is caused by the development of pith inside the stem. The solid regions of the stem resist sawfly infestation and cause high rates of larval mortality (Holmes and Peterson 1962). How wheat with solid stems resist infestation or cause sawfly mortality is not clearly known, however several studies have been conducted to determine the cause of resistance and mortality. One study analyzed

whether female sawfly had a reduced preference for laying eggs in solid stems. Farstad (1951) observed fewer eggs were laid in solid stem versus hollow stem wheat, however if the only available host was solid stem wheat, the sawfly would deposit eggs into it as well. Other studies focused on how the egg and larva inside the host might be affected by solid stems. A study by McGinnis and Kasting (1961) analyzed whether pith was deficient in essential nutrients causing the larvae to die from malnutrition. The study found no significant differences in dry matter or nitrogen content between pith in solid stem varieties and the tissue found in walls of hollow stem wheat. They believed solid stem wheat kills larvae by desiccation. Holmes and Peterson (1960, 1961) studied the susceptibility of eggs to destruction in solid stems, and they also reported that eggs and larvae appeared to be vulnerable to desiccation. Holmes and Peterson (1962) also suggested that the pith might impede larvae movement, causing starvation due to lack of cells to ingest. The highest sawfly mortality rates in solid stem wheat have been shown to occur after the larva has fully matured (Wallace and McNeal 1966). This could be due to the impediment of larvae movement by the pith, frass, and nodal plates (Farstad 1940, Holmes and Peterson 1962, Morrill et al. 1994). The restricted movement prevents the larvae from reaching the base of the stem, which exposes them to freezing temperatures during the winter, resulting in nearly 100% mortality (Morrill et al. 1994).

Environmental Effects

Environmental factors can affect the degree of stem solidness, which potentially reduces sawfly resistance. Platt (1941) and Platt et al. (1948) reported stem solidness was affected by changes in light, temperature, moisture, and plant spacing. Holmes et al.

(1960) found that shading from the two-leaf to boot stage reduced the solidness of the bottom internode. Other research on 'Rescue' showed that in the greenhouse, 4,000 foot-candles of supplemental light maintained stem solidness, but 1,500 foot-candles of supplemental light did not (Roberts and Tyrell 1961). Further studies conducted by Luginbill and McNeal (1954) reported the effect of fertilizers on 'Rescue'. Phosphorous applied alone caused increased sawfly stem cuttings, whereas potassium applied with both phosphorous and nitrogen reduced sawfly cutting. Nitrogen applied alone had no significant effect on sawfly cutting.

Stem Solidness Inheritance

Stem solidness is considered to be a highly heritable trait. A study conducted by Lebsock and Koch (1968) reported stem solidness heritability estimates in wheat ranging from 60% to 95%. Another study by McNeal and Berg (1979) reported 73% heritability for stem solidness. The number of genes that control the development of solid stems and whether the genes are recessive or dominant is uncertain. Engledow and Hutchinson (1925) conducted a stem solidness inheritance study, which concluded the solid stem trait was dominant and controlled by one gene. Another study by Platt et al. (1941) reported, however, that three recessive genes were the controlling factors for stem solidness. Putnam (1942) studied the inheritance of stem solidness in tetraploid wheat. He indicated that stem solidness was controlled by one partially dominant gene. A recent study in durum that was conducted by Clarke et al. (2002) reported a single dominant gene controls stem solidness.

McNeal (1956) studied inheritance of stem solidness by crossing 'Rescue' (solid stem) with 'Thatcher' (hollow stem). He found that 'Thatcher' and 'Rescue' were different by one major gene and several modifying genes, which affected stem solidness. The major gene was found to have an effect equal to two and one-half times that of all minor modifying genes. A study by McKenzie (1965) agreed with the study by McNeal (1956) concerning the presence of a single major gene and several minor genes. McKenzie (1965) studied inheritance of stem solidness by crossing two hollow stemmed ('Red Bobs' and 'Redman') and two solid stemmed ('C.T.715' and 'S-615') spring wheat cultivars. He reported one major gene and three minor genes were influencing stem solidness.

Further research, conducted by McNeal et al. (1957), examined F_2 progeny from crosses made between 'Rescue' and four solid stem wheat accessions from Portugal. They reported that each Portuguese wheat accession contained the same major gene for solid stem expression that was found in 'Rescue'. However, three of the Portuguese accessions varied slightly for the level of stem solidness of 'Rescue'. McNeal attributed the variation to the addition or loss of minor genes that affect stem solidness. Wallace et al. (1969) reinforced McNeal's hypothesis when he studied a group of solid stem Portuguese spring wheat accessions and reported that the accessions may possess different or additional genes from those found is 'S-615', the source of Rescue's stem solidness.

Cytogenetic analysis has further indicated that there are several genes controlling stem solidness. Larson (1952, 1959a) compared monosomic F_2 lines derived from

crossing 'Chinese Spring' (hollow stem) X 'S-615' (solid stem) with normal F_2 lines for solid stems. She found in 'Chinese Spring' that chromosomes 2A, 2D, 6D, and 7D carry genes for hollow stem and chromosome 4B has a gene for stem solidness. No genes for stem solidness were detected in S-615, leading Larson to postulate solid stem genes were probably recessive. Further analysis by Larson and MacDonald in 1959b, using monosomic lines of 'S-615', showed that chromosomes 3B, 3D, 5A, 5B, and 5D carried genes for solid stem expression, and chromosomes 2D, 6D, and 7D have genes for hollow stem. Lines monosomic for 3B and 3D were less solid in the top internode, and lines monosomic for 5A, 5B, and 5D were less solid in the bottom four internodes.

In 1962, Larson and MacDonald reported the development of monosomic lines of 'Rescue'. They found 'Rescue' has fewer chromosomes affecting solid stem development than 'S-615'. Chromosomes 3D, 5B, and 5D did not make the stem more solid and chromosomes 2D and 7D did not make the stem more hollow as in 'S-615'. It was revealed, however, that chromosome 3B has a very important gene for stem solidness (cited by Wallace and McNeal 1966). The presence of a major gene on chromosome 3B was reinforced by Larson and MacDonald (1963). They reported results from an analysis of F8 lines that were selected from F5 hexaploid plants of a 'Rescue' (*T. aestivum*) X 'Golden Ball' (*T. durum*) cross. Their work also suggested a major gene for stem solidness on chromosome 3B.

Pleiotrophic Effects of Solid Stems

Even though solid stem wheat is the best form of wheat stem sawfly control, producers are reluctant to grow the resistant varieties because of yield loss compared to

their hollow stem counterparts (Weiss and Morrill 1992). There was concern that solid

stems were related to low yields (Wallace and McNeal 1966). McNeal et al. (1965)

reported solid stems and yield were negatively correlated (-0.846 and –0.825) in two tests

of backcross lines derived from a 'Thatcher' X 'Rescue' cross. Other studies however,

have indicated there is no relationship between yield and stem solidness (Lebsock and

Koch 1968, McNeal and Berg 1979, Hayat et al. 1995). Hayat et al. (1995) attributed the

low yield in solid stem wheat to the poor genetic background contributed by the solid

stem source, rather than pleiotropy or deleterious linkage.

Microsatellites

Microsatellites, or simple sequence repeats (SSR's), are found interspersed in the

genomes of all eukaryotes and have emerged as an important source of co-dominant

genetic markers (Wang et al. 1994). They are a class of sequences consisting of tandem

repeats, such as $(GT)_n$ or $(CT)_n$, with a basic motif of less then six base pair (Litt and

Luty 1989). It was observed that microsatellites show a high frequency of variation, or

polymorphism in the number of repeats in different individuals, probably due to slippage

during DNA replication (Tautz et al. 1986). Polymorphism can be observed at a

specific locus using polymerase chain reaction (PCR), where primers are developed that

flank the tandem repeat sequence allowing the amplification of a specific microsatellite

locus. Microsatellites have been shown to be highly informative and locus specific

(Condit and Hubbell 1991, Wu and Tanksley 1993, Smulders et al. 1997).

Wheat has a very limited intraspecific level of polymorphism compared to other plant species (Chao et al. 1989, Kam-Morgan et al. 1989, Liu et al. 1990, Cadalen et al. 1997). Microsatellites, however, have a higher level of polymorphism and informativeness in wheat then any other marker system (Plaschke et al. 1995, Roder et al. 1995, Bryan et al. 1997, Roder et al. 1998). Several microsatellite maps have been constructed, revealing an even distribution of microsatellite loci along all chromosome arms, thus providing excellent coverage of the wheat genome (Korzun et al. 1997, Peil et al. 1998, Roder et al. 1998).

Use of Microsatellites in Identifying Genes

The development of microsatellite markers and maps has provided a useful tool for identifying genetic markers associated with agronomic and grain quality genes and quantitative trait loci (QTL) in wheat. A study, reported by Korzun et al. (1998), identified a microsatellite locus, *wms261*, that is 0.6 cM distal to the *Rht8* dwarfing gene on chromosome 2DS. Another study conducted by Prasad et al. (1999) analyzed 100 recombinant inbred lines and screened them with 232 microsatellite primer pairs. They detected a significant association between a microsatellite locus, *wmc41*, and a QTL for protein content, which accounted for 18.73% of the variation. Varshney et al. (2000) looked for associations between grain weight and microsatellite markers. From their analysis, microsatellite *Xwmc333* was found to be associated with a grain weight QTL on chromosome 1AS, which accounted for 15.09% of the variation for grain weight.

Microsatellites have also been used to identify genetic markers linked to disease and insect host plant resistance genes in wheat. Huang et al. (2000) identified a microsatellite marker associated with the powdery mildew resistance gene *Pm24*. The microsatellite locus, *Xgwm337*, located on chromosome 1D, was found to be 2.4 cM from *Pm24*. *Xgwm337* was shown to be diagnostic and therefore potentially useful for pyramiding two or more genes for powdery mildew resistance in a single genotype. Liu et al. (2001) used microsatellites to identify markers linked to Russian wheat aphid resistance genes. Microsatellite *Xgwm111*, located on wheat chromosome 7DS, was reported to be tightly linked to the Russian wheat aphid resistance genes *Dn1, Dn2, Dn5,* and *Dnx*. Another microsatellite marker, *Xgwm635*, located on the long arm of chromosome 7D marked the location of the Russian wheat aphid resistant gene *Dn8*. Lastly, a microsatellite locus *Xgwm642* marked and identified a Russian wheat aphid resistant gene *Dn9* on chromosome 1DL .

Marker Assisted Selection (MAS)

Once markers identifying genes of interest have been found, molecular genetics can be integrated with traditional methods of artificial selection of phenotypes by applying marker-assisted selection (Lande and Thompson 1990). Cultivar improvement predominantly has resulted from phenotypic selection wherein superior genotypes have been identified only through replicated testing in diverse environments. Plant breeders have been restricted to the use of phenotypic selection, because little is known of the genetic identity and chromosome location of most genes controlling most important

agronomic traits. Molecular marker technology offers the tools needed to identify, select, and combine favorable alleles via genotypic selection. Marker assisted selection could aid in the development of resistant cultivars by producing genotypes with more stable and durable resistance.

CHAPTER 3

MATERIALS AND METHODS

Plant Materials

A doubled haploid (DH) mapping population for stem solidness was derived from a cross of two hard red winter wheats, PI 593889 ('Rampart'), a solid stem genotype and PI 632433 ('Jerry'), a hollow stem genotype (Knox et al. 2000). The DH mapping population contained 96 lines generated from the F_1 generation.

In 2001, the DH population was planted at Bozeman, MT. The elevation at the experimental site is 1,439 m and the soil is an Amsterdam silt loam. The population was planted in single row non-replicated plots for seed increase. The plots were 1.5 m long with row spacing of 60 cm. The seeding rate varied among the lines. Planting occurred on 10 October 2000 and harvest occurred on 06 August 2001. Precipitation received from 01 October 2000 to 02 July 2001 was 311 mm. Preliminary stem solidness data were obtained.

In 2002, the 96 DH winter wheat lines and four check varieties ('Rampart', 'Jerry', 'Judith', and 'Norstar') were planted in a 10 X 10 lattice design with three replications at two Montana locations: Bozeman and Moccasin. At Bozeman, the experimental site was the same as 2001. The plots had four rows and were 3.3 m long with row spacing of 30 cm. The seeding rate was 67.2 kg ha^{-1}. Planting occurred on 30 September 2001 and harvest was on 16 August 2002. Precipitation received from 01 October 2000 to 02 July 2001 was 316 mm. Traits evaluated at Bozeman included stem

solidness, yield, test weight, protein content, emergence, winter survival, heading date, height, and lodging.

Moccasin has an elevation of 1,307 m and the soil is a Judith clay loam. The plots had five rows and were 2.4 m long with row spacing of 30 cm. The seeding rate was 67.2 kg ha^{-1}. Planting occurred on 24 September 2001 and harvest occurred on 09 August 2002. Precipitation received from 01 October 2000 to 02 July 2001 was 235 mm. Measured traits included stem solidness, yield, test weight, protein content, emergence, winter survival, heading date, and height.

In 2002, the 96 DH lines were also planted for observation in a randomized complete block single row design at Williston, ND. The Williston site has an elevation of 640 m and the soil is a Max loam. The plots were 2 m long with row spacing of 30 cm. Planting occurred on 11 September 2001 but they were not harvested. Precipitation received from 01 October 2000 to 02 July 2001 was 208 mm. Measured traits were stem solidness and winter survival.

To evaluate for stem solidness, ten stems were randomly selected from each plot. The stems were cross sectionally cut in the center of five internodes. The level of pith at each internode was rated on a previously established scale ranging from one to five; one was considered hollow and five was solid (Fig. 1) (O'Keefe et al. 1960, Wallace et al. 1973). Ratings for each of the five internodes were summed providing a total stem solidness score ranging from 5 – 25, where five indicated hollow and 25 was solid.