



Field evaluation of transgenic and classical sources of wheat streak mosaic virus resistance  
by Gail Louise Sharp

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in  
Agronomy

Montana State University

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Abstract:

Wheat streak mosaic virus (WSMV) continues to be an important disease in wheat (*Triticum aestivum* L.). The development of wheat cultivars that are resistant to WSMV, yet competitive in yield under non-diseased conditions, would benefit Montana producers. This field study was conducted to compare classical and transgenic sources of resistance to WSMV. Tolerance to WSMV has been observed in Montana-adapted cultivars. Additionally, germplasm with resistance to WSMV from a translocated segment of chromosome 4Ai-2 from *Thinopyrum intermedium* has been used in breeding programs at Montana State University. The *Thinopyrum* segment contains the resistance gene *Wsm1*. Transgenic wheat lines were also developed by incorporating viral coat protein or replicase sequences from WSMV into wheat. A replicated field trial was conducted at Bozeman, Montana over a two-year period to evaluate the effectiveness from these different sources of resistance to mechanical inoculation of WSMV. Adapted cultivars differed in their ability to tolerate WSMV with mean reductions in yield ranging from 41 to 74 percent. Incorporation of the replicase or coat protein gene from WSMV did not provide resistance to viral infection. Wheat-*Thinopyrum* lines PCR positive for the *Wsm 1* gene had significantly reduced yield losses due to WSMV with decreases in yield ranging from 5 to 39 percent in inoculated plots. Yields of *Wsm 1* lines in the absence of disease were 21 percent less than yields of near isogenic lines not having the *Wsm 1* gene. Advanced *Wsm 1* breeding lines that have comparable yields have been developed and provide an attractive alternative for resistance to WSMV for Montana producers.

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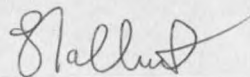
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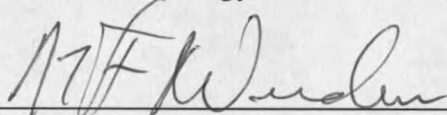
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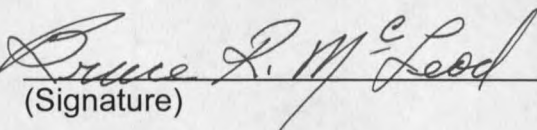
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## ABSTRACT

Wheat streak mosaic virus (WSMV) continues to be an important disease in wheat (*Triticum aestivum* L.). The development of wheat cultivars that are resistant to WSMV, yet competitive in yield under non-diseased conditions, would benefit Montana producers. This field study was conducted to compare classical and transgenic sources of resistance to WSMV. Tolerance to WSMV has been observed in Montana-adapted cultivars. Additionally, germplasm with resistance to WSMV from a translocated segment of chromosome 4Ai-2 from *Thinopyrum intermedium* has been used in breeding programs at Montana State University. The *Thinopyrum* segment contains the resistance gene *Wsm1*. Transgenic wheat lines were also developed by incorporating viral coat protein or replicase sequences from WSMV into wheat. A replicated field trial was conducted at Bozeman, Montana over a two-year period to evaluate the effectiveness from these different sources of resistance to mechanical inoculation of WSMV. Adapted cultivars differed in their ability to tolerate WSMV with mean reductions in yield ranging from 41 to 74 percent. Incorporation of the replicase or coat protein gene from WSMV did not provide resistance to viral infection. Wheat-*Thinopyrum* lines PCR positive for the *Wsm1* gene had significantly reduced yield losses due to WSMV with decreases in yield ranging from 5 to 39 percent in inoculated plots. Yields of *Wsm1* lines in the absence of disease were 21 percent less than yields of near isogenic lines not having the *Wsm1* gene. Advanced *Wsm1* breeding lines that have comparable yields have been developed and provide an attractive alternative for resistance to WSMV for Montana producers.

## CHAPTER 1

## INTRODUCTION

Wheat streak mosaic virus (WSMV) is an important disease in wheat (*Triticum aestivum* L.). The disease occurs in many areas of the world including Canada, Europe, and Russia, and is becoming increasingly more common in the Great Plains of North America (Weiss, 1987). WSMV is vectored by the wheat curl mite (*Aceria tulipae*) as it feeds while moving from one green plant to another. The virus is a flexuous rod of single stranded RNA 700 nm long and 15 nm wide. Symptoms of the disease include light green and yellow streaking of the leaves as well as curling of the leaves due to mite activity. Infected plants typically exhibit stunted growth, reduced tillering, and sterile or partially filled heads. WSMV has long been a concern in winter wheat production areas and changes in farming practices have caused growing concerns in spring wheat production as well. The seeding of winter wheat after spring wheat provides a natural "green bridge" for the vector. Additionally, conservation tillage and the promotion of grassland through the Conservation Reserve Program lead to increases in weedy and alternate host habitats for mite populations.

Since cultural practices do not provide adequate control for the spread of disease, host plant resistance is an attractive control strategy. To date, no resistance has been discovered in cultivated wheat. Resistance does, however, exist in wild relatives of wheat (Friebe et al., 1991), and the process of

introgressing the resistance gene into agronomically acceptable backgrounds has been accomplished (Friebe et al., 1996). The development of wheat-*Thinopyrum intermedium* (formerly *Agropyron intermedium*) crosses by the Kansas State University Wheat Genetics Resource Center has provided an excellent source for resistance to WSMV. The most promising line, CI 17884, contained a translocation whereby the short arm of chromosome 4D from wheat was replaced by the short arm of chromosome 4 from *Thinopyrum*. The *Thinopyrum* segment contains the resistance gene *Wsm1* (Friebe et al., 1996). Improvements to CI 17884 have been accomplished by backcrossing the germplasm line with 'Karl' hard red winter wheat (Gill et al., 1995). KS93WGRC27 is the bulked, selfed progeny of a Karl\*4/CI17884 plant that was homozygous for the *Thinopyrum* translocation. This germplasm was used by Montana State University (MSU) breeders to obtain lines whereby the *Wsm1* gene was incorporated into Montana-adapted cultivars. Talbert et al. (1996) developed polymerase chain reaction (PCR) markers to facilitate detection of *Wsm1* in wheat lines.

Additionally, Sivamani et al. (2000a and 2000b) developed potential resistance to WSMV in experimental lines by incorporating viral transgenes into wheat. Wheat plants of 'Hi-Line' (Lanning et al., 1992) and 'Bobwhite' were stably transformed with a viral replicase gene (Nib) or a viral coat protein (CP) gene via biolistic particle bombardments of immature wheat embryos.

In addition to *Wsm1* and viral transgene resistance, varying degrees of tolerance to WSMV have also been observed in cultivated wheat cultivars (Carroll et al., 1982; Shahwan and Hill, 1984; Siefers and Martin, 1988; and M. Young, *personal communication*). Siefers and Martin (1988) refer to tolerance as low-level resistance. Cultivars classified as tolerant become infected with WSMV but symptoms are less severe, plant virus levels are lower, and yield reductions are less than in susceptible cultivars. In replicated field trials in 1998, Young et al. initiated trials to evaluate WSMV titer and yield loss. Their findings indicated that Montana-adapted cultivars, when mechanically inoculated with the same WSMV isolate, exhibited yield reductions ranging from 18 to 61 percent and disease symptoms ranging from mild to severe.

Our objective with this study is to evaluate the effectiveness of viral mediated resistance to WSMV in field trials in comparison to that achieved with the *Thinopyrum* translocation. We conducted a field study in 1999 and 2000, utilizing wheat-*Thinopyrum* germplasm lines, transgenic lines, and Montana-adapted cultivars. Entries were planted in replicated field trials and mechanically inoculated with WSMV. Disease symptoms, virus titers, cereal quality, and morphological characteristics were obtained and statistically analyzed.

## CHAPTER 2

## LITERATURE REVIEW

Wheat is unique in the fact that it is the only grain that when ground and mixed with water and yeast can be baked into bread. This quality and the fact that whole grain can be easily stored and shipped make wheat an important dietary staple providing 20 percent of total dietary calories and protein for the world's population ( Betschart, 1988; and Bushuk, 1998). Wheat is an important commodity since it is widely adapted to a range of climatic conditions (Orth and Shellenberger, 1988) and is grown in all parts of the world except for the hottest tropical regions. Additionally, the gluten and starch components can be separated and sold as food additives. When grain is of poor quality or sprouting damage occurs, the grain can be safely fed to livestock increasing the number of end uses for wheat.

It is widely accepted that wheat originated and was first cultivated 10,000 to 8,000 B. C. in areas of the Middle East, Syria, and Palestine (Orth and Shellenberger, 1988; Hancock, 1992; and Feldman et al., 1995). Phylogenetic studies regarding cultivated wheat bear similarities to many of the wild grains found in this region. Modern wheat is derived from the genomes of *Triticum monococcum*, *Triticum tauschii*, and an unknown wild species. Doubling of the chromosome number from a cross between *T. monococcum* and the unknown species gave rise to a tetraploid species, *Triticum turgidum*, durum wheat.

*T. turgidum* and *T. tauschii* then combined and chromosomes doubled to form the hexaploid cultivated wheat, *T. aestivum*. The 'D' genome from *T. tauschii* is responsible for the protein components that are important in bread wheat (Poehlman and Sleper, 1995). Although hexaploid, the bread wheats act as diploids due to the *Ph1* gene on the 5B chromosome that suppresses homoeologous pairing. In the absence of the *Ph1* gene, multivalents between the three genomes will form.

Worldwide, wheat production accounted for 225 Mha in 1990 and is the world's leading crop plant (Feldman et al., 1995). On a local level, agriculture is the largest industry in Montana and wheat production accounts for 5.5 million acres and nearly 32 percent of agricultural sales (Montana Agriculture Statistics, 1999). Wheat and wheat products were the leading exports making up 72 percent of Montana's agricultural exports. Montana ranks second behind North Dakota in spring wheat production and fourth in all wheat production. In 1999, a total of 154.3 million bushels were harvested in the state.

Wheat streak mosaic virus (WSMV) is an important disease in wheat and is vectored by the wheat curl mite. The disease was first reported in Montana in 1954 on winter wheat (Bamford et al., 1996). Severe outbreaks of the disease occurred in 1964 and 1981. Epidemics in Montana in 1993 and 1994 were responsible for losses of 30 percent or more (Jacobsen and Mikkelsen, 1999) in the Golden Triangle area of Montana and an estimated \$12.7 million statewide in 1993 (Fowler, 1998). Yield losses can approach 100 percent if infection occurs

before tillering and infections during jointing can result in sterile florets or low test weights. Economic losses due to WSMV in Kansas averaged 13 million bushels annually 1971 to 1976 (Shahwan and Hill, 1984). In 1974, yield losses were estimated at 30 million bushels.

Wheat streak mosaic virus was described in 1937 by McKinney. The disease also infects barley (*Hordeum vulgare*), oats (*Avena sativa*), rye (*Secale cereale*), corn (*Zea mays*), and millets (*Panicum*, *Setaria*, and *Echinochloa* spp.). Many wild grasses also serve as hosts for the virus (Wiese, 1987 and Brakke, 1971). Infected wheat plants are stunted and leaves have yellow streaks that are parallel and discontinuous. Wheat streak had previously been assigned to the family Potyviridae of the Genus *Rymovirus* but recent phylogenetic studies now place the virus in the genus *Tritimovirus* (Stenger et al., 1998 and Fauquet and Mayo, 1999). The complete 9,384-nucleotide sequence of WSMV has been determined and reassignment to the new genus is based on sequence similarities to brome streak mosaic virus and sweet potato mild mottle virus.

Genetic differences in wheat streak mosaic virus isolates have been reported by Carroll et al. (1982). Eight WSMV isolates were collected from throughout Montana and analyzed for disease severity on Michigan Amber wheat. Seven of the isolates produced mild symptoms while the isolate collected from Conrad, MT produced severe symptoms. The 'Conrad' isolate was used to inoculate entries in this field study.

Variability among WSMV isolates was also investigated by Montana et al. (1996). Differences among the isolates were determined using the various serological techniques of enzyme-linked immunosorbent assay (ELISA), protein fingerprinting, Western blot, and serological specific electron microscopy. Sequencing of the viral genome done by McNeil et al. (1996) suggested three major and many minor lineages of WSMV in Nebraska. Because of the wide genetic variability of WSMV isolates it was necessary to find a single assay that could detect the various isolates.

Based on previous studies (Edwards and Cooper, 1985; Koenig, 1981; Lommel et al., 1982; and Sherwood, 1987) it was determined that indirect enzyme-linked immunosorbent assay (ELISA) would best quantify WSMV levels in infected wheat tissues. Indirect ELISA is sensitive, detecting viruses in low concentration. Specificity of indirect ELISA methods is decreased meaning that various isolates of a virus can be adequately detected using the same reaction reagents. Plants having titer values of three to four times that of uninfected leaf tissue are regarded as positive for WSMV infection (Khetarpal and Kumar, 1995; Siefers and Martin, 1988).

No resistance to WSMV has been discovered in cultivated wheat but resistance does, however, exist in wild relatives of wheat (Friebe et al., 1991). The transfer of alien genes into wheat was attempted by a variety of methods including radiation to disrupt the chromosomes and facilitate translocations at the broken ends of the chromosomes. Wang et al. (1977) used a *Ph* mutant to

induce homoeologous pairing between wheat and *Thinopyrum intermedium*. Lay et al. (1971) used an octaploid derivative of 'Carsten V' (*Triticum aestivum* L. Em. Thell), TA25, that was developed in Germany. TA25 was crossed with numerous cultivars of common wheat. Irradiated progenies were male-sterile and used as female parents in subsequent crosses with common wheat. A series of backcrosses to 'Lathrop' spring wheat produced a 42 chromosome substitution line that was later registered as CI 15092. This germplasm was further improved by numerous crosses to obtain CI 17884 (Wells et al., 1973 and 1982).

C-banding and *in situ* hybridization analysis of CI 17884 identified a compensating translocation where the complete short arm of chromosome 4 from *T. intermedium* was translocated to the long arm of chromosome 4D of wheat (Friebe et al., 1991). CI 17884 was backcrossed to 'Karl' winter wheat to produce KS93WGRC27 (Gill et al., 1995), which was used by breeding programs at Montana State University to introduce resistance to WSMV into Montana-adapted cultivars.

In the early 1980s expression of viral gene sequences in plants were suggested as a means of providing resistance by disrupting the life cycle of the virus (Hamilton, 1980 and Sanford and Johnston, 1985). Viral mediated resistance, or pathogen derived resistance, has since become a popular method for the development of resistant crop species. Based on this approach, Sivamani et al. (2000a and 2000b) transformed wheat plants with viral

sequences from WSMV. The nucleotide sequences of the WSMV Nib and CP genes were synthesized by PCR from cDNA clones of the 'Conrad' isolate of WSMV (Carroll et al., 1982). The sequences were ligated into vector plasmids containing the maize ubiquitin-1 (*ubi*) promoter and the nopaline synthase (*nos*) terminator regions, and an ampicillin resistance gene.

The plasmid pRQ101 contains the *bar* gene conferring resistance to glufosinate herbicide. This plasmid was used as a selectable marker for plants co-transformed with the Nib and CP plasmids. Initial greenhouse tests of the progeny indicated that some of the lines exhibited a delayed resistance phenotype characteristic of protein-mediated resistance (Sivamani et al., 2000a and 2000b).

The purpose of this study is to investigate the effectiveness of transgenic sources of resistance to WSMV and compare this to the level of resistance conferred by the *Wsm1* gene from *T. intermedium*.

## CHAPTER 3

## MATERIALS AND METHODS

Field Trial EvaluationPlant Material

Wsm1 Entries –The source of resistance, KS93WGRC27 (Gill et al., 1995), was crossed with Montana-adapted cultivars 'Amidon' (Frohberg, 1988) and 'McNeal' (Lanning et al., 1994). Resultant progenies were crossed with 'McNeal' or experimental line MT9328 and F<sub>1</sub> progenies were grown at the Arthur H. Post Field Research Farm near Bozeman, MT in 1995. Two subsequent generations were advanced in the greenhouse via single seed descent. Plants were tested for resistance by polymerase chain reaction (PCR) and by mechanical inoculation of seedlings with WSMV. Both resistant and susceptible populations were maintained for further testing. The F<sub>4</sub> generation was grown as head rows in 1996 and the F<sub>5</sub> generation as plant rows in 1997. Resistant and susceptible lines were grown and tested in the field during 1998 (Baley, 1999). Six wheat lines containing the *Wsm1* resistance gene and six sister lines without the gene were selected from F7 populations to be grown in the first year of this study (Table 1).

Transgenic Entries – Seven transgenic lines testing PCR positive for the viral replicase (NIb) or coat protein (CP) genes were obtained for the 1999 field

**Table 1.** Wheat lines evaluated for resistance to wheat streak mosaic virus at Bozeman, MT in 1999 and 2000.

Entry	Resistance Source	Pedigree*
4161R	<i>Wsm1</i>	McNeal/KS27//MT9328
4165R	<i>Wsm1</i>	McNeal/KS27//MT9328
4266R	<i>Wsm1</i>	Amidon/KS27//McNeal
4274R	<i>Wsm1</i>	Amidon/KS27//McNeal
4292R	<i>Wsm1</i>	Amidon/KS27//MT9328
4338R	<i>Wsm1</i>	Amidon/KS27//MT9328
4168S	No <i>Wsm1</i>	McNeal/KS27//MT9328
4199S	No <i>Wsm1</i>	McNeal/KS27//MT9328
4238S	No <i>Wsm1</i>	Amidon/KS27//McNeal
4241S	No <i>Wsm1</i>	Amidon/KS27//McNeal
4252S	No <i>Wsm1</i>	Amidon/KS27//McNeal
4316S	No <i>Wsm1</i>	Amidon/KS27//MT9328
CP20.2	Viral coat protein	Bobwhite
CPB4	Viral coat protein	Hi-Line
CP25.13**	Viral coat protein	Hi-Line
CP25.14**	Viral coat protein	Hi-Line
CP25.8	Viral coat protein	Hi-Line
Nlb4.4	Viral replicase gene	Hi-Line
Nlb4.8	Viral replicase gene	Hi-Line
Nlb7.17	Viral replicase gene	Hi-Line
Nlb7.26	Viral replicase gene	Hi-Line
Hi-Line	None	
Amidon	None	
Ernest	None	
Fergus	None	
Fortuna	None	
McNeal	None	
MTHW9420	None	
Rambo	None	
Scholar	None	

\* KS27 = KS93WGRC27, a Karl\*4/CI 17884 cross (Gill et al., 1995)

\*\* Additional transgenic lines included in the 2000 field trial only. All other entries were planted in both years.

trial. Six of the transgenic lines were derived from the cultivar 'Hi-Line', while the remaining transgenic line was derived from the cultivar, 'Bobwhite', which is not adapted in Montana, but was used in early transformation experiments since it was more amenable to regeneration after biolistic bombardment than other cultivars. Two additional transgenic lines, derived from 'Hi-Line', were field tested in 2000.

Transgenic wheat lines were planted in the greenhouse in January of 1999 in order to increase the amount of seed available for spring field planting. Upon emergence, transgenic lines were sprayed with glufosinate (0.01% ai) to eliminate plants not expressing the bar gene that confers resistance to glufosinate herbicide. Sivamani et al. (2000a and 2000b) showed co-segregation of the bar and replicase or coat protein genes in transformed plants. The additional CP entries used in the 2000 field trial were increased in the greenhouse in January of 2000.

Adapted Cultivar Entries – Nine adapted cultivars (including parents of the transgenic and *Wsm1* lines), exhibiting various degrees of tolerance to WSMV, were included in the study (Table 1).

#### Field and Location Conditions

Field trials were conducted at the Arthur H. Post Field Research Farm eight miles west of Bozeman, Montana in 1999 and 2000. The elevation is 1,439 m (4,772 ft) and the soil is an Amsterdam silt loam.

In 1999, the average temperature during the growing season (May 1 to August 31) was 15.6°C (60.2°F), with 16 cm (6.4 in.) of precipitation. Annual precipitation as measured from September through August was 35.6 cm (14.3 in.). The frost-free period was 109 days from June 9 to September 11, 1999. Maximum summer temperature was 36°C (97°F).

In 2000, the average temperature during the growing season was 16.7°C (62°F), with 16.2 cm (6.5 in.) of precipitation. Annual precipitation as measured from September through August was 32.2 cm (12.9 in.). The frost-free period was 116 days from June 1 to September 25, 2000. Maximum summer temperature was 36.6°C (97.8°F).

A single application of Curtail (2 1/2 pints per acre) herbicide was applied in 1999 at the five-leaf stage for control of broad-leaf weeds. Plots were hand weeded for the rest of the season. Available soil nitrogen of 135 pounds nitrogen per acre was supplemented with a pre-plant application of 400 pounds per acre of 25-10-10 blended fertilizer so that total available nitrogen available for the crop was approximately 235 pounds per acre.

In 2000, Bronate herbicide (1 1/2 pints per acre) was applied at the five-leaf stage for control of broad-leaf weeds. Available soil nitrogen was 110 pounds of nitrogen per acre with an additional 400 pounds 25-10-10 per acre added prior to planting.

### Experimental Design

In 1999, twenty-eight entries were planted in a randomized complete block design with three replications. Entries were planted in paired rows 3 meters long with row spacing of 30 cm. One row per entry was mechanically inoculated with WSMV and the second row was the non-inoculated control. In 2000, thirty entries were planted using the same experimental design as the previous year. Seed was derived from one replication of the non-inoculated row from the 28 entries in common with 1999. The two additional CP entries were increased in the greenhouse.

### Planting and Harvest Techniques

Small seed yields for some of the transgenic lines necessarily limited the size of field plots and the number of replications that could be planted. Consequently, seed was space planted in paired rows at Bozeman, Montana in 1999 for a total of 28 entries. Ninety seeds of each line were planted per row. Transgenic rows were sprayed with glufosinate, plant counts were made and paired rows from each experimental plot were thinned to the same number of plants so that the effects of WSMV could be evaluated.

The study was repeated in 2000 in essentially the same manner as the 1999 field trial, except for the addition of two new transgenic entries. Eight grams of seed were planted per row in 2000 for 28 of the entries. Coat protein lines, 25.13 and 25.14, grown in the greenhouse for seed increase in January of 2000 were inadvertently infested with viruliferous wheat curl mites and

subsequent infection with WSMV resulted in poor seed set. Mites present in the greenhouse had been feeding on mechanically inoculated wheat seedlings being grown for another study and became infected with WSMV. These two CP lines were planted at seven grams per row for the 2000 field trial. A greater abundance of seed also enabled us to plant four replicates of paired rows in 2000. In 2000, we did not spray the transgenic rows with glufosinate nor were rows thinned to a uniform number of plants.

Planting occurred on May 12, 1999 and May 3, 2000. Harvest occurred in August of both years. Individual rows were cut with a hand sickle and threshed with a stationary Vogel thresher.

#### Inoculation and Evaluation of Viral Resistance

Virus Identification – Positive identification of WSMV in inoculum sources was accomplished with the help of Sue Brumfield. Infected leaf tissue from greenhouse plants was prepared for electron microscopy using 300 mesh electron microscope grids treated with WSMV polyclonal antibody. Positive identification of WSMV was made based on the morphology of virus particles viewed under the electron microscope.

Inoculation – At the two- to three-leaf stage, one row from each plot was hand inoculated with WSMV. Inoculum was prepared from infected greenhouse seedlings that had been inoculated with the 'Conrad' strain of WSMV. Field inoculum was prepared by grinding diseased leaf tissue in a Waring blender with

phosphate buffer pH 6 (1g infected leaf tissue/10 mL buffer) in a modification of the method described by Carroll et al. (1982) and Shahwan and Hill (1984). A small amount of carborundum was added to facilitate leaf injury and viral introduction during application.

In 1999, we did not mock inoculate control rows with buffer and carborundum and this oversight was remedied in 2000.

Symptom Expression – Disease symptoms were rated on a scale of 0-5, with 0 denoting no visible signs of disease and 5 denoting severe symptoms, based on the method of Shahwan and Hill (1984) as noted in Table 2.

**Table 2.** Rating system used to evaluate symptoms of wheat streak mosaic virus in spring wheat lines.

Rating	Description
0	No disease symptoms
1	Light green streaking
2	Some yellow streaking
3	Mixed light green and yellow stunting with a noticeable decrease in height and number of heads forming
4	Pronounced stunting and yellow streaks
5	90% total destruction of plant material in the plot

Viral Replication Level – Virus level in infected wheat tissue was determined by use of protein A sandwich enzyme-linked immunosorbent assay (PAS-ELISA) as described by Edwards and Cooper (1985). The method used two layers of protein A combined with the antibody-antigen-antibody sandwich method of virus detection. The first layer was protein A (diluted 1:1000 in

carbonate coating buffer) adhered to the stationary phase, a plastic 96-well microtiter plate. Application of protein A is thought to increase the efficiency of bonding to the antibody (diluted 1:1000 in PBS-Tween), which was the next layer. The antigen, macerated WSMV infected leaf tissue, was then added and allowed to incubate overnight at 4°C. A second antibody layer was applied followed by the second application of protein A that was conjugated to the enzyme, alkaline phosphatase, which detected the second antibody layer. With the exception of the antigen layer, all reagent layers were allowed to incubate at room temperature for two hours. Three rinses with PBS-Tween were made before addition of each subsequent layer. The addition of p-nitrophenyl phosphate substrate was the final step of the ELISA procedure. This substrate is colorless when freshly prepared but turns yellow upon hydrolysis by alkaline phosphatase and is quantified spectrophotometrically at a wavelength of 405 nm.

Polyclonal antibodies to the WSMV virus had been previously prepared (Baley, 1999) by injecting purified WSMV into two rabbits at the Animal Resource Center, MSU-Bozeman. Leaf tissue infected with the 'Conrad' strain of WSMV was ground and virus extracted and purified based on the method of Brakke and Ball (1968). Antibody produced in one rabbit was used to test 1999 field samples, while antibody from the second rabbit was used to test 2000 field samples.

At 20 and 40 days post inoculation the newest leaf from ten plants per entry were analyzed for virus level using PAS-ELISA. Approximately 3 cm of leaf tissue was collected from each of seven inoculated plants and three non-inoculated plants and stored at -20°C. Samples were ground under liquid nitrogen in a 1.5 ml microcentrifuge tube with the aid of an electric drill and plastic Kontes pestle. A solution of PBS-Tween (800 µl) was added to the sample and vortexed. A 100-µl sample was then added to the microtiter plate and incubated overnight. Subsequent layers of reagents were added to complete the PAS-ELISA as described above. Optical density of ELISA samples was measured on a Kinetic Microplate Reader (Molecular Devices Corp., Sunnyvale, CA) at 405 nm after a 20-minute incubation with p-nitrophenyl phosphate (diluted 1:1000 in diethanolamine substrate buffer). Three negative control samples were included for each entry as well as one or more blanks consisting of PBS-Tween buffer. The negative controls were taken from non-inoculated control rows in each entry.

In this study, positive assessment of infection using optical density readings was accomplished by establishing the positive-negative threshold as three times the absorbancy reading of the negative controls (Khetarpal and Kumar, 1995).

#### Morphological Evaluation

Each row, both inoculated and non-inoculated, was evaluated for six morphological characteristics in 1999 and 2000.

Heading Date – Heading date for each row was recorded as the number of days from January 1 when 50% of the heads had emerged from the flag leaf. The date was recorded in Julian days.

Plant Height – Height of each row was taken as the average of two measurements made from the soil surface to the top of the spikes, excluding awns, of 3-5 main tillers gathered together.

Yield – Grain yield from each row was expressed as g m<sup>2</sup>.

1000 Kernel Weight – Grain yields from single rows were not sufficient to obtain test weights. Instead, a Contador E electronic seed counter (Hoffman Mfg., Inc.) was used to count a random volume of 30 mL of seed. An empty film canister was pulled through the center of each grain sample and the number of seeds removed was counted and weighed. The weight of 1000 kernels was calculated.

#### Cereal Quality Evaluation

Milling – Whole flour meal was prepared in a UDY Cyclone Mill fitted with a 0.5 mm screen. A random sample of 25 to 30 grams of seed was ground and the flour was placed in an envelope until further testing. All quality evaluations were conducted using approved methods (AACC, 1995).

Flour Moisture – Flour moisture was determined according to AACC Method 44-15A. Two to three grams of flour were placed in aluminum dishes

and heated in an oven for one hour at 130°C and allowed to cool in a desiccator. Flour weight was recorded before and after heating and percent moisture was calculated as the weight lost due to oven heating.

Processing of grain in the UDY Cyclone Mill tends to dry all flours to approximately the same moisture percentage. Therefore, oven moisture was not performed on all flour samples in either year. Twenty random samples from each replication, ten from the inoculated row and ten from the non-inoculated row were selected for oven moisture tests. In all, approximately one-third of the samples were tested for moisture each year.

Protein Quality – For the SDS sedimentation test, approximately 0.42 grams of flour meal was vortexed in five mL of blue water (0.01g Methylene Blue dye L<sup>-1</sup> water). Contents were again vortexed for five seconds at two-minute and four-minute intervals. Immediately after the last vortex, five mL of SDS-lactic acid solution were added and cylinders were inverted at timed intervals. The SDS-lactic acid solution was prepared by mixing 20 g SDS in 1 L water and adding 20 mL of stock diluted lactic acid (one part lactic acid plus eight parts water by volume). After the last inversion, cylinders were placed in a rack and sediment was allowed to settle for 20 minutes. The volume of sediment was measured in mL.

Grain Protein – Grain protein was measured on flour samples using a LECO-328 or a LECO FP-528 protein/nitrogen analyzer. A 0.2 gram sample was

wrapped in aluminum foil and dropped into the combustion chamber. The sample was burned in the presence of oxygen to yield oxides of nitrogen, which were catalytically reduced to nitrogen. Percent crude protein was calculated as percent nitrogen multiplied by 5.70 (AACC Method 46-30). Protein was adjusted for moisture content and reported at 14% moisture using the following formula:

$$\frac{(\text{LECO Protein Value}) \times (100-14)}{(100 - \text{Flour Moisture})}$$

### Molecular Marker Evaluation

#### DNA Extraction

Young leaves from transgenic and *Wsm1* entries were harvested and total genomic DNA was extracted following the method described by Lassner et al., (1989) or Riede and Anderson (1996). The latter method, with modifications, is described below and was preferred since the use of 2-mercaptoethanol was avoided and the extraction buffer was more convenient to prepare.

A 3-cm leaf section was ground under liquid nitrogen using an electric drill and Kontes pestle in a 1.5 mL microcentrifuge tube. After grinding, 750 µl of extraction buffer was added [0.5 M NaCl, 0.1 M Tris-HCl pH 8.0, 0.05 M ethylenediaminetetra-acetic acid (EDTA), 8.4 g L<sup>-1</sup> sodium dodecyl sulfate (SDS), 3.8 g L<sup>-1</sup> sodium bisulfite] and vortexed till suspended. Samples were incubated at 65°C for 45 minutes and mixed by gentle inversion every 10 minutes. Samples were cooled slightly and 750 µl of chloroform were added. Samples

were mixed till homogeneous and centrifuged for 10 minutes at 14000 g. Approximately 500 µl of the upper phase was drawn off with a pipette and added to a new 1.5 mL tube containing 1 mL of cold 95% ethanol. DNA was precipitated by gentle inversion of the tube and pelleted by centrifuging for 10 seconds. The DNA was washed with 1 mL of 70% ethanol and resuspended in 100µl TE.

### PCR Reaction Conditions

PCR amplifications were performed in 50 µl reactions consisting of 10X Promega reaction buffer, 50 µM of dNTP's, 1.5 mM MgCl<sub>2</sub>, 400 nM of left and right primers, 0.15 µl of Taq polymerase, and 100 ng of genomic DNA. The PCR was performed in a PTC-100 programmable thermocycler (MJ Research, Inc.) using the following conditions; initial denaturation of the DNA at 94°C for 4 minutes; 29 cycles of 94°C for 1 minute, annealing at 45°C for 1 minute, and extension at 72°C for 1 minute; a final extension of 7 minutes at 72°C; 4°C for 4 minutes; and an infinite hold at 10°C.

### PCR Primers

For detection of the *Wsm1* gene, the sequence tagged site primers, STSJ15, developed by Talbert et al. (1996) was used. This primer amplifies a 500 base pair segment associated with the translocation from *T. intermedium*.

For entries containing the coat protein or replicase gene of WSMV, primers designed and reported by Sivamani et al. (2000a and 2000b) were used.

The nucleotide sequences of the primers flank the 5' and 3' ends of the corresponding gene sequences of WSMV and PCR produces diagnostic bands approximately 1.3 kilobase pairs for each transgene.

#### PCR Product Evaluation

PCR products were separated on 0.7% polyacrylamide gels with 0.5% Tris-borate EDTA running buffer or on 1.0% agarose gels with 1.0% Tris-borate EDTA running buffer. The gels were stained with ethidium bromide and the DNA was visualized with UV light and photographed.

#### Statistical Analysis

Analysis of variance was computed for each measured trait using Statistical Analysis Systems (SAS) version 7.0 (SAS Institute, 1998). Entry means were computed for each year and combined for the two years. Entry means were compared using Fisher's least significant difference (LSD). Contrasts were used to compare the average of selected entries with the average of other grouped entries. For example, the six *Wsm1* lines were grouped and their average was compared with the average of the six sister lines not containing the *Wsm1* gene to see if there is a physiological cost to having the *Wsm1* gene in the wheat plant. Coat protein lines and replicase lines were grouped and compared. Replications were treated as random effects while entry and year were treated as fixed effects using a linear model.

## CHAPTER 4

## RESULTS

Field Trial EvaluationInoculation and Evaluation of Viral Resistance

Virus Identification – Analysis of virus particles prepared for viewing under electron microscopy revealed only flexuous rods approximately 700 nm long and 15 nm wide. It was determined that WSMV was intact and sufficient in number to establish infection in field trials and that no contaminants were present in the preparation.

Symptom Expression – Symptoms ranged from 0 to 5 on inoculated entries. Symptoms noted at four, six, and eight weeks post inoculation are reported for each year as well as the combined analysis for the two years (Table 3). Additional symptoms noted in 1999 for two, three, five, and nine weeks post inoculation are reported in Table 4.

Two of the wheat-*Thinopyrum* lines PCR positive for the presence of the *Wsm1* gene exhibited no symptoms while four of the lines developed mild symptoms in 1999. In 2000, all *Wsm1* lines remained symptom free throughout the growing season. The susceptible wheat-*Thinopyrum* entries exhibited similar or more severe symptoms than 'Amidon' or 'McNeal' in both years.

**Table 3.** Symptom expression means for spring wheat lines inoculated with wheat streak mosaic virus at Bozeman, MT in 1999 and 2000 at four, six, and eight weeks post inoculation.

Entry	----- 1999 -----			----- 2000 -----			---- Combined ----		
	4 wks	6 wks	8 wks	4 wks	6 wks	8 wks	4 wks	6 wks	8 wks
4161R	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4165R	0.67	0.67	1.33	0.00	0.00	0.00	0.29	0.29	0.57
4266R	1.33	0.67	2.33	0.00	0.00	0.00	0.57	0.29	1.00
4274R	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4292R	0.33	0.00	0.33	0.00	0.00	0.00	0.14	0.00	0.14
4338R	0.67	0.00	0.00	0.00	0.00	0.00	0.29	0.00	0.00
4168S	4.67	4.67	4.33	3.75	3.50	3.50	4.14	4.00	3.86
4199S	5.00	4.67	5.00	4.50	4.50	4.75	4.71	4.57	4.86
4238S	3.33	3.33	3.33	3.00	2.50	2.75	3.14	2.86	3.00
4241S	3.00	3.00	2.00	3.00	3.50	3.75	3.00	3.29	3.00
4252S	5.00	5.00	5.00	4.50	4.25	4.25	4.71	4.57	4.57
4316S	4.00	4.00	4.00	3.25	3.00	3.00	3.57	3.43	3.43
CP20.2	2.67	2.33	2.33	4.00	3.50	3.75	3.43	3.00	3.14
CP25.13				2.50	2.50	2.50			
CP25.14				3.25	3.00	3.25			
CP25.8	3.00	3.00	3.33	3.50	3.50	4.00	3.29	3.29	3.71
CPB4	3.00	3.00	3.67	2.75	3.25	2.75	2.86	3.14	3.14
Nlb4.4	3.00	2.67	3.00	2.00	2.25	2.00	2.43	2.43	2.43
Nlb4.8	3.00	3.00	3.00	3.00	3.50	3.50	3.00	3.29	3.29
Nlb7.17	3.00	3.00	3.00	2.50	1.75	2.25	2.71	2.29	2.57
Nlb7.26	3.00	3.00	3.00	3.25	2.75	3.00	3.14	2.86	3.00
Hi-Line	3.00	2.67	3.33	2.25	2.25	2.75	2.57	2.43	3.00
Amidon	3.00	2.00	2.33	2.75	2.75	2.75	2.86	2.43	2.57
Ernest	4.67	4.33	5.00	4.00	4.00	3.75	4.29	4.14	4.29
Fergus	3.00	3.67	3.67	2.50	2.50	2.50	2.71	3.00	3.00
Fortuna	3.33	3.00	3.00	3.25	3.00	3.00	3.29	3.00	3.00
MTHW9420	4.00	3.67	3.33	3.50	3.75	3.75	3.71	3.71	3.57
McNeal	2.67	2.33	2.00	2.50	1.50	1.50	2.57	1.86	1.71
Rambo	4.33	4.00	4.00	3.25	3.25	3.50	3.71	3.57	3.71
Scholar	4.00	4.00	3.00	3.00	3.00	3.00	3.43	3.43	3.00
LSD (0.05)	0.78	0.72	0.64	1.36	1.72	1.72	0.85	1.02	1.02

Symptoms are rated; 0 = no symptoms and 5 = severe symptoms

**Table 4.** Symptom expression means for spring wheat lines inoculated with wheat streak mosaic virus at Bozeman, MT in 1999 at two to nine weeks post inoculation.

Entry	2 wks	3 wks	4 wks	5 wks	6 wks	8 wks	9 wks
4161R	0.00	0.00	0.00	0.00	0.00	0.00	0.33
4165R	0.33	0.33	0.67	0.67	0.67	1.33	1.33
4266R	0.00	0.00	1.33	1.33	0.67	2.33	2.67
4274R	0.00	0.00	0.00	0.00	0.00	0.00	0.67
4292R	0.67	0.00	0.33	0.00	0.00	0.33	0.67
4338R	0.00	0.00	0.67	0.00	0.00	0.00	0.67
4168S	1.67	1.67	4.67	4.67	4.67	4.33	4.67
4199S	0.67	2.00	5.00	4.67	4.67	5.00	5.00
4238S	0.00	1.00	3.33	3.33	3.33	3.33	3.33
4241S	1.33	1.00	3.00	3.00	3.00	2.00	3.00
4252S	1.67	2.00	5.00	5.00	5.00	5.00	5.00
4316S	0.67	1.00	4.00	4.00	4.00	4.00	4.67
CP20.2	1.00	1.00	2.67	2.67	2.33	2.33	2.67
CP25.8	1.33	1.00	3.00	3.00	3.00	3.33	3.00
CPB4	1.00	1.67	3.00	3.00	3.00	3.67	3.33
Nlb4.4	0.33	1.00	3.00	2.67	2.67	3.00	2.67
Nlb4.8	1.00	1.00	3.00	3.00	3.00	3.00	3.67
Nlb7.17	0.33	1.00	3.00	3.00	3.00	3.00	3.33
Nlb7.26	0.00	1.00	3.00	3.00	3.00	3.00	3.00
Hi-Line	0.00	1.00	3.00	3.00	2.67	3.33	3.33
Amidon	1.00	1.00	3.00	2.67	2.00	2.33	2.67
Ernest	1.00	1.00	4.67	4.33	4.33	5.00	5.00
Fergus	0.33	1.00	3.00	3.67	3.67	3.67	3.67
Fortuna	0.67	1.00	3.33	3.00	3.00	3.00	3.67
MTHW9420	1.33	1.00	4.00	4.00	3.67	3.33	4.00
McNeal	1.00	1.00	2.67	2.67	2.33	2.00	2.33
Rambo	0.67	1.00	4.33	4.00	4.00	4.00	4.00
Scholar	1.67	1.00	4.00	4.00	4.00	3.00	3.00
LSD (0.05)	0.76	0.31	0.78	0.62	0.72	0.64	0.77

Symptoms are rated as 0-5; 0 = no symptoms and 5 = severe symptoms

Transgenic lines did not differ significantly from 'Hi-Line' for symptom expression with the exception of CP 20.2 that had the 'Bobwhite' parental background. CP 20.2 differed from 'Hi-Line' at eight weeks post inoculation in 1999, where symptoms in CP 20.2 were less severe than in 'Hi-Line', and at four weeks in 2000, where symptoms were more severe than in 'Hi-Line'. CP 20.2 had been regarded as susceptible in greenhouse trials (Sivamani et al., 2000b). Other observation dates indicated no significant differences between the two entries. At two and three weeks post inoculation in 1999, several of the other transgenic lines appeared more severely affected than 'Hi-Line', but later observation dates revealed no significant differences between the transgenics and 'Hi-Line'. N1b 4.4 and CP 25.14, which had shown recovery type resistance in greenhouse trials, (Sivamani et al., 2000a and 2000b), developed symptoms consistent with that of 'Hi-Line' and other susceptible entries in this field trial. Viruliferous wheat curl mites inadvertently infested the CP lines, 25.13 and 25.14, that had been planted for seed increase in the greenhouse in January of 2000. Subsequent infection with WSMV showed moderate symptom expression similar to that observed in the field trial.

Among adapted cultivars, 'Ernest' consistently showed more severe symptoms than other cultivars. In 1999, 'Fergus', MT9420, 'Rambo', and 'Scholar' were on some observation dates more severely affected than 'Hi-Line'. In 2000, 'Ernest' was the only cultivar that differed significantly from other cultivars.

Viral Replication Level – Virus titer as quantified by optical density readings of PAS-ELISA at 20 days post inoculation indicate similar high levels of virus accumulation in all entries with the exception of the six *Wsm1* entries (Table 5). The positive-negative threshold for distinguishing diseased and non-diseased plants was three times the average absorbancy of the negative control plants (Khetarpal and Kumar, 1995). Using this guideline the six *Wsm1* lines were classified as resistant to WSMV while all other entries were classified as susceptible.

In both years, virus levels of resistant wheat-*Thinopyrum* lines were significantly lower than parental lines while susceptible wheat-*Thinopyrum* lines were no different than parental lines. Transgenic lines did not differ significantly from 'Hi-Line', and adapted cultivars did not differ from one another in the amount of virus present in the plant.

Titer values from the two lines infected during greenhouse seed increase, CP 25.13 and CP 25.14, ranged from 0.900 to 1.045 while the titer for negative control plants was 0.040 (unpublished data). Negative control tissue for ELISA of greenhouse material had been collected from plants grown in a different greenhouse where no wheat curl mites were observed.

At 40 days post inoculation, optical density values for *Wsm1* entries increased relative to the values obtained at 20 days post inoculation indicating viral accumulation occurring at the later sampling date (Table 5). In 1999, significant differences were detected between resistant *Wsm1* lines and sister

**Table 5.** Optical density means for ELISA on spring wheat lines inoculated with wheat streak mosaic virus at Bozeman, MT in 1999 and 2000 at 20 and 40 days post inoculation (dpi).

Entry	20 dpi			40 dpi			WSMV Reaction
	1999	2000	Avg	1999	2000	Avg	
4161R	0.13	0.01	0.06	0.40	0.15	0.26	R
4165R	0.11	0.04	0.07	0.48	0.39	0.43	R
4266R	0.13	0.01	0.06	0.40	0.62	0.53	R
4274R	0.11	0.01	0.05	0.71	0.27	0.46	R
4292R	0.05	0.01	0.03	0.08	0.17	0.13	R
4338R	0.06	0.01	0.03	0.40	0.16	0.27	R
4168S	1.72	0.34	0.93	1.24	0.40	0.76	S
4199S	1.71	0.33	0.92	0.88	0.11	0.44	S
4238S	1.68	0.23	0.85	1.31	0.55	0.87	S
4241S	1.71	0.40	0.96	1.25	0.69	0.93	S
4252S	1.77	0.40	0.99	0.74	0.15	0.40	S
4316S	1.70	0.26	0.88	1.04	0.61	0.79	S
CP20.2	1.79	0.40	0.99	1.36	0.76	1.02	S
CP25.13		0.28			0.56		S
CP25.14		0.35			0.81		S
CP25.8	1.70	0.49	1.01	1.11	0.74	0.90	S
CPB4	1.84	0.43	1.03	1.23	0.63	0.89	S
NIb4.4	1.72	0.16	0.83	1.26	0.33	0.73	S
NIb4.8	1.59	0.29	0.49	1.34	0.41	0.81	S
NIb7.17	1.53	0.27	0.81	1.52	0.57	0.98	S
NIb7.26	1.76	0.40	0.98	1.40	0.75	1.03	S
Hi-Line	1.81	0.28	0.94	1.43	0.59	0.95	S
Amidon	1.47	0.41	0.86	1.28	0.50	0.83	S
Ernest	1.78	0.38	0.98	1.65	0.72	1.12	S
Fergus	1.45	0.16	0.71	0.98	0.47	0.69	S
Fortuna	1.69	0.41	0.96	1.35	0.57	0.91	S
McNeal	1.65	0.21	0.82	1.25	0.49	0.82	S
MTHW9420	1.62	0.30	0.87	1.35	0.39	0.80	S
Rambo	1.74	0.33	0.93	1.22	0.69	0.91	S
Scholar	1.77	0.30	0.93	1.21	0.61	0.87	S
3 X Control	0.15	0.04	0.08	0.22	0.11	0.15	
LSD (0.05)	0.52	0.18	0.24	0.40	0.48	0.32	

Optical density is recorded in absorbance units at 405 nm.

lines at 40 days post inoculation. In 2000 however, differences between resistant and susceptible wheat-*Thinopyrum* lines were not statistically significant at 40 days post inoculation.

### Morphological Evaluation

Heading Date – In 1999, heading was delayed one to nine days in susceptible lines with no delay in heading for resistant lines (Table 6). In 2000, heading was delayed in susceptible lines by only one to two days. 'Hi-Line' generally headed sooner than transgenic lines, but only at a significance level of 7 percent. Nlb 4.8 and CP 25.8 were the two lines that differed significantly from 'Hi-Line' using Fisher's LSD. Among adapted cultivars, WSMV delayed heading considerably in 'Ernest', 'Rambo', and 'Scholar', but only in 1999. In 2000, heading delay was virtually the same for all cultivars.

Plant Height – Plant height was reduced in susceptible lines by 18 to 49 percent (14 to 39 cm) in 1999, while the height of resistant lines was reduced by only 2 to 8 percent (1 to 6 cm) (Table 7). In 2000, the height of inoculated susceptible entries was reduced by 4 to 21 percent (3 to 18 cm) while the height of inoculated resistant entries was reduced by 1 to 5 percent (1 to 4 cm).

In the absence of disease, resistant *Wsm1* lines were generally shorter than their parental lines but did not show significant stunting in the presence of WSMV. Susceptible wheat-*Thinopyrum* lines were generally shorter than parent lines both in the absence and presence of disease.

**Table 6.** Heading date means for spring wheat lines with and without wheat streak mosaic virus at Bozeman, MT in 1999 and 2000.

Entry	Heading Date * 1999			Heading Date 2000			Combined Years		Delay due to WSMV (Days)
	Inoc	Non	Delay due to WSMV (Days)	Inoc	Non	Delay due to WSMV (Days)	Inoc	Non	
4161R	193	193	0	185	185	0	188	188	0
4165R	195	195	0	189	189	0	192	192	0
4266R	195	195	0	190	189	1	192	192	0
4274R	193	193	0	189	189	0	190	191	0
4292R	195	195	0	189	189	0	191	191	0
4338R	195	195	0	189	189	0	191	191	0
4168S	201	194	7	187	187	1	193	190	3
4199S	203	194	9	189	188	2	195	190	5
4238S	195	191	4	183	182	2	188	186	2
4241S	195	192	3	184	182	2	189	186	2
4252S	202	195	7	190	189	1	195	192	3
4316S	203	197	6	190	189	1	196	192	3
CP20.2	196	194	1	188	188	1	191	191	1
CP25.13				188	187	2			
CP25.14				188	188	1			
CP25.8	197	195	1	188	188	1	192	191	1
CPB4	195	193	2	188	187	1	191	189	2
Nlb4.4	195	193	2	187	187	1	191	189	1
Nlb4.8	198	196	2	190	189	1	193	192	1
Nlb7.17	195	193	2	188	188	1	191	190	1
Nlb7.26	195	193	2	187	185	2	190	189	2
Hi-Line	195	193	2	187	186	1	190	189	1
Amidon	197	195	2	188	186	2	192	190	2
Ernest	201	194	7	187	187	1	193	190	3
Fergus	195	192	3	184	184	1	189	187	2
Fortuna	197	194	3	187	186	1	191	189	2
McNeal	196	194	2	189	189	0	192	191	1
MTHW9420	195	193	2	187	185	2	190	188	2
Rambo	202	196	6	190	189	1	195	192	3
Scholar	200	195	5	189	189	1	194	191	2
LSD (0.05)	2	1	2	2	1	2	1	1	1

\* Heading date is recorded as number of days from January 1.

**Table 7.** Height means for spring wheat lines with and without wheat streak mosaic virus inoculation at Bozeman, MT in 1999 and 2000.

Entry	Height 1990 (cm)			Height 2000 (cm)			Combined Years (cm)		
	Inoc	Non	Reduc. due to WSMV (%)	Inoc	Non	Reduc. due to WSMV (%)	Inoc	Non	Reduc. due to WSMV (%)
4161R	70.5	69.3	-1.7	75.8	77.0	1.3	73.5	73.7	0.0
4165R	55.2	58.7	6.1	67.0	70.0	3.7	61.9	65.1	4.8
4266R	64.8	66.7	2.2	72.0	75.8	4.6	68.9	71.9	3.6
4274R	78.8	81.0	2.2	86.4	90.8	4.4	83.1	86.6	3.5
4292R	65.3	67.0	2.1	73.1	75.3	2.5	69.8	71.7	2.3
4338R	60.2	66.0	8.3	68.6	70.8	2.6	65.0	68.7	5.1
4168S	40.2	75.0	46.2	74.4	86.8	13.7	59.0	81.7	27.6
4199S	41.2	78.0	47.0	72.9	89.3	18.1	59.3	84.4	30.5
4238S	50.8	72.3	29.9	69.3	78.3	11.3	61.4	75.7	19.3
4241S	42.5	63.3	32.3	63.0	72.5	12.8	54.2	68.6	21.1
4252S	37.7	72.0	47.4	64.3	76.3	15.6	52.9	74.4	29.2
4316S	41.3	72.7	42.7	69.4	87.8	20.6	57.4	81.3	30.1
CP20.2	57.3	70.7	18.4	67.0	74.0	9.3	62.9	72.6	13.2
CP25.13				64.8	77.3	15.7			
CP25.14				64.1	75.8	15.2			
CP25.8	45.7	66.7	31.4	65.5	75.8	13.4	57.0	71.9	21.1
CPB4	46.0	64.3	27.7	71.8	78.0	7.6	60.7	72.1	16.2
NIb4.4	53.7	68.0	21.3	70.8	76.3	6.8	63.4	72.7	13.0
NIb4.8	49.3	67.7	26.1	70.5	85.0	16.6	61.4	77.6	20.7
NIb7.17	47.3	64.0	25.6	64.4	69.0	6.1	57.1	66.9	14.4
NIb7.26	47.5	66.3	28.1	69.3	78.5	11.5	59.9	73.3	18.6
Hi-Line	49.5	71.7	30.6	71.6	77.0	6.6	62.1	74.7	16.9
Amidon	56.7	79.7	28.6	86.6	92.8	6.2	73.8	87.1	15.8
Ernest	40.0	79.0	49.2	76.4	88.5	13.8	60.8	84.4	29.0
Fergus	52.8	71.7	25.9	68.3	72.8	5.9	61.6	72.3	14.5
Fortuna	48.3	85.0	43.0	79.6	93.5	14.7	66.2	89.9	26.8
McNeal	57.5	73.7	21.5	76.6	79.8	3.8	68.4	77.1	11.4
MTHW9420	47.7	66.0	27.4	63.5	73.8	13.7	56.7	70.4	19.6
Rambo	41.5	70.0	40.7	71.1	78.3	8.4	58.4	74.7	22.2
Scholar	50.8	77.0	33.8	77.8	89.0	12.3	66.2	83.9	21.5
LSD (0.05)	10.0	5.1	13.5	11.0	2.9	13.3	7.2	2.7	8.8

Transgenic lines were not statistically different from 'Hi-Line' for plant height. Among adapted cultivars, 'Amidon', 'Ernest', 'Fortuna', and 'Scholar' were generally taller than other cultivars but stunting due to WSMV was significant only in 'Ernest'.

Yield – Yield losses due to WSMV infection in 1999 ranged from 54 to 97 percent in susceptible lines (Table 8). Yield reduction in resistant lines ranged from 5 to 56 percent when compared with non-inoculated controls. In 2000, yield losses ranged from 20 to 82 percent in susceptible lines, and from 6 to 28 percent in the resistant lines.

Yield results for 1999 are reported at g/m<sup>2</sup>. Because rows were thinned after application of glufosinate, and they did not have comparable numbers of plants, yield was also calculated on a per plant basis. Analysis of variance for yield on a per plant basis did not reveal differences other than those indicated from analysis of yields reported at g/m<sup>2</sup>. Results in 2000 are more readily compared since plant numbers among lines and rows were comparable.

In the presence of disease, the resistant *Wsm1* lines yielded significantly higher than parental lines, and significantly higher than sister lines in both years. However, in the absence of disease, the *Wsm1* lines yielded 12 to 33 percent lower than sister lines and 7 to 33 percent lower than 'Amidon'. Yields were not significantly lower than 'McNeal' or other adapted cultivars.

**Table 8.** Yield means for spring wheat lines with and without wheat streak mosaic virus inoculation at Bozeman, MT in 1999 and 2000.

Entry	Yield 1999 (g m <sup>-2</sup> )			Reduc. due to WSMV (%)	Yield 2000 (g m <sup>-2</sup> )			Reduc. due to WSMV (%)	Combined Years (g m <sup>-2</sup> )		Reduc. due to WSMV (%)
	Inoc	Non			Inoc	Non			Inoc	Non	
4161R	275	309	7	304	343	11	291	328	9		
4165R	168	255	34	254	358	28	217	314	31		
4266R	155	350	56	310	421	27	244	391	39		
4274R	266	359	21	330	355	7	302	357	13		
4292R	236	339	32	286	303	6	264	318	17		
4338R	222	239	5	326	346	6	282	300	5		
4168S	30	430	93	163	403	58	106	415	73		
4199S	18	492	97	72	407	82	49	443	88		
4238S	125	466	73	200	398	45	168	427	57		
4241S	102	425	75	171	366	52	141	391	62		
4252S	29	509	93	104	404	75	72	449	82		
4316S	59	422	86	189	437	53	133	431	67		
CP20.2	166	359	54	151	372	60	157	366	57		
CP25.13				214	330	33					
CP25.14				139	309	54					
CP25.8	67	272	78	123	324	62	99	302	69		
CPB4	86	344	74	210	356	40	157	351	54		
Nlb4.4	155	462	66	238	345	30	202	395	46		
Nlb4.8	38	199	81	129	262	52	90	235	64		
Nlb7.17	79	292	73	213	289	25	156	290	45		
Nlb7.26	149	454	67	208	387	46	182	416	55		
Hi-Line	170	512	66	233	359	35	206	424	48		
Amidon	94	370	75	268	453	41	194	417	55		
Ernest	22	537	96	174	424	58	109	472	74		
Fergus	117	478	74	228	358	36	181	410	52		
Fortuna	88	346	72	200	395	49	152	374	59		
McNeal	146	462	69	282	354	20	223	400	41		
MTHW9420	58	368	85	162	371	56	117	370	68		
Rambo	44	389	89	166	357	52	114	370	68		
Scholar	97	497	81	243	438	43	180	463	59		
LSD (0.05)	69	134	20	100	56	29	66	64	19		

In both years, most transgenic lines performed equal to 'Hi-Line', while Nlb 4.8 yielded significantly less than 'Hi-Line' both in the presence and absence of disease. Nlb 4.8 had been regarded as susceptible in greenhouse trials and was susceptible in this field trial. Yield losses expressed as a percentage, however, reveal no differences between the transgenics and 'Hi-Line' suggesting that the presence of these genes in a 'Hi-Line' background do not provide resistance to WSMV.

In the presence of WSMV, adapted cultivars did not differ significantly in yield. In the absence of disease, however, 'Ernest' and 'Scholar' provided the highest yields.

1000 Kernel Weight – Decreases in kernel weight for susceptible lines ranged from 18 to 38 percent in 1999 (6 to 14 g) (Table 9). Decreases in kernel weights for resistant lines ranged from 6 to 25 percent (2 to 8 g), in the same year.

In the absence of disease, resistant *Wsm1* lines had 1000 kernel weights that were significantly lower than those of parental lines while susceptible wheat-*Thinopyrum* lines were better than parental lines. Transgenic lines performed equal to 'Hi-Line'. Adapted cultivars infected with WSMV had similar kernel weights.

**Table 9.** 1000 kernel weight means for spring wheat lines with and without WSMV inoculation at Bozeman, MT in 1999 and 2000.

Entry	1000kwt 1999			Reduc. due to WSMV (%)	1000kwt 2000			Reduc. due to WSMV (%)	Combined Years (g)		Reduc. due to WSMV (%)
	Inoc	Non	(g)		Inoc	Non	(g)		Inoc	Non	
4161R	29.8	32.8	9.2	24.9	25.5	2.5	27.0	28.6	5.3		
4165R	26.1	30.2	13.6	21.1	23.5	10.1	23.3	26.4	11.6		
4266R	23.6	31.5	25.1	22.5	25.0	9.9	23.0	27.8	16.4		
4274R	31.3	35.4	11.5	25.9	27.4	5.4	28.2	30.8	8.0		
4292R	29.6	32.1	8.0	25.3	26.4	4.0	27.1	28.8	5.7		
4338R	27.0	28.6	5.7	22.1	22.7	2.6	24.2	25.2	3.9		
4168S	21.1	33.9	37.7	23.4	26.0	9.6	22.4	29.4	21.6		
4199S	24.4	32.4	24.6	19.8	22.3	11.5	21.8	26.7	17.1		
4238S	31.1	42.8	27.0	24.6	27.8	11.1	27.4	34.2	17.9		
4241S	29.7	38.4	22.7	21.0	23.1	8.7	24.7	29.7	14.7		
4252S	24.2	36.1	32.7	23.5	27.4	14.5	23.8	31.1	22.3		
4316S	26.2	38.8	32.3	23.7	28.8	17.3	24.8	33.1	23.8		
CP20.2	25.4	31.1	18.2	21.7	22.8	5.0	23.3	26.4	10.7		
CP25.13				20.3	2.9	11.5					
CP25.14				18.5	21.8	15.1					
CP25.8	24.3	34.1	28.9	19.1	21.9	12.7	21.3	27.1	19.6		
CPB4	24.8	35.1	29.3	20.9	23.5	10.8	22.6	28.5	18.7		
NIb4.4	26.4	36.1	26.7	22.1	23.8	7.1	23.9	29.1	15.5		
NIb4.8	22.8	29.3	21.1	18.2	21.0	13.6	20.1	24.5	17.3		
NIb7.17	25.9	34.4	24.8	20.6	22.6	9.0	22.9	27.7	15.8		
NIb7.26	26.3	36.7	28.2	20.0	23.8	15.5	22.7	29.3	21.0		
Hi-Line	26.5	35.7	25.8	20.6	22.2	6.9	23.1	28.0	15.0		
Amidon	25.8	33.7	23.5	23.6	26.9	12.2	24.5	29.8	17.0		
Ernest	22.9	34.8	34.1	23.0	25.4	9.3	23.0	29.4	19.9		
Fergus	27.4	41.7	34.2	23.5	26.2	9.5	25.2	32.8	20.1		
Fortuna	35.0	43.7	19.6	25.3	30.2	16.0	29.5	36.0	17.6		
McNeal	27.0	33.5	19.3	21.4	23.8	9.7	23.8	27.9	13.8		
MTHW9420	23.8	35.2	32.1	17.7	21.2	16.0	20.3	27.2	22.9		
Rambo	25.6	36.6	30.8	23.2	23.1	-0.8	24.1	28.8	12.7		
Scholar	25.7	34.5	25.5	22.8	26.1	12.3	24.0	29.7	17.9		
LSD (0.05)	3.8	1.9	11.3	2.9	1.7	12.5	2.3	1.3	8.6		

### Cereal Quality Evaluation

Protein Quality – The transgenic lines, Nlb 4.8 and CP 20.2 had significantly lower SDS sedimentation volume than 'Hi-Line' under diseased conditions (Table 11) in both years. 'Amidon' was significantly lower in sedimentation volume than 'Hi-Line' both with and without disease. 'McNeal' was consistently higher in sedimentation volume, but 'McNeal' progeny, 4161R and 4165R, had sedimentation volumes significantly lower than that of 'McNeal'.

Grain Protein – In 1999 and 2000, changes in grain protein due to infection by WSMV did not differ significantly in susceptible or resistant wheat-*Thinopyrum* lines (Table 10). Transgenic lines did differ significantly from 'Hi-Line', but only Nlb 4.8 differed in both years. Grain protein in Nlb 4.8 was higher than that of 'Hi-Line' both under diseased and non-diseased conditions. Of the adapted cultivars, 'McNeal' had the highest grain protein in the absence of WSMV and although higher in protein when infected with WSMV, it was not significantly higher.

### Molecular Marker Evaluation

#### PCR Product Evaluation

The *Wsm1* lines regarded as resistant to WSMV had the characteristic 500 base pair band from the translocated segment of *T. intermedium* upon PCR with the STSJ15 primers while the six sister lines regarded as susceptible showed no band.

**Table 10.** Grain protein means for spring wheat lines with and without wheat streak mosaic virus inoculation at Bozeman, MT in 1999 and 2000.

Entry	Protein 1999 (%)			Protein 2000 (%)			Combined Years (%)		
	Inoc	Non	Inc. due to WSMV (%)	Inoc	Non	Inc. due to WSMV (%)	Inoc	Non	Inc. due to WSMV (%)
4161R	17.3	16.6	4.1	17.2	16.6	3.5	17.2	16.6	3.8
4165R	16.8	16.8	-0.1	17.1	16.4	4.3	16.9	16.6	2.4
4266R	16.1	15.1	6.7	16.9	16.6	8.3	16.5	15.4	7.6
4274R	16.9	15.9	6.3	17.3	16.5	5.0	17.1	16.3	5.5
4292R	16.0	16.0	-0.4	16.5	16.2	1.8	16.3	16.1	0.9
4338R	15.8	15.7	0.6	15.3	14.9	3.0	15.5	15.2	2.0
4168S	16.2	16.5	-1.5	17.7	16.9	4.7	17.1	16.7	2.0
4199S	15.2	14.7	3.7	15.6	15.2	3.1	15.5	15.0	3.3
4238S	15.5	16.2	-4.2	16.9	15.7	7.9	16.3	15.9	2.7
4241S	18.1	17.4	4.4	18.6	17.1	8.7	18.4	17.2	6.8
4252S	17.0	16.0	6.7	17.7	16.4	8.2	17.4	16.2	0.5
4316S	14.4	14.3	1.0	17.1	15.3	12.2	15.9	14.9	7.4
CP20.2	14.8	13.8	7.1	16.6	15.7	5.6	15.8	14.9	6.3
CP25.13				17.7	16.3	8.3			
CP25.14				18.5	16.5	12.1			
CP25.8	17.3	17.5	-1.2	18.7	16.6	12.9	18.1	17.0	6.8
CPB4	17.0	16.8	1.2	17.6	16.1	9.7	17.3	16.4	6.1
NIb4.4	17.2	16.4	5.2	17.4	16.5	5.8	17.3	16.4	5.5
NIb4.8	18.6	17.9	3.9	18.7	17.6	6.6	18.7	17.7	5.5
NIb7.17	16.7	16.7	0.0	17.6	16.5	7.0	17.2	16.6	4.1
NIb7.26	16.3	16.3	0.1	17.9	16.1	11.6	17.3	16.2	6.7
Hi-Line	16.5	15.6	5.9	17.4	16.2	7.3	17.0	16.0	6.7
Amidon	17.0	16.2	5.3	17.2	15.7	9.4	17.1	15.9	7.6
Ernest	17.5	16.5	6.2	17.5	16.2	0.0	17.5	16.3	7.2
Fergus	15.0	15.4	-2.4	17.1	16.4	4.1	16.2	16.0	1.3
Fortuna	16.1	16.8	-4.4	16.6	15.8	5.4	16.4	16.2	1.2
McNeal	17.4	16.5	5.7	18.2	17.0	6.7	17.9	16.8	6.2
MTHW9420	15.0	15.1	-0.6	17.7	16.4	8.0	16.6	15.9	4.3
Rambo	14.1	14.8	-4.6	16.5	15.8	4.9	15.5	15.4	0.9
Scholar	16.3	16.9	-3.5	18.3	16.8	8.8	17.4	16.8	3.5
LSD (0.05)	1.1	0.8	8.3	0.9	0.6	6.1	0.7	0.5	4.9

**Table 11.** SDS sedimentation means as a measure of protein quality for spring wheat lines with and without WSMV inoculation at Bozeman, MT in 1999 and 2000.

Entry	SDSS 1999 (ml)			SDSS 2000 (ml)			Combined Years (ml)		
	Inoc	Non	Inc. due to WSMV (%)	Inoc	Non	Inc. due to WSMV (%)	Inoc	Non	Inc. due to WSMV (%)
4161R	5.0	5.4	-6.7	6.0	6.3	-4.9	5.6	5.9	-5.7
4165R	5.9	6.8	-12.6	6.7	6.6	2.3	6.4	6.7	-4.1
4266R	7.8	6.7	13.0	6.9	7.6	-8.2	7.3	7.2	2.5
4274R	6.3	6.5	-2.7	7.5	7.2	4.1	7.0	6.9	1.2
4292R	5.9	5.7	3.2	6.0	5.9	2.2	5.9	5.8	2.6
4338R	7.0	6.9	2.5	7.3	6.8	0.4	7.2	6.8	5.3
4168S	7.3	6.3	16.4	7.9	7.5	6.2	7.7	7.0	10.5
4199S	7.4	7.3	1.3	7.4	7.2	2.5	7.4	7.3	2.0
4238S	6.9	5.2	33.9	7.0	6.7	6.1	7.0	6.1	18.0
4241S	6.7	5.4	25.8	6.3	6.3	4.2	6.5	5.9	13.5
4252S	7.9	6.9	18.2	7.4	7.5	-0.9	7.6	7.2	7.3
4316S	6.3	6.6	-3.1	7.1	7.7	-7.3	6.8	7.2	-5.5
CP20.2	4.3	4.3	-1.3	4.2	4.7	-11.4	4.2	4.6	-7.1
CP25.13				6.3	6.7	-4.5			
CP25.14				6.0	7.6	-21.4			
CP25.8	6.2	5.6	11.3	5.3	6.4	-17.2	5.7	6.1	-5.0
CPB4	6.8	5.4	31.2	6.2	6.5	1.0	6.4	6.0	14.0
NIb4.4	6.5	5.0	29.9	6.8	6.1	11.7	6.6	5.6	19.5
NIb4.8	3.8	4.9	-21.4	4.4	4.7	-7.3	4.1	4.8	-13.4
NIb7.17	6.5	4.7	38.7	6.4	7.0	-6.6	6.5	6.0	12.8
NIb7.26	6.5	6.1	12.8	6.2	6.7	-3.8	6.3	6.4	3.3
Hi-Line	6.6	5.3	24.4	7.1	6.5	10.7	6.9	6.0	16.6
Amidon	4.1	4.0	3.5	5.4	5.3	3.8	4.8	4.7	3.7
Ernest	5.9	5.8	2.5	6.7	7.0	-2.6	6.4	6.4	-0.4
Fergus	5.8	6.1	-5.4	6.5	6.5	0.4	6.2	6.3	-2.1
Fortuna	4.9	4.3	15.9	6.0	5.1	19.7	5.5	4.8	18.1
McNeal	7.7	8.1	-4.5	7.4	7.7	-2.8	7.5	7.8	-3.5
MTHW9420	6.1	5.6	12.8	5.5	5.7	-2.8	5.8	5.6	3.9
Rambo	6.1	6.4	-3.6	6.2	6.6	-5.0	6.2	6.5	-4.4
Scholar	4.8	4.4	8.1	4.9	5.2	-6.0	4.8	4.9	0.0
LSD (0.05)	0.9	1.2	22.0	0.9	0.9	22.8	0.6	0.7	16.0

Transgenic plants grown in the greenhouse for seed increase in 1999 were sprayed with glufosinate and found to be segregating for herbicide resistance. To verify co-segregation of herbicide resistance and viral transgenes as reported by Sivamani et al., (2000a and 2000b), we performed PCR on two of the transgenic lines for detection of the replicase or coat protein transgene. In both N1b 4.4 and CP B4, the presence or absence of a 1.3-kilobase diagnostic band was consistent with the reaction to glufosinate (unpublished data). Accordingly, selection of seed for the field trial was based on the herbicide reaction of greenhouse plants.

In 2000, DNA was extracted from a random sample of plants from CP 25.13 and 25.14 lines and analyzed for presence of the coat protein transgene via PCR analysis. All of the plants sampled from CP 25.13 were positive for the presence of a 1.3-kilobase pair fragment. Plants from CP 25.14 were segregating for the presence of the transgene.

After inadvertent infestation by viruliferous wheat curl mites, CP 25.13 and CP 25.14 plants were observed to develop symptoms characteristic of WSMV. Fourteen random plants were selected and symptoms ranging from none to mild were recorded. DNA from these plants was extracted and PCR using coat protein primers was performed. Plants positive for symptoms and ELISA titer had the 1.3-kilobase pair diagnostic band upon PCR analysis.

## CHAPTER 5

## DISCUSSION

Adapted cultivars were all susceptible to WSMV. Virus titers in new leaf tissue collected from cultivars at both 20 and 40 days post inoculation indicated a high level of virus accumulation regardless of symptom expression (Tables 3 and 5). 'Ernest' was the most susceptible cultivar exhibiting the most severe disease symptoms and the highest reduction in yield (Table 8). In the absence of WSMV, 'Ernest' was the highest yielding cultivar. 'Amidon', 'Scholar', and 'Hi-Line' also had high yields in the absence of WSMV.

A cultivar's ability to survive till maturity and produce seed while infected with disease may be interpreted as tolerance. Tolerance is also described as a low-level resistance (Siefers and Martin, 1988) whereby plants are infected and develop symptoms but yield is reduced less than in susceptible cultivars. Results of our field trial suggest that Montana-adapted cultivars differ in their ability to tolerate WSMV. 'Ernest' may be regarded as susceptible while most other cultivars grown in this trial exhibit some tolerance to WSMV. This agrees with previous field results obtained by Young (Table 12) where virus accumulation in all adapted cultivars was high regardless of symptom expression or decreases in yield due to disease. Our findings support the hypothesis that current breeding programs select for tolerance to WSMV rather than resistance. Our findings also indicate that symptom expression is not predictive of yield loss.

**Table 12.** Yield reduction in spring wheat due to wheat streak mosaic virus and corresponding symptom and ELISA data. Studies conducted in 1998 and 1999 in Bozeman or Conrad, Montana.\*

Entry	Bozeman 1998			Percent Reduction		
	Virus Titer	Symptom Expression	Percent Reduction	1999 Conrad	1999 Bozeman	Average
Conan	–	–	21	4	12	12
Scholar	0.558	Mild	35	12	13	20
McNeal	0.699	Severe	30	22	13	22
Fortuna	0.691	Moderate	30	15	20	22
Amidon	0.699	Severe	47	8	11	22
Rambo	0.699	Severe	28	28	19	25
Ernest	0.699	Severe	54	17	19	30
Fergus	0.691	Moderate	18	36	48	34
Olaf	0.699	Severe	61	28	29	39
MTHW9420	0.691	Moderate	38	31	60	43

\* Data based on personal communication with M. Young

Transgenic lines did not differ significantly from 'Hi-Line' in parameters measured, except N1b 4.8. All transgenic lines developed symptoms and had high virus titers suggesting that the transgenes do not confer resistance to WSMV (Tables 3 and 5). The yield of N1b 4.8 was significantly lower than that of 'Hi-Line' while grain protein was significantly higher both with and without disease. The SDS sedimentation volume was also lower in N1b 4.8. Data suggests that somaclonal variation due to the transformation process may have had a negative impact on the performance of this wheat line.

The recovery type resistance demonstrated by N1b 4.4 and CP 25.14 in earlier greenhouse studies (Sivamani et al., 2000a and 2000b) was not observed under field conditions. Leaf tissue sampled for ELISA at 20 and 40 days post

inoculation was from the newest unfurled leaf suggesting that spread of WSMV was systemic throughout the plant. It is possible that conditions in the field differed enough from conditions in greenhouse trials to have affected expression of the transgene. It is also possible that expression of the transgenes was silenced in subsequent generations of transgenic seedlings. This has been suggested in other studies (J. Sherwood, *personal communication*).

Greenhouse trials of transgenic wheat containing constitutive expression of puroindoline genes had initially shown moderate resistance to *Tilletia tritici* that causes common bunt of wheat. Through successive generations a decrease in resistance to the pathogen was observed even though presence of the transgene was confirmed by PCR. The expression vector used in the transformation of wheat seedlings with a puroindoline gene was directed by the maize ubiquitin promoter as was the vector used for the introduction of a coat protein or replicase gene from WSMV. It has been suggested that the maize ubiquitin promoter is not a reliable promoter for use in transforming wheat and a more effective promoter is being sought for use in transformations involving the expression of puroindoline genes in wheat tissue.

Wheat-*Thinopyrum* lines PCR positive for the *Wsm1* gene were resistant to WSMV as indicated by lack of symptoms and negative virus titer (Tables 3 and 5). The six susceptible sister lines lacking the *Wsm1* gene developed moderate to severe symptoms and had a high level of virus accumulation. In the absence of disease the average yield of *Wsm1* lines over the two years was 21

percent less than sister lines (Table 13). This may suggest a penalty due to presence of the *Wsm1* gene. Baley (1999) demonstrated a 5 percent reduction in the yield of *Wsm1* lines in the absence of WSMV when compared to non-inoculated susceptible lines.

**Table 13.** Means of selected traits in spring wheat groups with and without WSMV inoculation. Analysis is combined for 1999 and 2000.

Entry	Heading (Days)		Height (cm)		Yield (g m <sup>-2</sup> )		1000 kwt (g)		Protein (%)		SDSS (ml)	
	Inoc	Non	Inoc	Non	Inoc	Non	Inoc	Non	Inoc	Non	Inoc	Non
<i>Wsm1</i>	191*	191*	70*	73*	267*	335*	25.5*	28.0*	16.6	16.0	6.6*	6.6
Susceptible**	193	189	57	78	111	426	24.1	30.7	16.8	16.0	7.1	6.8
Amidon	192	190	74	87	194	417	24.5	29.8	17.1	15.9	4.8	4.7
McNeal	192	191	68	77	223	400	23.8	27.9	17.9	16.8	7.5	7.8
Transgenic	191	190	60	72	149	336	22.4	27.5	17.4	16.5	5.7	5.6
Hi-Line	190	189	62	75	206	424	23.1	28.0	17.0	16.0	6.9	6.0
Adapted	192	190	64	79	164	411	24.2	30.0	16.8	16.1	6.0	5.9

\* Significant at the probability level of 0.01

\*\* Susceptible = the six susceptible wheat-*Thinopyrum* lines

In 1999, reduction in yields of lines inoculated with WSMV ranged from 5 to 97 percent. We did not mock inoculate control rows in 1999 and a portion of the yield decrease between inoculated and non-inoculated rows may have been due to the failure to include an appropriate control. In 2000, we mock inoculated control rows and were therefore able to determine yield reduction due solely to infection by WSMV.

In 1999, it was noted that four of the *Wsm1* lines exhibited symptoms consistent with WSMV and yield reductions of 5 to 56 percent. These lines had

negative virus titers at 20 days post inoculation. This suggests that disease symptoms attributed to WSMV may have been due to the presence of a second pathogen. Yield reductions in other lines in 1999 were much greater than reductions due to WSMV in previous field trials (Table 12). This further supports the possibility of a second pathogen present in the 1999 field study.

Viruses causing WSMV like symptoms include barley yellow dwarf virus, septoria leaf and glume blotch, tan spot, and wheat spot mosaic. It is possible that another strain of WSMV not detected by antibodies with ELISA was present in the field as well. Nitrogen and sulfur deficiencies and herbicide injury can also cause similar symptoms. One virus found to be associated with WSMV and also vectored by the wheat curl mite is high plains virus (HPV) (Jensen et al., 1996). This virus was first detected in 1993 and by 1995, was known to exist in ten states. Examination of infected leaf tissue revealed the presence of spherical or ovoid double-membrane-bound vesicles 100 to 200 nm in diameter. HPV was almost always found in wheat and corn plants along with WSMV. Since both diseases are vectored by the wheat curl mite, infections of HPV alone were seldom found. HPV can be reliably diagnosed by ELISA and antiserum to the virus is available. Jensen et al. (1996) observed that simultaneous infection of plants by WSMV and HPV produced symptom expression much too severe for WSMV alone. In retrospect, it would have been useful to test plants in 1999 for the presence of a second pathogen. Since antibodies for HPV are available, this would have been easily tested in the 1999 field trial.

At 40 days post inoculation, virus titers of *Wsm1* entries were higher than titers obtained at 20 days post inoculation. This was not expected in resistant lines. A review of available literature regarding the temperature sensitivity of *Wsm1* derived resistance revealed that resistance is effective at 20°C but not at 25°C (Siefers et al., 1995). In their study, CI 17884 was used as a parent in a randomly mated, recurrently selected population. Progeny were symptom free and negative in ELISA at 20°C, but were positive in ELISA at 27°C. All lines that developed WSMV symptoms and titers carried only the short arm of the *T. intermedium* chromosome 4Ai-2. Progeny lines that remained resistant at 27°C had been derived from crosses involving CI 15092 that has the short arm of 4Ai-2 in addition to the long arm of 4Ai-2. Genes on the long arm allow effective resistance to WSMV at the higher temperature. The source of resistance to WSMV used in this study is from CI 17884, thus temperature sensitivity could be a contributing factor to virus accumulation and yield loss in *Wsm1* entries.

Heating interferes with the resistance mechanisms (Pfannenstiel and Niblett, 1978) so that symptoms and positive virus titers occur at elevated temperatures. Under field conditions, plants may remain resistant when exposed to high daytime temperatures because long, cool nights interrupt the heating cycle.

The average temperature during grain fill in July of 1999 and 2000 was near 20°C and reached 36°C on July 29, 1999 and July 31, 2000. Increased temperatures may have contributed to high virus titers at 40 days post

inoculation since leaf samples were collected for ELISA on July 14, 1999 and July 24, 2000. Virus accumulation occurring in plants during the grain fill period may have reduced yields of the inoculated *Wsm1* lines however, the delay of symptom onset significantly decreased the loss due to WSMV.

Siefers et al. (1995) concluded that the resistance to WSMV conferred by the translocated segment of the short arm of chromosome 4Ai-2 is temperature sensitive, but stable enough to provide protection from WSMV under field conditions. Although our study supports this conclusion, our data indicates that *Wsm1* lines may incur some yield loss. This loss in yield however, is significantly less than the reduction in yield of susceptible lines.

Yields of *Wsm1* lines in the absence of disease were 21 percent less than yields of near isogenic lines not having the *Wsm1* gene (Table 13). Advanced *Wsm1* breeding lines that have comparable yields (Lanning, *personal communication*) have been developed and provide an attractive alternative for resistance to WSMV for Montana producers.

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