EVALUATION OF SUSCEPTIBILITY TO WHEAT STREAK MOSAIC VIRUS AMONG SMALL GRAINS AND ALTERNATIVE HOSTS IN THE GREAT PLAINS

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TABLE OF CONTENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES-CONTINUED

Table Page

LIST OF TABLES-CONTINUED

Table Page

LIST OF TABLES-CONTINUED

Table Page

ABSTRACT

Wheat streak mosaic virus (WSMV), endemic in small grains production areas of the Great Plains, causes yield losses of wheat 2 to 5% annually. Yield loss in individual fields can reach 100%. Control relies on cultural practices to control the vector, the wheat curl mite (*Aceria tosichella* Keifer, WCM), and the use of resistant or tolerant varieties. WSMV and WCM depend on living tissue for survival and reproduction, including common grassy weeds. Little is known about the relative importance of these weeds as alternative hosts of WSMV. The purpose of these studies was to evaluate the risk of infection with WSMV in commonly grown wheat varieties and various grassy weed species, information useful to understanding WSMV epidemiology and control. Winter wheat, spring wheat and barley varieties in Montana were evaluated in the field by measuring the effect of fall vs. spring inoculation and variety on incidence, symptom severity, and yield components. Winter wheat varieties from five states, and spring wheat and barley varieties from Montana were tested for incidence and absorbance in greenhouse. Fall-inoculated winter wheat had less effect of WSMV inoculation compared to spring-inoculated winter wheat. Yields of spring wheat varieties were largely reduced by WSMV inoculation. There was no correlation between yield and incidence or symptom severity. In greenhouse studies, the highest incidence was observed in varieties from Idaho and Nebraska, whereas the highest relative absorbance was observed in varieties from Montana. In 2008 and 2009, surveys of common grassy weeds were conducted. Grass species from croplands in six states were selected and mechanically inoculated to determine the susceptibility to WSMV. Grassy weeds were also evaluated as a source of WSMV by measuring transmission efficiency with virulifeous WCM. *Bromus tectorum* was the most prevalent grassy weed and the most frequent viral host. *Aegilops cylindrica*, and *Avena fatua* had the highest incidence and relative absorbance. There were no differences in the susceptibility of grass species to WSMV by their state of origin. WCM transmission study indicated infected grass species had lower transmission efficiency than from infected wheat. These studies will benefit producers in Montana to assess their risk of WSMV based on variety selection and the presence of grassy weeds.

INTRODUCTION

Biology and Agricultural Importance of Wheat Streak Mosaic Virus

Wheat streak mosaic virus (WSMV) is a widely prevalent and important virus in small grain production systems worldwide (Wiese, 1987). WSMV is a type member of the genus *Tritimovirus* which belongs to the plant virus family *Potyviridae*. The virus is described as non-enveloped, filamentous, 700 nm long (Brakke, 1971), approximately 8500 nucleotides, monopartite, and composed of positive sense single stranded RNA (ssRNA). The viral genomic RNA functions as messenger RNA for translation by host ribosomes (Brakke, 1971). The viral genome is translated into one large, single protein (polyprotein) that self-cleaves into functional proteins including viral RNA-dependent RNA polymerase, coat protein, and the multifunctional helper component-protease (HC-Pro; Stenger *et al*, 1998). HC-Pro of *Potyvirus*, the largest genus in family *Potyviridae*, has been shown to mediate attachment of virus particles to the vector stylet and is involved in disease synergism with unrelated viruses during co-infections and suppression of post transcriptional gene silencing (Maia *et al*, 1996; Stenger *et al*, 2007). The HC-Pro of WSMV is required for vector transmission but dispensable for suppression of host defense mechanisms (Stenger *et al*, 2007).

The 'wheat streak mosaic (WSM) complex,' which includes WSMV, *Wheat mosaic virus* (WMoV, formerly called *High Plains virus*, HPV), and *Triticum mosaic virus* (TriMV), causes annual yield losses of approximately 2 to 5 % in the Great Plains region (Appel *et al*, 2007; Shahawan and Hill, 1984; Wiese, 1987). According to Appel *et*

al (2007), over the last two decades WSM has been the second most important wheat disease after leaf rust in terms of yield loss in Kansas. In Montana, statewide outbreaks of WSM have been recorded in 1964, 1981, 1993, and 1994 (Bamford *et al*, 1996 and Burrows *et al*, 2009).

Yield loss in individual fields due to WSM can be severe - near 100% (McNeil *et al*, 1996; Riesselman, 1993) - if early infection occurs on susceptible varieties. Depending on the variety, 25 to 80% yield loss has been recorded in Montana (Riesselman and Carlson, 1994). Shawan and Hill (1984) reported yield loss of 50.2 to 91.4% in winter wheat varieties in Colorado. Yield loss on spring wheat varieties due to WSMV infection in North Dakota was estimated at 31.9 to 98.7% (Edwards and McMullen, 1988). WSMV is consistently present in north central Montana (Golden Triangle) where approximately 40% of all wheat, primarily winter wheat, in Montana is produced, (USDA-NASS, 2009). The disease can be severe in the northeastern portion of the state that grows primarily spring and durum wheat.

Symptoms of WSM

WSM symptoms are characterized by a yellow leaf streaking or stippled pattern and stunting, head sterility, low test weights, and poor tillering (Murray *et al*, 2005). Early infection can be confused with nutrient deficiency, drought, root rots, and early stages of fungal foliar diseases. Symptoms commonly appear first on the edge of the field as the virus and vector, the wheat curl mite (WCM, *Aceria tosichella* Keifer), move into the crop from ditches and infected crops that are adjacent or upwind. In general, early fall

infection by WSMV in winter wheat causes the most significant damage to yield and provides the greatest amount of inoculum for spread in the spring (McMullen and Waldstein, 2010; Hunger, 2004; Thomas and Hein, 2003). Fall infection is hard to diagnose because the symptoms may not be observed until temperatures warm up in the spring.

Movement of WSMV

WSMV has three mechanisms to move from plant to plant: mechanical, seed, and the wheat curl mite vector. Mechanical transmission of the virus occurs when viruliferous plant sap enters a susceptible host through damage caused by human activities such as moving equipment, by herbivores, or from plants rubbing together. This pathway is not considered the primary mechanism of virus spread in nature; however, it is routinely performed in the lab to establish, transmit, and maintain virus cultures. Seed transmission was reported at 0.1% in maize (Hill *et al*, 1974) and 0.5 to 1.5 percent in wheat (Jones *et al*, 2005). Although seed transmission serves as a way for the virus to travel into new production areas, wheat varieties in the United States have not yet been tested for their seed transmission ability of the WSMV. Seed certification for absence of WSMV is currently required for exporting wheat and/or corn seed to Argentina, Australia, New Zealand, Taiwan, and Korea (*Julie Klapp, APHIS-PPQ, personal communication January 22, 2010*).

The primary transmission mechanism for WSMV is by the WCM, an eriophyid mite less than 0.3 mm in length with two pairs of legs (Manson and Oldfield, 1996;

Murray *et al*, 2005). The WCM prefers to feed on the upper leaf surface adjacent to the leaf sheath. Heavy infestations cause the leaf margin to curl inwards. Female eriophyid mites typically exist as deutogynes (diapausing, migratory forms) and protogyne (reproductive forms), which slightly differ in morphology (Manson and Oldfield, 1996). WCM produce larger, more robust forms than normal in response to favorable temperatures, environmental conditions and food supply (Manson and Oldfield, 1996; Somsen, 1996). WCM can overwinter at any stage of their life cycle inside the curled leaf and become active and reproduce during warm days in mid-winter. An adult WCM can survive winter temperatures of -15ºC to -20ºC for 2 days, and eggs can survive up to 8 days (Somsen and Sill, 1970). WCM can survive in sub-freezing temperatures for 3 months (Somsen and Sill, 1970). After the eggs hatch, mites pass through two developmental stages to become adults (Manson and Oldfield, 1996).

The typical life span of the mites is 8 to 10 days (Somsen and Sill, 1970). Mites lay 12 to 20 eggs (Del Rosario and Sill, 1958; Somsen and Sill, 1970), which leads to rapid increase of the mite populations. Mites do not physically mate with each other; males leave sacs called spermatophores on the leaf surface to fertilize eggs as females walk around. Females reared in the absence of males produce males by arrhenotoky (Oldfield and Michalska, 1996). Mites migrate up the plant to the youngest leaves as their population increases. As the plant matures, the mites move to glumes and are carried by wind currents to a new host (Jiang, 2005). If hail or another seed-dehiscence event occurs near grain maturity, mites can move onto the resulting volunteer crop (Gibson and Painter, 1956; Staples and Allington, 1956).

WSMV and the WCM are both dependent on living plants for survival (Somsen and Hill, 1970). This 'green bridge' occurs when susceptible hosts are present in or near a field between harvesting of one crop and the planting of the next crop. The 'green bridge' can occur due to delayed fall harvests, lack of adequate time for volunteer and grassy weed control between harvest and planting, and the desire to plant crops early to obtain good yield, adequate growth before winter, and minimum heat stress from hot weather (Matz, 1991).

WSMV is thought to be transmitted by WCM in a semi-persistent manner (Stenger *et al*, 2005), although recent data suggests it may be circulative (G. Hein, *personal communication*; Siriwetwiawat, 2006). Semi-persistent transmission is one of several types of plant virus transmission mechanisms. The others include non-persistent, circulative (or persistent) -nonpropagative, and circulative-propagative. Non-persistent transmission is also referred to as 'stylet-borne' since the virus particles attach to mouthparts of an arthropod vector (Slykhuis, 1955). Acquisition is rapid (seconds to minutes), and retention is short (minutes to hours). With circulative transmission, the virus must be transported through the gut and haemolymph of the vector to the salivary glands. Vectors must feed on the plant for more extended periods (minutes to hours) to acquire the virus and the ability to transmit is retained for hours to days, even the life of the insect. Propagative viruses replicate within the insect, while nonpropagative viruses do not. Semi-persistent transmission can be described as intermediate between non-persistent and circulative transmission. The virus is acquired in minutes to hours, while retention time is days to the next molt (Ng and Falk, 2006). Semi-persistent viruses

are associated with the foregut of the vector and tend to increase in transmission efficiency as vector acquisition feeding time increases (Gray and Banerjee, 1999). In the case of WSMV, WCM can be viruliferous at all stages except the egg, with a minimum of 10 to 15 minutes of feeding on infected plant material. Adults may acquire the virus within the foregut and gut, but will not transmit the virus to the egg. Transmission efficiency is high (84 to 92%) if the mites acquire the virus from wheat (Sill and del Rosario, 1959; Staples and Allington, 1956). The mites remain viruliferous for up to 21 days (Del Rosario and Sill, 1958).

Alternative Hosts of WSMV

Grassy weed hosts of WSMV and the WCM are prevalent in the Great Plains region and include wild oat (*Avena fatua*), cheat (*Bromus secalinus*), downy brome (*Bromus tectorum*), jointed goat grass (*Aegilops cylindrica*), green foxtail (*Setaria viridis*), and Persian darnel (*Lolium persicum*) (Brey *et al*, 1998; Connin, 1956; Somsen and Sill, 1970; Townsend and Johnson, 1996). It is believed that neither WSMV nor WCM survive on broadleaf plants (Burrows *et al*, 2009; Somsen and Sill, 1970). Incidence of grassy weeds including volunteer crops has increased in the Great Plains for the last couple of decades due to the following: widespread changes in crop management practices including reduced tillage associated with continuous cereal planting (Anderson, 2005; Blackshaw, 1994; Dao, 1987; Douglas *et al*, 1990; Wicks, 1984); increased population of herbicide-resistant weed biotypes (Heap, 2005); control of broadleaf weeds with in-crop herbicide (Derksen *et al*, 2002); use of semi-dwarf cultivars (Challaiah *et al*,

1986; O'Donovan *et al*, 2000); and broadcast application of fertilizer, such as nitrogen, phosphorus, or both (Anderson, 1991; Thill *et al*, 1984; Wicks, 1984). Grassy weeds can be important in the maintenance of the 'green bridge' due to their abundance in wheat fields in the Great Plains region. Because winter annual grasses such as *Bromus* species and jointed goat grass have life cycles similar to winter wheat, it is believed they do not play an important role for bridging WSMV and WCM during the period between harvest and germination of wheat in the following crop season. Summer annual grasses such as wild oat and green foxtail and volunteer wheat are more likely candidates to be 'green bridge' hosts during such period.

Emergence of New Wheat Viruses

In addition to WSMV, wheat production in the Great Plains is threatened by the newly discovered wheat viruses including *Wheat mosaic virus* (WMoV, formerly known as High Plains virus, HPV) and *Triticum mosaic virus* (TriMV). Both viruses closely match the life cycle of WSMV and are transmitted by WCM. Co-infection of a single plant with multiple wheat viruses enhances symptom severity and potential yield loss (Mahmood *et al*, 1998; Stenger *et al*, 2007; Tatineni *et al*, 2010).

WMoV was identified in Colorado, Idaho, Kansas and Texas in 1993 and 1994 first in corn, then in winter wheat (Jensen *et al*, 1996). Host range studies of WMoV in Kansas showed grassy weed hosts of WMoV closely matched WSMV, such as cheat, oat (*Avena sativa*), rye (*Secale cereale*), and green foxtail (Seifers *et al*, 1998). TriMV was first isolated from a WSMV-resistant wheat variety in Kansas. The amino acid sequence

of TriMV is approximately 15 to 30% different from other *Potyviridae* including WSMV and is most closely related to *Sugarcane mosaic virus* (49 to 65% similarity; Tatineni *et al*, 2009). It is highly likely that grassy weed species are involved in the disease cycle of TriMV because its vector is the WCM and several grasses can serve as alternative hosts of TriMV (Table 1.1).

Table 1.1. Comparison of experimental host ranges of *Wheat streak mosaic virus* (WSMV), *Wheat mosaic virus* (WMoV), and *Triticum mosaic virus* (TriMV) on common grass species.

		Susceptibility[xyz]		
Scientific name	Common name	WSMV	WMoV	TriMV
Aegilops cylindrica	Jointed goat grass	S	N/A	+
Avena fatua	Wild oat	S	-	+
Bromus inermis	Smooth brome	I	-	-
B. japonicus	Japanese brome	S	-	N/A
B. secalinus	Rye brome (Cheat)	S	+	+
B. tectorum	Downy brome	S	-	+
Dactylis glomerata	Orchard grass	I	+	-
Echinochloa crusgalli	Barnyardgrass	S	-	-
Elymus repens	Quackgrass	I	N/A	-
Eragrostis cilianensis	Stinkgrass	S	-	-
Lolium perenne ssp. perenne	Perennial ryegrass	I	-	-
Poa pratensis	Kentucky bluegrass	I	-	-
Setaria glauca	Yellow foxtail	I	+	-
S. viridis	Green foxtail	S	-	+
Thinopyrum intermedium	Intermediate wheatgrass	I	N/A	-

[x] Data from Somsen, 1970; Seifers, 1998; and Seifers, 2010.
[y] S = susceptible and I = immune to mechanical inoculation.
[z] Susceptibility of each grass species to WMoV and TriMV is described as: + = infection from either mechanical inoculation (TriMV) or mite transmission (WMoV); - = not able to infect by mechanical (TriMV) or mite (WMoV) transmission. Species not tested for are marked as N/A, not applicable.

Summary and Project Goals

Currently, control methods for mite-transmitted wheat viruses rely on cultural practices including: altering planting date and destroying the 'green bridge' with herbicide application and tillage 2 to 3 weeks before planting a new cereal crop.Varieties resistant or tolerant to WSMV have been developed in states outside Montana by introducing the coat protein gene of WSMV or the *Wsm-1* gene derived from *Thinopyrum intermedium* (Sivamani *et al*, 2002; Tatineni *et al*, 2010). Poor replication of WSMV and TriMV was observed in Mace, a variety with *Wsm-1* released in Nebraska in 2007 (Graybosch, *et al*, 2009). Resistance to WSMV in 'Mace' is remarkable especially in cool environments (Tatineni *et al*, 2010). There are no widely planted varieties that are resistant to both WSMV and WCM. Additionally, these two new wheat viruses have the ability to infect WSMV-resistant varieties. The goals of this project were 1) to evaluate tolerance to WSMV in widely planted winter and spring wheat varieties in Montana and the Great Plain states, and 2) to determine the risk associated with grassy weed genotypes as sources of the virus and the wheat curl mite.

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EVALUATION OF WIDELY PLANTED WHEAT VARIETIES IN MONTANA AND THE GREAT PLAINS FOR SUSCEPTIBILITY TO WHEAT STREAK MOSAIC VIRUS

Abstract

Winter wheat, spring wheat and barley varieties widely planted in Montana and the Great Plains were evaluated in the field and greenhouse for their tolerance to a single *Wheat streak mosaic virus* (WSMV) isolate. Field trials measured the effects of inoculation timing and variety on disease incidence, disease severity and yield components. Fall inoculation of winter wheat was less efficient (average 6.1%) than spring inoculation (average 55.1%) as WSMV percent incidence measured by indirect enzyme linked immunosorbant assay (ELISA). Spring wheat varieties averaged 39.1% incidence of WSMV with greater yield due to WSMV (1701 kg/ha) than spring-inoculated (1161 kg/ha) or fall-inoculated (307 kg/ha) winter wheat. No winter or spring wheat variety was resistant to WSMV as measured by viral incidence, symptom severity, yield or seed quality. Barley varieties were resistant to WSMV mechanical inoculation (6.3% incidence) and yield loss (981 kg/ha). Total twenty two winter wheat varieties from five Great Plain states (Idaho, Montana, Nebraska, Oklahoma, and Texas) and three varieties each of spring wheat and barley from Montana were tested for susceptibility to WSMV in the greenhouse as measured by incidence and absorbance, which we assumed to represent the virus titer relative to a susceptible spring wheat variety. Among the winter wheat varieties, the highest viral incidence was observed in the varieties from Idaho (98.4%) and Nebraska (93.2%), while the highest relative

absorbance were observed in the varieties from Montana (86.5%). Varieties widely planted in Texas had relatively low viral incidence (71.2%) and relative absorbance (??). This study indicates that variety susceptibility as measured by yield cannot be predicted by visual symptoms or viral incidence, and varieties widely planted across the Great Plains vary in their susceptibility to a strain of WSMV from Montana.

Introduction

Wheat streak mosaic virus (WSMV) is widely prevalent in the small grain production system of the Great Plains region of the United States as well as worldwide (Appel *et al*, 2007; Burrows *et al*, 2009a; Wiese, 1987). Annual yield loss of wheat due to WSMV has been estimated at 2.0 to 5.0% in the Great Plains (Baley *et al*, 2001; Riesselman and Carlson, 1994; Wiese, 1987). Yield loss within individual fields can reach 100% (Edwards and McMullen, 1988; McNeil *et al*, 1996; Riesselman, 1993; Riesselman and Carlson, 1994; Shahwan and Hill, 1984). Yield losses from outbreaks of WSMV in Montana in 1993 were estimated at $12.7 million (Baley *et al*, 2001) and in 1995 at $35.0 million (Suszkiw, 2000). WSMV reduces yield, seed test weight, and milling quality (Finney and Sill, 1963; Murray *et al*, 2005).

Damage to winter wheat (*Triticum aestivum*) by WSMV is generally regarded to be most severe when infection occurs in the early fall and is associated with early planting or infected volunteer wheat (Young, 1998). Symptoms are generally not seen until spring when temperatures reach 20°C for 10 days (M. Langham, *personal communication*). Infection of plants with WSMV occurs when viruliferous wheat curl

mites (*Aceria tosichella* Keifer, WCM) move from volunteer wheat or grassy weeds into the crop.

There are no effective pesticides for management of WSMV or the WCM. The primary management for WSM is cultural and includes eliminating the 'green bridge,' altering planting date to avoid high mite activity, and using resistant varieties (Slykhuis *et al*, 1957; Somsen and Sill, 1970; Staples and Allington, 1956). Use of resistant varieties is generally recommended for control of WSMV especially where viral disease epidemics are frequent (Baley *et al*, 2001; De Wolf and Sloderbeck, 2009; Graybosch *et al*, 2009), but tolerance to WSMV varies by variety and the viral strain (Ogg and Anderson, 2009). In Montana, many varieties previously determined tolerant to WSMV were released before the early 1990's (WTARC, 2009a, 2009b; Young, 1998), and only a few of these varieties are still widely planted (Houska and Stringer, 2007, 2008; Ogg and Anderson, 2009). The *Wsm1* gene, from intermediate wheatgrass (*Thinopyrum intermedium*), has been developed as a major source of resistance to WSMV and possibly to *Triticum mosaic virus* (TriMV) (Friebe *et al*, 1996, 2009). Mace, which carries this gene, was recently released in Nebraska (Graybosch *et al*, 2009).

There is a need to provide growers facing high risk of disease with options for WSMV management. This study evaluated 1) field performance of widely planted winter wheat, spring wheat and barley varieties in Montana and 2) greenhouse performance of widely planted winter wheat varieties from five Great Plains states for WSMV.

Materials and methods

Source of Virus

Symptomatic winter and spring wheat samples were collected in 2007 from Conrad, Montana. An isolate of WSMV obtained from these plants (designated 'Conrad-I') was mechanically transferred to and maintained in a susceptible spring wheat 'Choteau'. The greenhouse was maintained with a 16h day: 8h night photoperiod and temperatures of 24±4°C (day) / 18±4°C (night) in the Plant Growth Center, Montana State University, Bozeman, Montana. The planting media used was 1:1 ratio of MSU mix (a 1:1:1 mix of mineral soil, Canadian sphagnum peat moss, Aquagro 2000G [Aquatrols, Paulsboro, NJ] and washed concrete sand steam pasteurized at 80°C for 45 min) and Sunshine Mix #2 Basic (Sun Gro Horticulture, Vancouver, BC, Canada). The isolate was periodically renewed from lyophilized stocks of the initial mechanical transmission, which were stored at -80°C.

For mechanical inoculation, symptomatic leaves were ground in a sample extraction bag (Agdia, Inc., Elkhart, IN) at 1:10 (weight (g) : volume (ml)) ratio with phosphate buffered saline (PBS, 136.9 mM sodium chloride (NaCl), 8.1 mM sodium phosphate dibasic (Na_2HPO_4), 1.5 mM of potassium phosphate monobasic (KH_2PO_4), and 2.9 mM of potassium chloride (KCl), pH 7.2). Approximately 1% (wt:vol) carborundum (fine, 320 grit, Alfa Aesar, Ward Hill, MA) was added as an abrasive. Wheat leaves were rubbed gently two or three times between gloved fingers dipped into the inoculum.

Identification of Virus

WSMV strain Conrad-I coat protein was sequenced by reverse transcription polymerase chain reaction (RT-PCR), and the sequence was deposited in GenBank as accession number HM535796. RNA was extracted from 50 mg of symptomatic leaves using the TRI reagent RT (Molecular Research Center, Cincinnati, OH) and re-suspended in 30 µl of DEPC-water according to manufacturer's instructions. The extracted RNA was reverse transcribed using SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA) with a gene specific reverse primer, C8908 (Myslik and Nassuth, 2001), according to manufacturer's instructions. The PCR reaction mixture consisted of 1X GoTaq Master mix (Promega Corporation, Madison, WI), 1.25 µM forward primer H8369 (Myslik and Nassuth, 2001), 1.25 µM reverse primer C8908, 1 µl of cDNA, and water for a final volume of 25 µl. PCR conditions were modified from Myslik and Nassuth (2001). Denaturation occurred at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 56°C for 30 sec, and extension at 72°C for 60 sec with a final extension at 72°C for 5 min (Myslik and Nassuth, 2001). The PCR amplification was performed by an iCycler Thermal Cycler (Bio-Rad Laboratories Ltd, Hemel Hempsted, UK). The product of PCR amplification (540 bp) was confirmed by gel electrophoresis with a 2% agarose gel containing ethidium bromide (1.5%). PCR fragments were extracted with a QIAquick Gel Extraction kit (Qiagen, Inc., Valencia CA) following manufacturer's instructions, and sequenced using the primers described above by Functional BioSciences (Madison, WI). Coat protein regions were compared to sequence collections in GenBank (National Center for Biotechnology Information (NCBI)) using

BLAST algorithm blastn. The closest similarity sequences include *Wheat streak mosaic virus* strain Sidney 81 (AF057533; E-value = 0.0; French and Stenger, 2005; Stenger *et al*, 1998).

Detection and Semi-Quantification of
Virus by Enzyme-Linked Immunosorbant Assay

Enzyme linked immunosorbant assay (ELISA) was used for virus detection and quantification. Anti-WSMV antiserum was purchased from the American Type Culture Collection (ATCC, Manassas, VA) and used at a working dilution of 1:3200 according to manufacturer's instructions. Leaf samples were weighed, and 0.05 to 0.1g of tissue was placed in a 10.2 X 15.2 cm plastic bag (Fisher Scientific, Pittsburgh, PA). A corresponding amount of 1X PBS was added to make a 1:10 dilution (wt:vol) and the sample was mechanically ground.

One hundred microliters of extracted sap were placed in each well of a 96-well BD Falcon micro titer plate (Fisher Scientific, Pittsburgh, PA) pre-coated with 100 µl of 1X carbonate buffer (0.05M sodium carbonate) for 2 h. Samples were loaded in duplicate or triplicate with 1X PBS as a background control, virus-free wheat samples maintained in the greenhouse as a negative control, and wheat inoculated with WSMV Conrad-I 2 weeks prior to the assay as a positive control. Overnight incubation at 4°C was followed by seven cycles of rinsing with 5 sec soaks with 200 µl of 1X PBST (1X PBS with 0.5% Tween 20, Agdia, Elkhart, IN) in a 96-well PW^{TM} microplate washer (Tecan, Durham, NC). WSMV antiserum diluted in ECI buffer (Agdia, Elkhart, IN) was added and the plate was incubated for 2 h in a sealed plastic bag at room temperature. The plate was

washed as described above, and 100 µl of goat anti-rabbit IgG alkaline phophatase conjugate (Sigma, St. Louis, MO) at 1:30,000 dilution (5 µg/ml) in ECI buffer was added to each well. After the 2 h incubation and the final wash, 100 µl p-nitrophenol (PNP) substrate (Agdia, Elkhart, IN) (Agdia, Elkhart, IN) in 1X PNP buffer (0.5mM of magnesium chloride hexahydrate ($MgCl_2(H_2O)_6$) and 1.0 M diethanolamine ($C_4H_{11}NO_2$) in 1000 ml of water, pH 9.8; Agdia, Elkhart, IN) was added to the wells. The plate was incubated for 1 h in the dark and absorbance (A_{405}) quantified using a microplate reader (SpectraMax Plus, Molecular Devices, Sunnywyle, CA). Absorbance values at least twice the value of the negative control were considered positive as performed by past study (Christian and Willis, 1993). Absorbance values of wheat varieties were translated to a value relative to the positive control in the same 96-well plate as described in the subsequent section.

Field Studies: Evaluation of Popular
Wheat Varieties in Montana to WSMV Infection

Winter wheat varieties widely grown in the Great Plains (Houska and Stringer, 2008) were planted at the Arthur H. Post Agronomy Research Farm, Bozeman, MT (Amsterdam-Quagle silt loam, pH7.5). Plots used for winter wheat were fallow the previous growing season, and maintained according to standard practices. Plots of winter wheat were planted at the rate of 60 seeds per m^2 on 24 Sep, 2008. Each plot consisted of four rows 6.0 m long and 0.3 m apart. Winter wheat varieties included ‘Genou’, ‘CDC Falcon’, ‘Rampart’, ‘Neeley’, ‘Ledger’, ‘Morgan’, ‘Jagalene’, ‘Tiber’, ‘Rocky’, ‘Pryor’, ‘Yellowstone’, and ‘MTV0734’, a breeding line heterozygous for *Wsm1*.

The experiment was arranged as a factorial design with four replications. The main effect was variety, which was fully randomized in each of four blocks. Subplots consisted of inoculation (not inoculated, fall inoculation, spring inoculation, and buffer inoculation) which were not randomized for simplicity of applying the treatments. The buffer inoculation consisted of PBS and carborundum. Results from the measured variables did not differ between no inoculation and buffer inoculation; therefore, the buffer inoculated data is excluded from the analysis.

Inoculum consisted of a 1:10 dilution sap from ground tissue of susceptible spring wheat 'Choteau' inoculated 2 weeks prior with WSMV strain Conrad-I in the greenhouse. Approximately 200 seeds of 'Choteau' were planted in 30.5 x 45.7 cm flats 2 to 3 cm deep in the planting media described above. Plants were mechanically inoculated with a 1:10 dilution (wt:vol) of WSMV inoculum at growth stage Feekes 2 to 3. Each plant was inoculated at least twice to ensure 100% infection and the majority of plants in each flat showed symptoms of severe leaf streaking when harvested for inoculation.

A high-pressure Husky siphon feed spray gun (HDS75000AV, Home Depot, Inc., Atlanta, GA) connected to air compressor (B335B-335TV, CompAir, Sidney, OH) was used to inoculate plants with WSMV. WSMV inoculum was prepared as described above with 0.5% carborundum sprayed at 80 psi at the rate of approximately 500 ml of inoculum per 6 m of row. We developed a jig with either polymerized vinyl chloride (PVC) pipe or two-by-four pieces of wood that helped to maintain a distance of approximately 20 cm between the spray gun nozzle and the wheat plant. Fall inoculation occurred on 29 October, 2008 and spring inoculation on 18 May, 2009. The plots were

regularly scouted for wheat curl mites with a pocket microscope (Carson, Hauppauge, NY) or 10X hand lens. No wheat curl mites were noted in the plots at any time during these experiments, and no WSMV symptoms were observed in the control rows.

Spring wheat and barley WSMV trials were established at the same location as the winter wheat trial described above. Plots were fertilized with nitrogen at a rate of 2.6 lb/bu x 100bu/a (J. Berg, *personal communication*). The experiment was designed as a 2-factor experiment with four replications. Each plot consisted of two rows. Variety plots (main plots) were planted 16 May 2009 in a completely randomized design in four blocks. Inoculations (subplots) were not randomized for simplicity of inoculation. The spring wheat varieties included 'Amidon', 'Choteau', 'Conan', 'Corbin', 'Ernest', 'Fortuna', 'Hank', 'McNeal', 'Reeder', and 'Scholar'. Barley varieties were 'Haxby' and 'Metcalfe'. Mechanical inoculation with WSMV occurred as described above on 9 June, 2009.

Disease Severity Assessments

Disease severity was rated in each row of winter wheat on 22 June, 2009 and in spring wheat plots on 24 July, 2009. The severity scale was 1 to 5: 1 = no symptoms, 2 = light, mild symptoms (light streak), 3 = light to moderate symptoms (definite yellow streak), 4 = moderate to severe symptoms (heavy streak), 5 = severe yellowing and streak (Seifers *et al*, 2006). These symptoms were then transformed into a disease severity scale (1 to 25) with the formula:

$$\text{Disease severity scale} = \sum[(1*n_1)+(2*n_2)+(3*n_3)+(4*n_4)+(5*n_5)]/n_t, \text{ (eq. 1)}$$

where n_x is the number of plots with the designated disease severity and n_t is the total number of observations.

Thirty flag leaves were randomly sampled from the center 1.0 m of each row on 25 June, 2009. Spring wheat plots were sampled for viral incidence on 19 July, 2009. Percent viral incidence was calculated for each plot using the number of WSMV ELISA-positive plants divided by the number of plants sampled.

Agronomic Variables

Height was measured on 10 randomly selected plants in the center 1.0 m of each row on 24 August, 2009. Heights were measured from the soil to the base of the head of the main tiller. No stunting due to WSMV was observed in the winter wheat plots so data were not taken.

Winter wheat plots were harvested on 27 August, 2009. Spring wheat plots were harvested on 18 September, 2009. Single rows were cut with a Suzue harvest-binder, EN25L-2 (Suzue Manufacturing, Japan). Wheat bundles were allowed to dry for 1 to 2 days before thrashing with a Vogel thrasher (custom built by Bill's Welding, Pullman, WA). Yield was adjusted to 13% moisture. Thousand kernel weight was measured after counting seeds with a 850-2 Electric Counter (The Old Mill Company, Savage, MD). Protein and moisture content measurements were performed by the Cereal Quality Lab at Montana State University, Bozeman according to standard practices.

Greenhouse Studies:
Susceptibility of Widely Planted Wheat
Varieties from Five Great Plains States to WSMV

Greenhouse experiments were conducted to determine variation in susceptibility of popular wheat varieties from the Great Plains represented by incidence and measurable

relative absorbance. Two experiments for incidence and relative absorbance each were conducted a total of six times. Each replication for incidence contained one pot and for relative absorbance contained two pots.

For the experiment of the viral incidence, three to six popular varieties were selected from five states in the Great Plains: Idaho, Montana, Nebraska, Oklahoma, and Texas (Table 2.1). Three popular spring wheat and barley varieties in Montana were also selected for analysis of incidence and relative absorbance. The WSMV-resistant winter wheat cultivar, ‘Mace’ (Graybosch et al, 2009), was included as well. ‘Mace’ was not included in means of percent incidence or relative absorbance of variety pooled by state of origin because it was not yet widely planted throughout the region when we selected the variety for our experiments (USDA-NASS, 2009b).

Table 2.1. Wheat varieties used in greenhouse studies of *Wheat streak mosaic virus* (WSMV) incidence and relative severity and their state of origin[y].

Variety	State	Variety	State
Winter wheat		*Winter wheat*	
Brundage	Idaho	Duster	Oklahoma
Promontory	Idaho	Endurance	Oklahoma
Utah 100	Idaho	Fuller	Oklahoma
Westbred 470	Idaho	Jagger	Oklahoma
Weston	Idaho	Moreland	Oklahoma
		OK Bullet	Oklahoma
CDC Falcon	Montana		
Genou	Montana	T105	Texas
Rampart	Montana	T111	Texas
Yellowstone	Montana	T112	Texas
Jagalene	Nebraska		
Mace[z]	Nebraska		
Millenium	Nebraska		
Overland	Nebraska		
Spring wheat		*Barley*	
Choteau	Montana	Harrington	Montana
McNeal	Montana	Haxby	Montana
Reeder	Montana	Metcalfe	Montana

[y] State refers to where the variety is widely planted or the state of origin in the case of 'Mace'.
[z] 'Mace' has the WSMV resistance gene, *Wsm1* (Graybosch *et al*, 2009).

Ten to twelve seeds of each wheat variety were planted 2 cm deep in 18 cm diameter pots filled with the planting medium already described. The pots were arranged randomly in the greenhouse with 16h day: 8h night photoperiod and temperatures of 24±4°C day / 18±4°C night in the greenhouse of the Plant Growth Center, Montana State University, Bozeman, MT. Plants were thinned to ten per pot by removing late-germinated or stunted plants. This insured uniformity in growth stage for mechanical inoculation and to have similar leaf tissue mass at the time of sampling.

Plants were mechanically inoculated by hand-rubbing with infectious sap at

Feekes 2 to 3 growth stage with the procedure described previously. A susceptible spring wheat ‘Choteau’ was included in each experiment as a positive control mechanically inoculated and maintained in the greenhouse, different from the one included as the sample. It was selected because it showed consistently high incidence and strong WSM symptom expression in a preliminary study (data not shown). Buffer- (1X PBS) inoculated ‘Choteau’ was also included in each experiment as negative controls. The youngest fully expanded leaves on eight uniformly-sized plants from each pot were selected 14-days post inoculation and tested for viral incidence experiment.

The collected leaf samples were weighed and ground with 1x PBS at 1:10 dilution (wt;vol). All samples were processed for ELISA as described above with duplicate plates. Individual leaf samples for the disease incidence were loaded over multiple plates due to limited numbers of wells on 96-well plate. Each plate had background controls (1x PBS), negative controls (uninfected ’Choteau’), and the positive control (‘Choteau’ WSMV mechanically inoculated for each experiment).

The experiment for the relative absorbance was performed in a similar manner as described above. A minor change was made on the sampling from each pot. A 2.5 to 5 cm leaf piece from the youngest fully expanded leaves from the plants were sampled separately from the incidence experiment and pooled in a plastic bag. Pooled samples of each variety were tested by ELISA as described above but on the same plate. To normalize the absorbance data, the average absorbance value of the buffer controls was subtracted from the absorbance value for each sample and control. Following the subtraction, the relative absorbance was calculated as an estimate of WSMV titer of the

sample relative to the positive control for each plate and averaged over duplicate plates: Relative absorbance for each plate = (Absorbance of the individual well / absorbance of positive control on the corresponding plate) * 100 (eq.2)

During the experiments, pots containing healthy wheat plants 'Choteau' were placed around the experimental pots to check for spread of the virus by wheat curl mites within the greenhouse. No contamination was observed for any of the experiments included in this study.

Statistical Analysis

Analysis of variance (ANOVA) was used to describe different reactions of each variety to mechanical inoculation of WSMV in terms of symptom severity, incidence, and yield. Additionally, protein content, test weight, and 1000 kernel weight were analyzed by time of the inoculation. Proc GLM in Statistical Analysis System (SAS v.9.2, Cary, NC) was used in the field and greenhouse experiments using replication as the error term. Residuals for experimental data were examined for assumption of normality with the Shapiro-Wilk's test (Shapiro, 1955) and homogeneity of variances by the Levene's test (Levene, 1960). Data were log transformed as necessary although untransformed data are presented in the tables. For field experiments, visual estimates of symptom severity of each variety were rank transformed (Conover and Iman, 1976), and incidence data were log transformed after addition of 1 to convert samples with 0% incidence to be applicable. The yield variables were paired within plots for analysis over variety:

$$\text{Variable}_{\text{paired}} = \text{Variable}_{\text{control}} - \text{Variable}_{\text{inoculated}} \quad \text{(eq.3)}$$

where $\text{variable}_{\text{inoculated}}$ represented the fall or spring inoculated rows respectively in the

winter wheat experiment and the spring inoculated row in the spring wheat experiment. The equation (eq.3) gave a positive paired value for loss. Paired values were multiplied by -1 for use in tables to indicate the negative values of yield loss. The difference between the effects of timing of the inoculation was also analyzed by PROC GLM (SAS v.9.2, Cary, NC) for protein content, test weight, a thousand kernel weight, and raw (unpaired) yield values. However, statistical inference was limited for the timing of inoculation due to the lack of randomization of inoculation within each plot. Percent incidence and paired yield were analyzed using PROC GLM (SAS v.9.2, Cary, NC) to compare among varieties within each inoculation treatment. Entry means for each PROC GLM analysis over variety or inoculation were subsequently compared by Fisher's least significant difference (LSD) at significance level of $P = 0.1$ because of high variance in the field trial

For the greenhouse studies, PROC GLM (SAS v.9.2, Cary, NC) was used to compute ANOVA for percent incidence and relative severity individual wheat varieties and state of origin as two distinct explanatory variables. Both response variables were log transformed as described above. The analysis using the state of origin as explanatory variable was to examine if the varieties from particular state tend to be susceptible or resistant to WSMV. The other analysis using individual variety as explanatory variable was to examine any particular varieties were very susceptible. Mace was not included in the analysis over state of origin because it is recently released, but was included in the analysis over individual variety as a representative of a WSMV-resistant variety. Barley varieties were excluded from the analysis because of very low viral incidence as

described in the following result section. Because the study has multiple experiments, they were designated as an error factor in the analysis. Entry means for each analysis were subsequently compared by Fisher's least significant difference (LSD) at significance level of P = 0.05.

Results

Symptom Severity of Winter and Spring Wheat Varieties Inoculated with WSMV in the Field

Symptoms in fall-inoculated winter wheat varieties were significantly less severe than spring inoculations (P_{2009} <0.01; Table 2.2). Typical WSM symptoms including yellowing and streaking of leaves did not appear in the fall-inoculated plots until late spring (D. Ito, *personal observation*). Spring-inoculated varieties expressed symptoms as mild to moderate streaking on upper leaves.

Table 2.2. Disease severity in Montana winter wheat varieties mechanically inoculated with *Wheat streak mosaic virus* (WSMV) in fall or spring bassed on visual symptoms [xyz].

	Disease severity scale (1-25)				
Variety	Fall	(SE)	Spring		(SE)
CDC Falcon	2.9	0.4	10.8	a	1.0
Genou	2.0	1.0	4.6	cd	0.6
Jagalene	1.8	0.8	5.3	bcd	1.3
Ledgar	3.0	1.2	6.8	b	1.2
Morgan	5.4	0.4	11.9	a	0.6
MTV0734	3.8	1.6	5.9	bc	0.6
Neeley	1.8	0.4	6.5	bc	1.0
Pryor	2.4	0.9	4.0	d	0.0
Rampart	3.5	1.2	14.1	a	1.4
Rocky	1.4	0.4	6.5	b	0.0
Tiber	3.1	0.9	6.8	b	0.8
Yellowstone	1.8	0.4	3.6	d	0.4
Mean	2.7	0.3	7.2		0.5

[x] Symptom severity was rated as 1 = no symptoms to 5 = severe yellowing and streak. Disease severity scale was calculated as described in the materials and methods. Fall represents fall inoculation and spring represents spring inoculation.
[y] Means followed by the same letter are not significantly different at P = 0.10. Data were analyzed by rank transformed ANOVA, and means compared using Fisher's LSD.
[z] SE, standard error.

Symptom severity differed among winter wheat varieties and timing of the inoculation in 2009 (Table 2.2). Symptom severity appeared mild in fall-inoculated plots and varieties did not differ (P = 0.19). In spring-inoculated plots, 'CDC Falcon', 'Morgan', and 'Rampart' were the most symptomatic, and 'Pryor' and 'Yellowstone' were the least symptomatic (P < 0.01).

Spring wheat and barley varieties differed in symptom severity (P < 0.01, Table 2.3). 'Haxby', 'Metcalfe' and 'McNeal' expressed relatively low symptom severity, while Ernest expressed relatively more severe symptoms (Table 2.3). Height was

decreased in spring wheat varieties by WSMV inoculation an average of 4.3 cm (Table 2.4). Little stunting was observed in barley (Table 2.4) or winter wheat (data not shown).

Symptoms of WSM were more severe in spring wheat (Table 2.3) than winter wheat varieties (Table 2.2). Barley showed little to no symptoms of WSMV infection (Table 2.3).

Table 2.3. Disease severity in Montana spring wheat and barley varieties mechanically inoculated with *Wheat streak mosaic virus* (WSMV) [xyz].

	Disease severity scale (1-25)		
Variety			(SE)
Spring wheat			
Amidon	18.8	abc	3.6
Choteau	20.8	abc	1.6
Conan	16.5	abcd	3.0
Corbin	15.6	bcd	1.9
Ernest	21.6	a	1.1
Fortuna	19.4	abc	2.2
Hank	14.5	cd	1.8
Mcneal	8.4	de	3.1
Reeder	17.4	abc	1.9
Scholar	20.5	ab	1.8
Barley			
Haxby	2.1	e	0.7
Metcalfe	2.1	e	0.7
Mean $_{\text{spring wheat}}$	17.4		0.9
Mean $_{\text{barley}}$	2.1		0.5

[x] Symptom severity was visually rated as 1 = no symptoms to 5 = severe yellowing and streak. Disease severity scale was calculated as described in the materials and methods.
[y] Means followed by the same letter are not significantly different at P = 0.10. Means were analyzed by ANOVA over variety after rank and log transformations for each response variable and means were compared using Fisher's LSD.
[z] SE, standard error

Table 2.4. The effect of mechanical inoculation of *Wheat streak mosaic virus* (WSMV) on height (cm) of Montana spring wheat and barley varieties [xyz].

	Height (cm)		Difference (cm)		
Variety	Control	(SE)	Inoculated		(SE)
Spring wheat					
Amidon	84.0	1.5	-8.7	bc	0.5
Choteau	66.6	1.5	-3.9	bc	1.1
Conan	68.9	0.7	-3.0	bc	1.7
Corbin	69.6	1.7	-4.4	bc	0.3
Ernest	81.9	1.1	-11.6	c	1.5
Fortuna	80.8	3.6	-4.7	bc	4.3
Hank	65.5	0.9	12.1	a	9.0
McNeal	72.0	1.1	-6.2	bc	0.5
Reeder	74.6	3.1	-5.7	bc	2.9
Scholar	78.9	1.3	-7.1	bc	2.0
Barley					
Haxby	63.7	2.1	-2.0	b	1.7
Metcalfe	75.9	1.3	-1.7	b	1.6
Mean $_{\text{spring wheat}}$	74.3	1.2	-4.3		1.4
Mean $_{\text{barley}}$	69.8	2.6	-1.9		1.1

[x] Height was measured from soil level to the base of the wheat head of arbitrarily selected plants in the center 1.0 m of each row (n = 20 plants/plot).
[y] Means followed by the same letter are not significantly different at P = 0.10. Data from paired plots were analyzed by ANOVA, and means were compared using Fisher's LSD.
[z] SE, standard error.

Incidence of WSMV in Cereal Varieties

Disease incidence was higher in the spring-inoculated winter wheat plots (mean = 55.1%) than the fall-inoculated plots (mean = 6.1%, Table 2.5). Differences among varieties were observed in fall-inoculated plots (P = 0.09, Table 2.5), but not spring inoculation. 'Morgan' had the highest WSMV incidence, while 'Genou', 'Pryor' and 'Tiber' had the lowest WSM incidence (Table 2.5). No WSM was found in control plots.

Table 2.5. Incidence (%) of Montana winter wheat varieties mechanically inoculated with *Wheat streak mosaic virus* (WSMV)[xyz].

	Incidence (%)				
Variety	Fall		(SE)	Spring	(SE)
CDC Falcon	5.0	bc	2.2	57.3	7.7
Genou	3.4	c	0.0	59.2	6.4
Jagalene	6.7	abc	2.7	58.3	10.2
Ledgar	4.2	bc	1.6	40.8	8.1
Morgan	12.5	a	5.2	66.7	3.0
MTV0734	9.2	abc	2.5	48.3	11.7
Neeley	7.5	abc	1.6	60.0	3.3
Pryor	2.5	c	0.8	54.2	5.7
Rampart	10.2	ab	2.5	64.2	5.5
Rocky	5.0	bc	2.9	46.7	4.9
Tiber	2.5	c	0.8	63.3	7.6
Yellowstone	4.2	bc	1.6	42.1	5.8
Mean	6.1		0.8	55.1	2.2

[x] WSMV incidence was measured as the percentage of ELISA- positive flag leaves (n = 30) in inoculated plots. Fall represents fall inoculation and spring represents spring inoculation.
[y] Means followed by the same letter are not significantly different at P = 0.10. Data were log transformed before ANOVA and means comparison by Fisher's LSD.
[z] SE, standard error.

The average incidence of WSM in spring wheat varieties was 33.6% (Table 2.6). Differences among varieties were observed ($P < 0.01$). 'Amidon', 'Corbin' and 'McNeal' had the highest incidence of WSM, whereas Hank had the lowest incidence among the spring wheat varieties. Barley varieties had a low incidence of WSM (≤ 10.0%).

Table 2.6. Incidence (%) of *Wheat streak mosaic virus* (WSMV) in mechanically inoculated spring wheat and barley varieties in Montana.

	Incidence (%)		
Variety			(SE)
Spring wheat			
Amidon	50.0	a	4.7
Choteau	35.0	bcd	6.7
Conan	31.7	de	5.5
Corbin	49.2	a	9.3
Ernest	29.2	de	3.7
Fortuna	45.8	abc	2.5
Hank	19.2	ef	1.6
Mcneal	51.7	a	4.8
Reeder	32.5	cde	3.4
Scholar	46.7	ab	5.3
Barley			
Haxby	10.0	fg	1.9
Metcalfe	2.5	g	1.6
Mean $_{\text{spring wheat}}$	39.1		2.2
Mean $_{\text{barley}}$	6.3		1.8

[x] WSMV incidence was measured as the percentage of ELISA- positive flag leaves (n = 30) in mechanically inoculated plots.
[y] Means followed by the same letter are not significantly different at P = 0.10. Data were log transformed before ANOVA. Means comparisons were calculated with Fisher's LSD.
[z] SE, standard error.

Effects of WSMV on Agronomic Variables in Winter Wheat

Winter wheat yield was affected by variety and timing of inoculation ($P_{variety} < 0.01$; $P_{inoculation} < 0.01$; Table 2.7, 2.8). Fall inoculation reduced yield an average of 307kg/ha (5.1%) and spring inoculation reduced yield 1161kg/ha (19.1%) compared to control plots. The largest yield losses due to WSMV inoculation were consistently seen in 'Yellowstone' and 'Neeley'. 'Jagalene' and 'Ledger' consistently had the lowest yield losses due to WSMV.

WSMV inoculation had variable effects on seed quality as measured by protein content, test weight, and 1000 kernel weight. Protein content increased approximately 0.3% in spring-inoculated winter wheat ($P < 0.01$; Table 2.8) whereas test weight ($P < 0.01$) and thousand kernel weight ($P < 0.01$) decreased in spring-inoculated plots. Interaction between variety and timing of the inoculation was observed for protein ($P = 0.03$) and test weight ($P < 0.01$)

Table 2.7. The effect of variety and time of *Wheat streak mosaic virus* (WSMV) mechanical inoculation on yield (kg/ha) of winter wheat in Bozeman, Montana[xyz].

	Yield (kg/ha)		Yield compared to control (kg)					
Variety	Control	(SE)	Fall		(SE)	Spring		(SE)
CDC Falcon	5314	246	118	abc	99	-972	bcd	155
Genou	6147	145	116	abc	164	-935	bc	271
Jagalene	4366	454	986	a	734	-6	a	246
Ledger	5468	280	407	ab	239	-449	ab	226
MTV0734	5288	188	-777	cde	98	-1044	bcd	55
Morgan	6065	269	-377	bcd	146	-1394	cd	266
Neeley	7517	183	-1013	de	428	-2622	e	148
Pryor	7304	292	-86	bcd	184	-1071	bcd	186
Rampart	5701	169	-112	bcd	187	-903	bc	194
Rocky	5348	171	-643	cde	135	-1046	bcd	252
Tiber	6363	313	-847	de	491	-1691	cd	740
Yellowstone	7955	527	-1455	e	310	-1797	de	221
Mean	6070	165	-307		126	-1161		119

[x] Control, uninoculated rows; Fall, fall inoculated rows; Spring, spring inoculated rows.
[y] Means within each column followed by same letter are not significantly different from each other at $P = 0.10$. Means were analyzed by ANOVA over variety after yield of control and the inoculated plots were paired (see materials and methods), and compared by Fisher's LSD.
[z] SE, standard error.

Table 2.8. The effect of timing of mechanical inoculation of *Wheat streak mosaic virus* (WSMV) on seed quality in winter wheat[xyz].

	Yield (kg/ha)			Protein (%)			TWT (kg/hL)			TKW (g)		
Inoculation			(SE)			(SE)			(SE)			(SE)
Control	6069.8	a	165.5	13.4	c	0.1	79.7	a	0.1	34.9	b	0.5
Fall	5763.0	b	133.5	13.5	b	0.1	79.7	a	0.2	35.2	ab	0.5
Spring	4909.0	c	121.8	13.7	a	0.1	77.8	b	0.2	33.2	c	0.4

[x] Ctrl, uninoculated rows; Fall, fall inoculated rows; Spring, spring inoculated rows.
[y] Means followed by the same letter within each column are not significantly different at P=0.10. Means were analyzed by ANOVA and means separations calculated using Fisher's LSD.
[z] TWT, test weight; TKW, 1000 kernel weight; SE, standard error

Effects of WSMV on
Agronomic Variables in Spring Wheat and Barley

Yield of spring wheat was reduced by WSMV inoculation an average of 1701kg/ha (41.3%; Table 2.9).Yield losses differed by variety (P = 0.07; Table 2.9), and inoculation reduced yield (P < 0.01; Table 2.10) 'Ernest' lost the greatest amount of yield due to inoculation with WSMV and 'Corbin' the least. Barley varieties 'Haxby' and 'Metcalfe' lost 981kg/ha (15.0%) of their yield due to inoculation with WSMV, lower than most spring wheat varieties.

WSMV had minor effects on seed quality. Test weight and a thousand kernel weight decreased 2.0g/hL (P < 0.01) and 2.5g, respectively, by WSMV inoculation (P < 0.01; Table 2.10). Protein content was increased 0.3% by inoculation (P < 0.01). An interaction between spring wheat variety and timing of the inoculation was observed for test weight (P = 0.06) and a thousand kernel weight (P = 0.09). WSMV inoculation did not alter protein content, test weight, or a thousand kernel weight of the barley varieties.

Table 2.9. Effect of variety and mechanical inoculation of *Wheat streak mosaic virus* (WSMV) on yield (kg/ha) in spring wheat and barley in Montana[yz].

	Yield (kg/ha)		Yield compared to control (kg)		
Variety	Control	(SE)	Inoculated		(SE)
Amidon	4783	600	-1849	bcde	460
Choteau	4035	353	-2096	de	218
Conan	3783	156	-1231	abc	521
Corbin	3779	205	-1204	ab	138
Ernest	4357	309	-2497	e	304
Fortuna	3558	394	-1500	abcd	198
Hank	4151	252	-1179	ab	152
McNeal	4067	552	-2048	cde	443
Reeder	4560	200	-1560	abcd	105
Scholar	4155	468	-1843	bcde	150
Haxby	6619	591	-881	a	637
Metcalfe	6466	237	-1081	ab	391
Mean $_{\text{spring wheat}}$	4123	119	-1701		108
Mean $_{\text{barley}}$	6542	296	-981		348

[y] Means of the varieties followed by same letter are not significantly different from each other at P = 0.10. Varieties were analyzed using ANOVA after yield of control and the inoculated plots were paired, and means comparisons calculated using Fisher's LSD.
[z] SE, standard error.

Table 2.10. The effect of timing of mechanical inoculation of *Wheat streak mosaic virus* (WSMV) on seed quality in spring wheat and barley[xyz].

	Yield (kg/ha)			Protein (%)			TWT (kg/hL)			TKW (g)		
Crop			(SE)			(SE)			(SE)			(SE)
Spring wheat												
Ctrl	4123.0	a	119.1	14.6	b	0.1	78.8	a	0.2	36.7	a	0.5
Inoculated	2422.4	b	112.3	14.9	a	0.1	76.8	b	0.2	34.2	b	0.5
Barley												
Control	6542.3	a	296.2	12.6		0.2	67.7		0.4	40.1		0.7
Inoculated	5561.2	b	277.1	12.5		0.2	67.7		0.4	38.8		1.0

[x] Ctrl, uninoculated rows.
[y] Means within a column and crop type followed by the same letter are not significantly different from each other at P = 0.10. Means were analyzed by ANOVA after yield of control and the inoculated plots were paired, and compared using Fisher's LSD.
[z] TWT, test weight; TKW, thousand kernel weight; SE, standard error.

Incidence and Relative Absorbance of
Winter Wheat Varieties from Five Great Plains States
and Spring Wheat and Barley Varieties from Montana to WSMV

Winter wheat varieties from five Great Plains states were inoculated with a Montana strain of WSMV in the greenhouse to determine how much variation in WSMV susceptibility there is in currently deployed germplasm. As a comparison we included Montana spring wheat varieties, which were known to be susceptible to WSMV and barley varieties which were not susceptible to WSMV by mechanical inoculation.

The average incidence of WSMV in the winter wheat varieties was 83.9% and spring wheat was 100%. The incidence of WSMV varied by both state of origin ($P < 0.01$; Table 2.11) and cereal variety ($P < 0.01$; Table 2.12). The winter wheat varieties can be classified into two groups according to state of origin: high and moderate incidence. Idaho and Nebraska varieties had the highest incidence followed by Oklahoma. The winter wheat varieties from Montana and Texas showed the lowest virus incidence. Spring wheat varieties widely grown in Montana had a very high incidence of WSMV (Table 2.10). Barley varieties had a consistently low incidence of WSMV (Table 2.11). One hundred percent infection was consistently found in ten cereal varieties: ‘Brundage’, ‘Choteau’, ’Duster’, ’Endurance’, ’McNeal’, ’Millennium’, ’Promontory’, ’Reeder’, and ’Utah 100’. Five varieties showed moderate (50 to 70%) incidence: ’Fuller’, ’Rampart’, ’T105’, ’T111’, and ’Yellowstone’. Varieties from each state were generally consistent in their susceptibility to infection with WSMV. However, Oklahoma contained varieties with high, moderate, and low incidences of WSMV (Table 2.12).

Table 2.11. Incidence (%) of mechanically inoculated *Wheat streak mosaic virus* (WSMV) in small grain varieties grouped by state and type of cereal crop[xyz].

State of origin	Incidence (%)		(SE)
Idaho	98.4	a	1.3
Montana (WW)	71.5	c	3.5
Nebraska	93.2	ab	2.5
Oklahoma	83.5	b	4.3
Texas	71.2	c	3.9
Montana (SW)	100	a	0
Montana (Barley)	2.0 (Haxby)		

[x] Percent incidence was measured by ELISA out of eight plants per pot. Total six replications were performed. Number of winter wheat (WW) varieties in each state were: ID = 5; MT = 4; NE = 4; OK = 6; TX = 3. Number of varieties for spring wheat (SW) = 4 and barley = 3.

[y] Means followed by same letter are not significantly different from each other at P = 0.05. Means were log transformed and analyzed by ANOVA. Means were compared using Fisher's LSD.

[z] SE, standard error.

Table 2.12. Incidence (%) of mechanically inoculated *Wheat streak mosaic virus* (WSMV) in major wheat varieties from five Great Plains states.

Variety	State	Incidence (%)[xy]		(SE)[z]	Variety	State	Incidence (%)[xy]		(SE)
Winter wheat					*Winter wheat*				
Brundage	Idaho	100.0	a	0.0	TAM 105	Texas	65.0	ef	8.2
Promontory	Idaho	100.0	a	0.0	TAM 111	Texas	69.0	cdef	5.4
Utah 100	Idaho	100.0	a	0.0	TAM 112	Texas	79.5	abcde	6.3
Westbred 470	Idaho	98.0	a	2.0					
Weston	Idaho	93.8	ab	6.2	*Spring wheat*				
CDC Falcon	Montana	71.0	bcdef	2.5	Choteau	Montana	100.0	a	0.0
Genou	Montana	87.7	abcd	4.6	McNeal	Montana	100.0	a	0.0
Rampart	Montana	66.8	def	6.2	Reeder	Montana	100.0	a	0.0
Yellowstone	Montana	60.7	f	6.2					
Jagalene	Nebraska	89.8	abc	3.8	*Barley*				
Mace	Nebraska	75.2	bcdef	8.6	Harrington		0.0		0.0
Millenium	Nebraska	100.0	a	0.0	Haxby		2.0		2.2
Overland	Nebraska	89.8	abc	5.9	Metcalfe		0.0		0.0
Duster	Oklahoma	100.0	a	0.0					
Endurance	Oklahoma	100.0	a	0.0	Choteau (Healthy)		0.0		0.0
Fuller	Oklahoma	52.3	g	16.2					
Jagger	Oklahoma	75.3	bcdef	10.2					
Moreland	Oklahoma	87.7	abcd	4.6					
OK Bullet	Oklahoma	85.7	abcd	7.4					

[x] Percent incidence was calculated as the percent of wheat plants determined virus-positive by enzyme linked immunosorbant assay out of eight plants from one pot per experiment. The pots were placed randomly in the greenhouse and the study was repeated six times.

[y] Means followed by same letter are not significantly different from each other at P = 0.05. Means were log transformed before analysis by ANOVA for individual variety, and compared using Fisher's LSD.

[z] SE, standard error

Relative absorbance was measured using ELISA absorbance values relative to a susceptible spring wheat variety ‘Choteau’ as a way to estimate relative viral titer. The relative susceptibility to WSMV varied by state of origin ($P = 0.01$; Table 2.13) and cereal variety ($P < 0.01$; Table 2.14). Among the states, Montana winter wheat varieties had relatively high ELISA absorbance on average, followed by Idaho. Varieties from Nebraska, Oklahoma, and Texas showed lower absorbance values. Each state except Texas included one or two varieties with a relative absorbance higher than 85% of the susceptible check, ’Choteau’. Relative absorbance of spring wheat varieties from Montana were comparable to Montana winter wheat varieties, and were higher than winter wheat from the other states. Although barley varieties had very low incidence of WSMV, one ’Haxby’ plant, which was positive for WSMV, had very high relative absorbance.

Winter wheat varieties, ’Genou’, ’Rampart’, ’Reeder’, and ’Westbred 470’ showed over 90% relative absorbance. ’Mace and ‘Jagger’ had the lowest relative absorbance (<60%). Many of the Montana winter and spring wheat varieties tended to have a relatively high absorbance (≥ 85%), with the exception of ‘Yellowstone’ (71.3%).

Table 2.13. Relative absorbance (%) of mechanically inoculated *Wheat streak mosaic virus* (WSMV) on small grain varieties grouped by state and type of cereal crop.

State of origin	Relative abs. (%)[xy]		(SE)[z]
Idaho	82.1	ab	2.3
Montana (WW)	86.5	a	2.6
Nebraska	77.2	b	4.2
Oklahoma	76.4	b	2.8
Texas	76.6	b	3.7
Montana (SW)	88.8	a	3.8
Montana (Barley)	93.6 (Haxby)		

[x] Relative absorbance (Relative abs.) was measured as the absorbance value of each variety relative to the absorbance value of Choteau spring wheat, where Choteau = 100%. Numbers of winter wheat (WW) varieties for each state were: ID = 5; MT = 4; NE = 4; OK = 6; TX = 3. Number of varieties for spring wheat (SW) = 4.

[y] Means followed by same letter are not significantly different from each other at P = 0.05. Means were analyzed by ANOVA after log transformation over the state of origin and compared using Fisher's LSD.

[z] SE, standard error.

Table 2.14. Relative absorbance (%) of mechanically inoculated *Wheat streak mosaic virus* (WSMV) in major wheat varieties from five Great Plains states.

Variety	State	Rel. abs.(%)[xy]		(SE)[z]	Variety	State	Rel.abs.(%)[xy]		(SE)
Winter wheat					*Winter wheat*				
Brundage	Idaho	81.9	abcde	6.9	TAM 105	Texas	61.0	cde	6.7
Promontory	Idaho	82.9	abcde	5.0	TAM 111	Texas	82.2	abcde	8.5
Utah 100	Idaho	74.4	bcde	4.3	TAM 112	Texas	78.9	abcde	8.7
Westbred 470	Idaho	91.2	abcd	6.1					
Weston	Idaho	80.0	abcde	3.5	*Spring wheat*				
CDC Falcon	Montana	85.4	abcd	4.4	Choteau	Montana	85.1	abcde	6.5
Genou	Montana	95.7	ab	3.4	McNeal	Montana	83.5	abcde	5.8
Rampart	Montana	93.6	abc	6.6	Reeder	Montana	100.3	a	7.3
Yellowstone	Montana	71.3	cde	3.2					
Jagalene	Nebraska	69.2	de	5.0	*Barley*				
Mace	Nebraska	44.6	f	4.7	Harrington		N/A		
Millenium	Nebraska	77.2	e	9.7	Haxby		93.6		
Overland	Nebraska	85.1	abcde	6.2	Metcalfe		N/A		
Duster	Oklahoma	80.2	abcde	8.8					
Endurance	Oklahoma	87.1	abcd	9.5					
Fuller	Oklahoma	74.4	cde	5.7					
Jagger	Oklahoma	59.1	f	8.7					
Moreland	Oklahoma	86.7	abcd	5.0					
OK Bullet	Oklahoma	73.6	cde	5.8					

[x] Relative absorbance (Rel abs.) was measured as percentage of absorbance value of each variety to the absorbance value of mechanically inoculated Choteau spring wheat where its absorbance value = 100%.

[y] Means followed by same letter are not significantly different from each other at P = 0.05. Means were log transformed before analysis by ANOVA for individual variety, and compared using Fisher's LSD.

[z] SE, standard error; N/A, not applicable.

Discussion

Our field study showed fall inoculation of winter wheat was not as efficient as spring inoculation based on the incidence. In the spring we observed yellow streaking of leaves in the lower canopy and mild symptoms in the upper canopy of fall-inoculated varieties. This supports our speculation that systemic movement of the virus is limited by cool fall temperatures and the short time period until plant dormancy in northern climates such as Montana. Winterkill is unlikely to be a primary driver of the observed low viral incidence, since no differential winterkill of inoculated plots was observed (data not shown), and viral incidence was not correlated with winter hardiness type (WTARC, 2009a). Early infection of the plant by WSMV allows the virus to have a longer period of time for multiplication within the plant, acquisition by the WCM vector, and subsequent spread within the field (Madden et al 2000). In a winter wheat cropping system this increases the source of inoculum for spread in the spring and summer. Fall infection is likely more significant in areas of the U.S. with a more extended fall planting period and warmer fall temperatures favoring viral replication, reproduction and movement of the WCM vector. For example in Oklahoma, fall inoculation of WSMV reduced yield and seed quality of wheat more than spring inoculation (Hunger *et al*, 1992).

Our study supports the recommendation for growers in Montana to delay planting in the fall within the range of planting date guidelines (USDA-NASS, 2010). Planting date guidelines for Montana were established in collaboration with the Risk Management Agency after the WSMV epidemics in the mid-1990s due to concerns about the ‘green bridge’ (J. Riesselman, *personal communication*). Delayed planting reduces the length of

the WCM-active period (Somsen and Sill, 1970) and limits the amount of time WSMV has to systemically infect the plant and overwinter (Murray *et al*, 2005). Planting times in northern climates are balanced by field access due to weather and soil conditions and the desire by growers to have a well-established crop going into the winter months (Hunger *et al*, 1992; Peel *et al* 1997). In southern states where winter wheat is planted as fall forage, growers balance pest concerns with economic concerns including forage quality and expected grain yields (Epplin *et al* 2000).

Spring inoculation of winter wheat with WSMV resulted in high symptom severity and viral incidence as well as reductions in yield and seed quality. Increased protein content likely was due to decreased kernel size, as overall test weight and thousand kernel weight were decreased. This has been seen in previous studies of WSMV (Finney and Sill, 1963; Riesselman and Carlson, 1994) and *Barley yellow dwarf virus* (BYDV; Edwards *et al*, 2001; Fitzgerald and Stoner, 1967). We considered the spring inoculation the most representative of the pathogenic effects of WSMV in Montana.

Spring wheat was highly susceptible to WSMV as measured by symptom expression, yield loss, and seed quality in the field. Spring wheat was also highly susceptible in greenhouse studies as measured by incidence and relative absorbance. Barley varieties were tolerant to WSMV according to field and greenhouse studies. Poor symptom development and low yield losses due to WSMV in barley have also been observed by researchers in Kentucky (Townsend and Johnson, 1996) and Kansas (Sill *et al*, 1964). Barley is also a poor host for the wheat curl mite (Gillespie *et al*, 1997, Schafer

et al, 1985; Slykhuis, 1955). Barley may be a good choice to be included in continuous cereal systems rather than planting only wheat.

Significant positive correlations among symptom severity, viral incidence, and yield loss were not observed in these studies (see Appendix B). The lack of a direct relationship between symptom expression, viral titer and yield loss has also been seen for BYDV in wheat, durum, oat and triticale (Comeau and Haber, 2002). A determination of pathogen tolerance in a crop variety must rely on consistent and measureable yield losses due to infection, not visual symptoms or viral incidence. A number of factors including temperature, available moisture, nutrition, crop stress, etc. contribute to yield effects due to disease (Qamar, 2003).

We were not able to predict winter wheat variety susceptibility based on the geographic origin of varieties. Our hypothesis was that varieties originating from states with more frequent virus epidemics, such as Oklahoma and Texas would be more susceptible to WSMV than varieties from states with infrequent epidemics such as Idaho and Montana. The difference in frequency of WSMV epidemics in those locations may is primarily influenced by cultural practices including continuous wheat production, which is approximately 75% in Oklahoma, 60% in Texas, 12% in Idaho, 6% in Nebraska, and 3% in Montana (Padgitt *et al*, 2000; USDA-NASS, 2009a). Continuous wheat production favors survival of the virus and the WCM vector.

Other factors contributing to epidemic frequency are the virulence of WSMV strains and the capacity of WCM biotypes to transmit WSMV. The variation of WSMV genotypes within a field can be as great as between counties within a state (McNeil *et al*,

1996). Variety susceptibility varies by virus strain (Carroll *et al*, 1982; Seifers *et al*, 1996 and data not shown). Field trials of spring wheat in 2010 were inoculated with two Montana strains of WSMV (Conrad-I and Huntley-I), and varieties reacted differently (data not shown). Preliminary greenhouse tests with strains of WSMV from Colorado, Wyoming, Nebraska and Texas indicate wheat varieties respond differently to these isolates than to the Conrad-I strain of WSMV (data not shown).

Temperature-sensitive resistance was identified in wheat germplasm lines (Seifers *et al*, 2007) in which resistance was lost at temperatures above 18□. As briefly mentioned in previous sections, WSMV-resistance genes were identified (Friebe *et al*, 1991; Haley *et al*, 2002; Lu *et al*, 2011; Seifers *et al* 1995) and introduced in Mace in Nebraska (Graybosch *et al*, 2009). Currently available resistance genes are single dominant. It is hard to predict how long the plant virus resistance will last in the field. García-Arenal and McDonald (2003) discussed that resistance to plant viruses tends to be durable and less likely overcome than resistance to fungal or bacterial pathogens. However, the durability in the field is influenced by multiple factors such as high mutation rates of RNA virus (Drake and Holland, 1999), population size and gene flow (McDonald and Linde, 2002), and genetic structure of the viral population (Fabre *et al*, 2009). Data presented here and preliminary data from ongoing experiments indicate a risk of different WSMV strains reacting differently to WSMV resistance.

WCM biotype differences in transmission efficiency of WSMV have been reported in Australia and Nebraska (Schiffer *et al*, 2008; Seifers *et al*, 2002). Variation in WCM populations can be as great on a wheat head as within a field (Siriwetwiwat, 2006).

WCM biotypes also vary in their response to WCM-resistance sources (Malik *et al*, 2003). Such variability makes it difficult for breeding programs to develop WCM resistance, although resistance has been identified in *Setaria cereale* (Sebesta, *et al*, 1994; Wood *et al*, 1995), *Aegilops tauschii* (Malik *et al*, 2003; Thomas and Conner, 1986), and *Thinopyrum ponticum* (Whelan and Hart, 1988) and incorporated into wheat lines. Resistance to an arthropod is very specific and target species can be easily replaced by the other species or biotypes (Dedryver, 2004). The lack of sustainability of a resistant variety to either the virus or the mite suggests breeding efforts should not be focus on just mite or virus resistance, but incorporate both aspects simultaneously to improve the durability of resistance.

In this study we used incidence, severity, or relative absorbance to infer whether varieties may be resistant to WSMV. Although we were not able to directly correlate these variables with yield loss, this is still valuable information since both variables can affect the spread of the virus within and between fields. Incidence will influence the size of the inoculum source for mite transmission. Viral titer in the host tissue which we inferred from the absorbance will influence the acquisition efficiency of WSMV by the mite vector. Both of these variables should be incorporated in models of disease risk (Madden *et al*, 2000). WCM local movement (crawling) is limited to approximately 3 cm/day and longer distance travel relies on wind (Coutts, 2008; Slykhuis, 1955). Transmission efficiency generally increases with increasing viral titer in systems such as BYDV (Gray *et al*, 1991; Power *et al*, 1991), *Bean yellow mosaic virus* (Swenson, 1962), *Maize dwarf mosaic virus* (Tu and Ford, 1971) and *Potato virus Y* (Simons, 1966).

Supplementary use of WSMV-resistant wheat varieties has potential to significantly reduce transmission efficiency of WCM and reduced subsequent incidence in the field (Harvey *et al*, 2005).

An understanding of the relative susceptibility of wheat varieties gives us a greater understanding of potential contributing factors to the frequency and severity of wheat viral epidemics, as well as identifying potential sources of resistance for future breeding efforts. Current control measures rely on cultural practices that may be difficult to achieve when a grower is balancing decisions about planting date with yield potential and timing of weed management. Yield loss will vary according to the variety, viral strain, time of infection, incidence of infected plants, and environmental factors. Variety selection is a tool for WSMV management, but cultural practices are key for management of wheat virus epidemics.

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RELATIVE SUSCEPTIBILITY AMONG ALTERNATIVE HOSTS PREVALENT IN THE GREAT PLAINS TO WHEAT STREAK MOSAIC VIRUS

Abstract

Grassy weeds are known to be hosts of *Wheat streak mosaic virus* (WSMV) and its vector, the wheat curl mite (WCM, *Aceria toshichella* Keifer). However, their relative importance in the 'green bridge' as a source of WSMV has not been quantitatively evaluated. A survey of common grassy weeds in small grain fields throughout Montana was conducted and plants were tested by indirect ELISA in 2008 and 2009. *Bromus tectorum* was the most prevalent grassy weed in the locations sampled and the most frequent viral host, with 6.0% infection by WSMV in 2008 (n = 125) and 15.0 % in 2009 (n=358). *Triticum mosaic virus* was identified in *B. tectorum* and *Avena fatua* during the 2009 survey. In addition to the field survey, widely prevalent grass species from croplands in Colorado, Idaho, Montana, Nebraska, Oklahoma, and Texas were selected. Each grass species was mechanically inoculated to determine the relative susceptibility to WSMV by incidence and absorbance relative to spring wheat (var. Choteau). The highest incidence and relative absorbance among the species tested were found in *Bromus secalinus*, *Aegilops cylindrica*, and *A. fatua*. There was no difference in the susceptibility of grass species to WSMV based on their state of origin. *Agropyron repens* was susceptible to WSMV although it had not previously been reported as a WSMV host. Mite transmission studies indicate that transmission efficiency from susceptible grass species was lower than from wheat, and a grass must be a host for both WSMV and the WCM in order to serve as an inoculum source.

Introduction

Control of *Wheat streak mosaic virus* (WSMV) relies on cultural practices to control the vector, the wheat curl mite (*Aceria tosichella* Keifer, WCM), and to a lesser extent on the use of resistant or tolerant crop varieties to WSMV (Burrows *et al*, 2009b; Townsend and Johnson, 1996). Both the virus and vector are dependent on the 'green bridge' because of their dependence on living tissue for survival and reproduction (Somsen and Sill, 1970). Volunteer wheat is considered the primary reservoir of the virus and mite, particularly between winter wheat harvest and planting of the new crop (Slykhuis, 1953; Somsen and Sill, 1970; Townsend and Johnson, 1996). Although many consider volunteer crops the principal reservoir of both WSMV and WCM, various grassy weeds have been shown to be alternative hosts for the virus and mite. Grassy weeds identified as important in the 'green bridge' in the Great Plains region include *Aegilops cylindrica*, *Avena fatua*, *Bromus secalinus*, *B. tectorum*, *Lolium persicum*, and *Setaria viridis* (Brey *et al*, 1998; Somsen and Sill, 1970; Townsend and Johnson, 1996).

Although there is information in the literature about the susceptibility of grass species to WSMV and the WCM, evaluations of susceptibility differ based on the source of information, materials sampled and methods used. Prior to this study, there were no published data on whether the WCM can transmit WSMV from susceptible grass species to crops. Increasing the complexity of the system, the WCM can reproduce on grasses that are immune to WSMV, while other grasses can serve as hosts of WSMV but not the WCM. It was unknown whether transmission can occur from a WSMV-susceptible plant that is not a reproductive host of the WCM (Brey *et al*, 1998; Christian and Willis, 1993;

Coutts *et al*, 2008). Summer annuals such as *Echinochloa crus-galli* and *S. viridis* have been considered relatively more important in facilitating the 'green bridge' because their life cycle fills a gap between winter wheat harvest and fall planting (Somsen and Sill, 1970). Winter annual grasses were thought to have a less significant role in WSMV epidemiology because their growth cycle closely matches winter wheat. The need to better understand disease cycles of wheat viruses was heightened by the discovery of two new viruses, *Wheat mosaic virus* (WMoV, formerly named *High Plains virus*) and *Triticum mosaic virus* (TriMV). Both of these viruses have disease cycles similar to WSMV, are transmitted by the WCM, and are able to infect grassy weeds (Seifers *et al*, 1997, 1998, 2002, 2010). Co-infection of a single plant with multiple wheat viruses has been reported and is known to enhance symptom severity and yield loss (Mahmood *et al*, 1998; Stenger *et al*, 2007). WSMV is dominant in the nine-state Great Plains region, but WMoV and TriMV have also been reported in these states (Burrows *et al*, 2009a).

This study focuses on alternative hosts of WSMV to evaluate the risk of various grassy weed species to serve as hosts of WSMV and the WCM vector. A field survey of grassy weeds in Montana identified *B .tectorum* as the most consistently prevalent and naturally infected grassy weed host of WSMV. We also evaluated grassy weed germplasm from six Great Plains states to evaluate their susceptibility to WSMV with mechanical inoculation and the capacity of WSMV-susceptible weeds to serve as sources for transmission by WCM to wheat. Susceptibility data generally agreed with previously published data, with the exception of *A. repens,* which had not previously been reported as susceptible to WSMV. Variation in relative susceptibility to WSMV based on state of

origin of the grass species was low, except in *A. cylindrica*. Grassy weed species can serve as a source for virus transmission to wheat if they can also support WCM reproduction.

Materials and Methods

Source of Virus

Symptomatic winter and spring wheat samples were collected in 2007 from Conrad, Montana. An isolate obtained from these plants (designated 'Conrad-I') was mechanically transferred to and maintained in a susceptible spring wheat variety, Choteau. The greenhouse was maintained with a 16h day: 8h night photoperiod and temperatures of 24±4°C (day) / 18±4°C (night) in the Plant Growth Center, Montana State University, Bozeman, Montana. The planting media used was 1:1 ratio of MSU mix (a 1:1:1 mix of mineral soil, Canadian sphagnum peat moss, Aquagro 2000G [Aquatrols, Paulsboro, NJ] and washed concrete sand steam pasteurized at 80°C for 45 min) and Sunshine Mix #2 Basic (Sun Gro Horticulture, Vancouver, BC, Canada). The isolate was periodically renewed from lyophilized stocks of the initial mechanical transmission stored at -80°C.

For mechanical inoculation, symptomatic leaves were ground in a sample extraction bag (Agdia, Inc., Elkhart, IN) at 1:10 (weight (g) : volume (ml)) ratio with phosphate buffered saline (PBS, 136.9 mM sodium chloride (NaCl), 8.1 mM sodium phosphate dibasic (Na_2HPO_4), 1.5 mM of potassium phosphate monobasic (KH_2PO_4), and 2.9 mM of potassium chloride (KCl), pH 7.2). Approximately 1% (wt:vol) of carborundum (fine, 320 grit, Alfa Aesar, Ward Hill, MA) was added as an abrasive.

Wheat leaves were gently rubbed two or three times between gloved fingers dipped into the inoculum.

Identification of Virus

WSMV strain Conrad-I coat protein was sequenced by reverse transcription polymerase chain reaction (RT-PCR), and the sequence was deposited in GenBank as accession number HM535796. RNA was extracted from 50 mg of symptomatic leaves using the TRI reagent RT (Molecular Research Center, Cincinnati, OH) and re-suspended in 30 □l of DEPC-water according to manufacturer's instructions. The extracted RNA was reverse transcribed using SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA) with a gene specific reverse primer, C8908 (Myslik and Nassuth, 2001), according to manufacturer's instructions. The PCR reaction mixture consisted of 1X GoTaq Master mix (Promega Corporation, Madison, WI), 1.25 µM forward primer H8369 (Myslik and Nassuth, 2001), 1.25 µM reverse primer C8908, 1 µl of cDNA, and water for a final volume of 25 µl. PCR conditions were modified from Myslik and Nassuth (2001). Denaturation occurred at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 56°C for 30 sec, and extension at 72°C for 60 sec with a final extension at 72°C for 5 min (Myslik and Nassuth, 2001). The PCR amplification was performed by an iCycler Thermal Cycler (Bio-Rad Laboratories Ltd, Hemel Hempsted, UK). The product of PCR amplification (540 bp) was confirmed by gel electrophoresis with a 2% agarose gel containing ethidium bromide (1.5%). PCR fragments were extracted with a QIAquick Gel Extraction kit (Qiagen, Inc., Valencia CA) following manufacturer's instructions, and sequenced using the primers described above by

Functional BioSciences (Madison, WI). Coat protein regions were compared to sequence collections in GenBank (National Center for Biotechnology Information (NCBI)) using BLAST algorithm blastn. The most similar sequences include *Wheat streak mosaic virus* strain Sidney 81 (AF057533; E-value = 0.0; French and Stenger, 2005; Stenger *et al*, 1998).

Detection and Semi-Quantification of
Virus by Enzyme-Linked Immunosorbant Assay

Enzyme linked immunosorbant assay (ELISA) was used for virus detection and quantification. Anti-WSMV antiserum was purchased from the American Type Culture Collection (ATCC, Manassas, VA) and used at a working dilution of 1:3200 according to manufacturer's instructions. TriMV antiserum was obtained from Jeff Ackerman, Texas along the TriMV infected wheat samples. TriMV antiserum was used at 1:1000 dilution. Leaf samples were weighed and 0.05 to 0.1g of tissue was placed in a 10.2 X 15.2 cm plastic bag (Fisher Scientific, Pittsburgh, PA). A corresponding amount of 1X PBS was added to make a 1:10 dilution (wt/vol) and the sample was macerated.

One hundred microliters of extracted sap were placed in each well of a 96-well BD Falcon microtiter plate (Fisher Scientific, Pittsburgh, PA) pre-coated with 100 µl of 1X carbonate buffer (0.05M sodium carbonate) for 2 h. Samples were loaded in duplicate or triplicate with 1X PBS as a background control, virus-free wheat samples maintained in the greenhouse as a negative control, and wheat inoculated with WSMV Conrad-I two weeks prior to the assay as a positive control. Overnight incubation at 4°C was followed by seven cycles of rinsing with 5 sec soaks with 200 µL of 1X PBST (1X PBS with 0.5%

Tween 20, Agdia, Elkhart, IN) in a 96-well PW^{TM} microplate washer (Tecan, Durham, NC). WSMV antiserum diluted in ECI buffer (Agdia, Elkhart, IN) was added and the plate was incubated for 2 h in a sealed plastic bag at room temperature. The plate was washed as described above, and 100 µl of goat anti-rabbit IgG alkaline phophatase conjugate (Sigma, St. Louis, MO) at 1:30,000 dilution (final concentration: 5 µg/ml) in ECI buffer was added to each well. After a 2 h incubation and final wash, 100 µL of p-nitrophenol (PNP) substrate (Agdia, Elkhart, IN) (Agdia, Elkhart, IN) in 1X PNP buffer (0.5mM of magnesium chloride hexahydrate ($MgCl_2(H_2O)_6$) and 1.0 M diethanolamine ($C_4H_{11}NO_2$), pH 9.8; Agdia, Elkhart, IN) was added to the wells. The plate was incubated for 1 h in the dark and absorbance (A_{405}) quantified using a microplate reader (SpectraMax Plus, Molecular Devices, Sunnywyle, CA). Absorbance values at least twice the value of the negative control were considered positive (Christian and Willis, 1993). Absorbance values of grassy weeds were translated to a value relative to the positive control in the same 96-well plate as described in appropriate section.

Field Studies: Field Surveys of
Small Grain Fields in Montana in 2008 and 2009

Sampling sites were selected according to reports of virus-like symptoms from Montana county extension agents, samples submitted for diagnosis to the Schutter Diagnostic Lab, Montana State University, Bozeman, MT, and visual observations (Table 3.1). Samples were taken from five counties representing 22.7% of the cereal grain acreage in Montana (USDA-NASS, 2009). Five to ten symptomatic leaf samples of the crop were randomly selected, pooled, and screened for WSMV, WMoV, TriMV, *Barley*

yellow dwarf virus – PAV and *Cereal yellow dwarf virus* - RPV with an ELISA kit provided by Agdia (Elkhart, IN) as a part of a regional wheat virus survey (Burrows *et al*, 2009a, 2009c). We identified the pooled samples infected by either WSMV or TriMV but not the remaining three viruses (data not shown). Individual samples were screened for WSMV and TriMV by ELISA as described in the previous section. Grassy weeds associated with these fields were collected as described below. The survey in 2008 was performed at one location in Pondera County on 26 June and at one location each in Chouteau and Pondera counties on 15 July (Table 3.1). The site of collection in Pondera County on the two separate dates was in the same field. In 2009, samples were collected from a total of twelve locations (Table 3.1).

Table 3.1. Locations sampled for *Wheat streak mosaic virus* (WSMV) in small grain crops and grassy weeds in Montana in 2008 and 2009.

Year	Site	Town	County	Crop	Date
2008	A	Conrad	Pondera	Winter wheat	26 June
	B	Fort Benton	Chouteau	Winter wheat	15 July
	C	Conrad	Pondera	Winter wheat	15 July
2009	1	Huntley	Yellowstone	Winter wheat	29 May
	2	Denton	Fergus	Winter wheat	7 July
	3	Denton	Fergus	Winter wheat	7 July
	4	Denton	Fergus	Winter wheat; spring wheat	7 July
	5	Denton	Fergus	Winter wheat	7 July
	6	Denton	Fergus	Winter wheat	7 July
	7	Denton	Fergus	Spring wheat	7 July
	8	Denton	Fergus	Winter wheat	7 July
	9	Ledger	Pondera	Barley	8 July
	10	Ledger	Pondera	Barley	8 July
	11	Ledger	Pondera	Barley	8 July
	12	Shelby	Toole	Winter wheat; spring wheat	8 July

To assess the overall incidence of WSMV in the symptomatic area, fifty to one hundred cereal crop leaves were sampled by walking in a zig-zag pattern from the edge into the field to five meters beyond the edge of the symptomatic area with two to three youngest, fully expanded leaves arbitrarily selected at each collection point. Symptoms in all fields were observed at the edge, not throughout the field. For grassy weeds, whole plants found in and adjacent to the symptomatic sampling area were carefully collected with the seed head when available and roots. Sample numbers of the grassy weeds varied by their prevalence in the field, with up to 50 to 100 plants sampled per field. All plant materials were stored in sealed plastic bags on ice until returned to the laboratory, then subsampled for identification and frozen at -20°C until processed for viral detection with indirect ELISA. The collected leaf samples were screened by ELISA as described above with WSMV only in 2008 and WSMV and TriMV in 2009. Antibody of TriMV (final concentration of 1mg/ml, 1:1000 working dilution [v:v]) was provided by Kansas State University. TriMV-infected wheat positive controls were provided by the Texas AgriLife Research and Extension Center, Amarillo, TX.

Greenhouse Studies:
Evaluation of the Relative Susceptibility of
Grassy Weeds Commonly Found in the Great Plain States to WSMV

The experiments were performed to compare susceptibility of common grassy weeds in the Great Plains by incidence and absorbance relative to wheat, which we assume is representative of viral titer. Two separate experiments were conducted for 1) incidence with five repetitions and 2) relative absorbance with six replications with exception of *S. viridis* which had four replications due to low germination. Each

replication for incidence contained one pot and for relative absorbance contained two pots.

For the experiment of the viral incidence, major grassy weed species commonly found in small grain fields were selected from Colorado, Idaho, Montana, Nebraska, Oklahoma, and Texas (Table 3.2). Southern states have greater risk of WSMV because of longer growing season and 'green bridge' periods. The grass species were selected according to their potential importance as grassy weeds in cereal production fields, biological characteristics, ability to support WSMV replication and mite reproduction according to the literature (Brey *et al*, 1998; Somsen and Sill, 1970; Townsend and Johnson, 1996), and local prevalence information provided by collaborators from each state.

Table 3.2. Grassy weed species used in this study and their state of origin[z].

Scientific name	Common name	State of origin
Aegilops cylindrica Host.	Jointed goatgrass	Colorado, Idaho, Montana, Nebraska
Agropyron cristatum (L.) Gaertn.	Crested wheatgrass	Montana
Agropyron repens (L.) P. Beauv; *(Elymus repens* (L.) Gould)	Quackgrass	Idaho, Montana
Avena fatua L.	Wild Oat	Idaho, Montana
Elymus canadensis L.	Canada wild rye	Texas
Elymus lanceolatus (Scribn. & J.G. Sm.) Gould	Thickspike wheatgrass	Montana
Elymus trachycaulus (L.) Gould ex Shinners	Slender wheatgrass	Montana
Bromus catharticus Vahl	Rescue grass	Oklahoma
Bromus inermis Leyss.	Smooth brome	Montana
Bromus japonicus Thunb.	Japanese brome	Texas
Bromus secalinus L.	Cheat (Rye brome)	Oklahoma
Bromus tectorum L.	Downy brome (Cheatgrass)	Colorado, Idaho, Montana, Nebraska, Oklahoma
Echinochloa crus-galli (L.) P. Beauv.	Barnyardgrass	Montana
Eragrostis cilianensis (All.) Vign. ex Janchen	Stinkgrass (Lovegrass)	Montana
Lolium sp.	Ryegrass	Oklahoma
Secale cereale L.	Rye	Colorado, Oklahoma
Setaria viridis (L.) P. Beauv.	Green foxtail	Idaho, Montana
Sorghum halepense (L.) Pers.	Johnsongrass	Texas

[z] Synonyms and common name were retrieved from USDA- NRCS Plant database, 2010

The grassy weeds were planted in 17.8 cm pots in the planting media described above. At least ten seeds of each grass species were planted approximately 2 cm in depth in three pots and watered as necessary. To facilitate germination, the palea and lemma were removed from *A. fatua* seeds (D. Morishita, *personal communication*). The pots were arranged as a completely random design with 16h day: 8 h night photoperiod and

temperatures of 24±4°C day / 18±4°C night in the greenhouse of the Plant Growth Center, Montana State University, Bozeman, MT.

The grassy weeds were mechanically inoculated with WSMV at the 2 to 3-leaf stage by hand-rubbing with infectious sap as previously described. Plants were inoculated on two consecutive days to ensure a high infection rate. A pot of spring wheat, 'Choteau', was also mechanically inoculated at 2 to 3-leaf stage the same day the grassy weeds were inoculated. It was selected because it showed consistently high incidence and strong WSMV symptom expression in a preliminary study (data not shown). A pot of buffer-(1X PBS) inoculated 'Choteau' was included in each replication as negative control. An additional four to five wheat pots were distributed around the experiment to check for WSMV spread. No contamination was observed for any of the experiments included in this study. Three weeks post-inoculation, the youngest fully expanded leaf on eight randomly selected plants were collected from each pot for virus incidence determination and placed in individual plastic bags.

The collected leaf samples were weighed and ground in 1x PBS with 1:10 dilution (wt:vol). All samples were processed for ELISA as described above with duplicate plates. Individual leaf samples for the viral incidence were loaded in multiple plates due to limited numbers of wells on 96-well plates. Each plate had two background controls (1x PBS), two negative controls (buffer-inoculated wheat, 'Choteau'), and a positive control ('Choteau', WSMV mechanically inoculated for each experiment).

The experiment for the relative absorbance was performed similar to what was described above, except each replication had two pots per grass species. Two- to 3-cm

leaf pieces from the youngest fully expanded leaves from the plants were sampled separately from the incidence experiment and pooled in a plastic bag. Pooled samples of each variety were tested by ELISA as described above but on the same plate. For each plate, the absorbance value of the background control (buffer only) was subtracted from each well. The relative absorbance of WSMV in a sample was calculated as the absorbance of the test sample divided by the absorbance of the positive control on the same 96-well plate multiplied by 100 to estimate viral titer as a percentage of the positive control, spring wheat variety Choteau. Calculated relative absorbance of each grassy weed species was averaged over duplicate plates.

Greenhouse Studies:
Ability of the Wheat Curl Mite to
Transmit WSMV from Alternate Hosts to Wheat

A wheat curl mite colony was established from a population collected at the Arthur H. Post Agronomy Research Farm, Bozeman, MT in September, 2007 and identified to species with the aid of the Insect Quarantine Laboratory at Montana State University and SEM. WCM were maintained on pathogen-free spring wheat, variety Choteau, planted in a 15.2 cm pot with the plant media previously described covered by a thin layer of play sand (approximately 0.7 cm) on the surface in an isolated growth chamber (16 h light at 25±0.5°C). The colonies were maintained in pots containing four to five plants each. Each pot was covered by an acrylic tube 10 cm in diameter with three to four holes, approximately 7.0 cm in diameter, sealed by Nylon lab pak mesh (25 micron, Sefar AG, Switzerland) and placed in a saucer. Plants were maintained by pouring water into the saucer as necessary. Once the population had established, ten to

twenty mite eggs were transferred to healthy ’Choteau‘ wheat at the two to three leaf stage with an eyelash brush. This process was repeated twice. The WSMV-free colony was transferred every month or when mite-infested plants started drying by inserting a 2.5 cm leaf piece colonized by WCM into a slit made on the youngest fully expanded leaf of a healthy wheat plant, growth stage of Feekes 2 to 3.

For transmission tests, mite chambers were constructed from a clear plastic 1-gal jug (United States Plastic Corp, Lima, OH) with two side holes (approximately 5.7 cm in diameter) sealed with Nylon lab pak mesh. Three to five 2.5-cm leaf pieces of WSMV-inoculated grassy weeds testing positive for WSMV were used as the source tissue. WSMV infected and healthy ’Choteau‘ spring wheat were included in each experiment as positive and negative controls, respectively. Each experiment was repeated four times. Small pieces of lightly moistened paper towels or filter papers were used to wrap one end of the source leaf pieces, which were placed in a sterile petri dish. The leaf pieces were maintained by adding water to the paper towel as needed. A 2.5-cm leaf piece from the WCM colony with at least 10 to 30 mites was placed into a slit cut into each of the grassy weed leaf pieces. The mites were allowed a 72-h acquisition access period (AAP). The presence of mites on the recipient leaflet was then confirmed under a dissecting scope. Each leaf piece of the grassy weed with WCM was placed into a slit on single healthy wheat plant ‘Choteau’ to reach a total of ten recipient plants per source plant. Ten recipient plants were planted in a 15.2-cm pot with the planting media described above. Recipient wheat plants were caged and maintained in the greenhouse or growth chamber, and the mites were allowed a 72-h inoculation access period (IAP)

before acaricide application (Temik 15G, Bayer Crop Science) at 5 to 10 granules per pot (Skare *et al*, 2003). The recipient wheat plant was not watered during the IAP to avoid extremely high humidity in the mite cage and molding of the mite-infested leaf pieces, which hindered mite movement to the recipient leaflet during preliminary observations. Forty eight h after acaricide application, the recipient plants were un-caged and allowed to develop symptoms for two to three weeks. Healthy wheat plants were distributed amongst the experimental pots to check for mite and virus spread. Plants were tested by indirect ELISA as the described in previous section. Transmission efficiency was calculated as the number of plants that become infected out of the total number inoculated with viruliferous mites.

Statistical Analysis

Analysis of variance (ANOVA) was computed for percent incidence and relative absorbance obtained from greenhouse studies using Statistical Analysis System (SAS v.9.2, Cary, NC). However, relative absorbance for the grassy weeds, which showed zero incidence, was excluded from the analysis. Residuals for experimental data were examined for normality by the Shapiro-Wilk's test (Shapiro and Wilk, 1965) and homogeneity of variances by the Levene test (Levene, 1960). Data were log transformed as necessary although untransformed data are presented in the tables. Proc GLM (SAS v.9.2, Cary, NC) was used for analysis over grassy weed species and subsequently over state of origin for the species from multiple states (Table 3.2). The analysis using the state of origin as explanatory variable was to examine potential regional differences within the grassy weed species. Replication was used as an error term within the grass

species. Entry means were compared using Fisher's least significant difference (LSD) at a significance level of P = 0.05.

Results

Field Survey for WSMV in Cereal Crops and Grassy Weeds

The predominant grassy weed species identified in the field survey of five Montana counties in 2008 and 2009 were *A. cristatum*, *B. tectorum*, *Hordeum jubatum*, *A. fatua*, and volunteer crops (Tables 3.3 and 3.4). Volunteer crops, *A. cristatum*, and *B. tectorum* were the most common grassy weeds throughout the region. Grassy weeds were the most often found on the field edge. Both crop and volunteer wheat and barley and a few plants of *B. tectorum* expressed viral-infection symptoms including chlorosis, streaking, and purpling of leaves. Symptoms were not observed on other grassy weeds.

Table 3.3. Detection of *Wheat streak mosaic virus* (WSMV) in crops and grassy weeds during field surveys of Montana in 2008.

Virus host		Site[yz]		
Scientific name	Common name	A	B	C
Triticum aestivum	Winter wheat (crop)	66/100	68/100	42/100
Agropyron cristatum	Crested wheatgrass	0/25	0/25	0/25
Avena fatua	Wild oat	1/25	0/25	0/50
Bromus tectorum	Downy brome	0/25		6/100
Hordeum jubatum	Foxtail barley	0/25	0/25	0/25
Triticum aestivum	Volunteer wheat	7/50		4/50

[y] Site A was in Pondera Co. on 26 June. Site B was in Chouteau Co. on 15 July. Site C was in Pondera Co. on 15 July.

[z] Number of plants ELISA-positive for WSMV/ total number of samples collected in each field. Sample numbers of each grass species varied by the species' prevalence in the field.

In 2008, an average of 58.7% of winter wheat plants from the three sites was positive for WSMV (Table 3.3). A wider diversity of grassy weed species was present at the Pondera Co. site than the Chouteau Co. site. An average of 4.0% of grassy weeds including volunteer crops was positive for WSMV. Volunteer wheat had the highest incidence of WSMV infection (avg. 5.0%), followed by *B. tectorum* (3.0%) and *A. fatua* (1.3%). No virus was detected in *A. cristatum* or *H. jubatum*.

In 2009, WSMV levels in the crops were highly variable, ranging from 0.0 to 57.8%, with an overall average of 14.1%. Spring wheat fields (29.3% avg. at three sites) had relatively more WSMV in the crop than winter wheat (17.3% avg. at eight sites) or barley (3.3% avg. at three sites). Volunteer crops were present at four of twelve sites, with an average WSMV incidence of 5.4%. *B. tectorum* was the most commonly identified grassy weed during the 2009 survey, being present at nine of the twelve sites, and had an average WSMV incidence of 10.5%. *A. cylindrica* (avg. 0.0%) and volunteer *T. aestivum* (avg. 3.2%) were present at three of twelve sites, and other grassy weeds were present at one site only (Table 3.4).

In 2009, TriMV was added to the survey (Table 3.5). TriMV levels in the crop were highly variable, ranging from 0.0 to 93.3%, with an average of 10.9% infection of the 13 crops at 11 sites. *B. tectorum*, the most commonly identified grassy weed, was infected with TriMV at four sites at low levels (avg. 6.6%). *A. cylindricae*, *B. inermis* and volunteer *T. aestivum* and *H. vulgare* were also found infected with TriMV in at least one site (Table 3.5). *A. cristatum* was not infected with TriMV (1 site).

Table 3.4. Detection of *Wheat streak mosaic virus* (WSMV) in small grain crops and grassy weeds in Montana during 2009[z]

		Sample collecting site					
Scientific name	Common name	1	2	3	4	5	6
Crop							
Triticum aestivum	Winter wheat	19/90	0/45	1/91	2/90	1/45	2/90
Triticum aestivum	Spring wheat				29/90		
Grassy weed							
Agropyron cristatum	Crested wheatgrass			0/45			
Avena fatua	Wild oat			0/84	0/2		
Bromus inermis	Smooth brome	0/20					
Bromus tectorum	Downy brome	20/90	2/18	0/7	0/45		9/45
Elymus sp.	Wild rye	0/5					
Poa pratensis	Kentucky bluegrass	3/20					
Triticum aestivum	Volunteer wheat		0/3			0/22	

		Sample collecting site					
Scientific name	Common name	7	8	9	10	11	12
Crop							
Hordeum vulgare	Barley			5/89	3/90	1/90	
Triticum aestivum	Winter wheat		32/90				52/90
Triticum aestivum	Spring wheat	26/90					11/45
Grassy weed							
Aegilops cylindricae	Jointed goatgrass			0/3	0/13		0/13
Bromus tectorum	Downy brome		3/59	0/28	0/16		18/50
Hordeum vulgare	Volunteer barley				3/25		
Triticum aestivum	Volunteer wheat			2/21			

[z] Leaf samples collected as zig-zag patterns from the field edge of each location (Table 3.1) were screened for WSMV. Results are presented as: the number of ELISA-positive samples for WSMV / total number of samples from each site. If blank, it was not tested nor collected.

Table 3.5. Detection of *Triticum mosaic virus* (TriMV) in small grain crops and grassy weeds in Montana during 2009[z].

		Sample collecting site					
Scientific name	Common name	1	2	3	4	5	6
Crop							
Triticum aestivum	Winter wheat		0/45	7/91	0/90	1/45	2/90
Triticum aestivum	Spring wheat				0/90		
Grassy weed							
Agropyron cristatum	Crested wheatgrass			0/45			
Avena fatua	Wild oat			19/84	0/2		
Bromus tectorum	Downy brome		0/18	0/7	13/90		0/45
Triticum aestivum	Volunteer wheat		0/3			0/22	

		Sample collecting site					
Scientific name	Common name	7	8	9	10	11	12
Crop							
Hordeum vulgare	Barley			1/89	0/90	22/90	
Triticum aestivum	Winter wheat		0/90				0/90
Triticum aestivum	Spring wheat	0/90					42/45
Grassy weed							
Aegilops cylindricae	Jointed goatgrass			0/3	1/13		0/13
Bromus tectorum	Downy brome		1/59	0/28	1/16		2/50
Hordeum vulgare	Volunteer barley				3/25		
Triticum aestivum	Volunteer wheat			1/21			

[z] Leaf samples collected as zig-zag patterns from the field edge of each location (Table 3.1) were screened for TriMV except for site 1 due to lack of appropriate antibody at the time. Results are presented as: the number of ELISA-positive samples for WSMV / total number of samples from each site. If blank, it was not tested nor collected.

The incidence of WSMV and TriMV in the crop and weed species were not correlated at any site. Co-infection of plants with WSMV and TriMV were identified in only a single plant in six of the seven sites except in spring wheat at site 12 (Table 3.6).

Table 3.6. Co-infection of plant species with *Wheat streak mosaic virus* (WSMV) and *Triticum mosaic virus* (TriMV) sampled in Montana during 2009[yz].

Year	Site	County	Virus host	Co-infection
2009	8	Fergus	*Bromus tectorum*	1/59
	9	Pondera	*Hordeum vulgare* (crop)	1/89
	12	Toole	*Triticum aestivum* (winter, crop)	11/45
	12	Toole	*Bromus tectorum*	1/50

[y] Only sites and sampled plant species where co-infection of WSMV and TriMV were detected by ELISA are presented.
[z] Number of plants ELISA-positive for WSMV and TriMV / total number of plant samples collected in each field. Sample numbers of each grass species varied by the species' prevalence in the field.

Evaluation of the Relative Susceptibility of Major Grassy Weeds from Six Great Plains States to a Strain of WSMV from Montana

Widely prevalent grassy weed species from six states in the Great Plains region were tested for susceptibility to a Montana strain of WSMV by mechanical inoculation (Table 3.7). Symptoms of WSMV varied, and were noted in only a few species. *A. cylindrica*, *A. fatua*, and *E. crus-galli* had typical WSMV symptoms of yellow leaf streaking. *B. japonicas*, *B. secalinus*, *B. tectorum*, *B. japonicus*, *B. secalinus*, and *B. tectorum* showed a mild leaf streaking. *E. canadensis*, *S. cereale*, and *Lolium sp.* mostly appeared asymptomatic. *S. viridis* also appeared asymptomatic, although viral symptoms may have been masked by purpling of plant tissue especially in older tissue in all plants of this species, regardless of whether they were mechanically inoculated with WSMV.

The average incidence of WSMV in the grass species tested in the greenhouse was 32.1%. No grass species except the positive control 'Choteau' spring wheat exhibited 100% incidence of infection by WSMV. The incidence varied by grass species ($P_{grass} < 0.01$; Table 3.7). *A. cylindrica* had the highest infection rate among the grasses tested followed by *A. fatua* and *B. secalinus*. Grassy weeds with moderately high (30-50%) incidence of WSMV included *S. cereale*, *B. japonicus*, and *B. tectorum*. Two summer annuals, *S. viridis* and *E. crus-galli* had a relatively low (<30%) incidence of WSMV infection. All perennial grasses were infrequently infected (<20%) including *A. repens, E. Canadensis,* and *Lolium* spp. *Sorghum halepense*, *B. catharticus*, *B. inermis*, *E. cilianensis*, *A. cristatum, E. trachycaulus*, and E. *lanceolatus* were not susceptible to WSMV. *A. cylindrica*, *A. fatua, A. repens*, *B. tectorum*, *S. cereale*, and *S. viridis* genotypes from multiple states were tested, and no significant differences in incidence were observed based on state of origin (Table 3.8).

Table 3.7. Relative susceptibility of grassy weed species from six Great Plains states to *Wheat streak mosaic virus* (WSMV) as measured by percent incidence (%) and relative absorbance (% of positive control))[wx].

Scientific name	Growth cycle[y]	Inc. (%)		(SE)[z]	Rel abs. (%)		(SE)
Aegilops cyrindlica	Winter annual	80.9	a	4.1	87.1	ab	5.2
Agropyron cristatum	Perennial	0	f	0			
Agropyron repens	Perennial	12.7	ef	4.7	38.1	ef	2.0
Avena fatua	Summer annual	53.9	b	8.6	104.3	a	5.3
Elymus canadensis	Perennial	4.8	f	2.8	22.3	f	3.0
Elymus lanceolatus	Perennial	0	f	0			
Elymus trachycaulus	Perennial	0	f	0			
Bromus catharticus	Winter annual	0	f	0			
Bromus intermis	Perennial	0	f	0			
Bromus japonicus	Winter annual	42.4	bcd	11.7	54.6	cde	7.0
Bromus secalinus	Winter annual	52.1	b	15.1	79.7	b	10.3
Bromus tectorum	Winter annual	32.6	cd	6.1	48.0	de	2.7
Echinochloa crus-galli	Summer annual	26.0	de	13.2	56.0	cd	4.2
Eragrostis cilianensis	Summer annual	0	f	0			
Lolium sp.		9.9	ef	2.6	26.5	f	3.6
Secale cereale	Winter annual	46.2	bc	8.7	72.2	bc	6.9
Setaria viridis	Summer annual	28.1	de	10.3	38.2	ef	6.9
Sorghum halapense	Perennial	0	f	0			
Triticum aestivum (negative control)		0	f	0			

[w] All grass species were tested by ELISA three weeks after mechanical inoculation of WSMV Montana isolate Conrad-I. Percent incidence (inc.) was determined from screening eight plants per pot. The inoculated Choteau exhibited 100% incidence. Relative absorbance (rel. abs.) was determined as average of pooled leaf samples from two pots, and those species with zero incidences were excluded from the statistical analysis. Calculation of the relative absorbance is described in the materials and methods. Plant species with zero incidence but detectable relative absorbance are due to high background from healthy wheat and some species of grassy weeds. Plants were considered ELISA-positive if the absorbance value minus the absorbance of the buffer was twice the value of the negative control.

[x] Means followed by the same letter within each column are not significantly different at P = 0.05. Data were log transformed and analyzed by ANOVA. Means comparisons were performed using a Fisher's LSD designating replications as a random factor.

[y] USDA-NRCS, 2010.

[z] SE, standard error

Table 3.8. Difference in incidence (%) and relative absorbance (% of positive control) among grassy weed species from multiple Great Plains states to a Montana strain of *Wheat streak mosaic virus* (WSMV)[xy].

Scientific name	State of origin	Inc. (%)	(SE)	Rel. abs. (%)		(SE)
Aegilops cylindrica	Colorado	85.6	4.6	105.6	a	6.7
	Idaho	81.8	4.1	103.1	a	12.1
	Montana	65.7	13.2	43.2	b	2.9
	Nebraska	90.5	4.7	96.6	a	5.4
Agropyron repens	Idaho	16.7	9.6	34.8		2.6
	Montana	9.4	4.4	40.0		2.7
Avena fatua	Idaho	65	11.6	104.5		6.9
	Montana	42.7	11.7	104.0		8.3
Bromus tectorum	Colorado	32.5	11.5	43.2		6.5
	Idaho	32	17.8	47.2		5.1
	Montana	27.6	10.2	48.3		7.0
	Nebraska	45.3	22.3	54.2		3.8
	Oklahoma	25.4	4.6	46.5		8.6
Secale cereale	Colorado	57.6	8.9	71.7		8.6
	Oklahoma	34.9	14.0	72.6		10.9
Setaria viridis	Idaho	33.3	18.3	34.4		8.1
	Montana	22.9	11.4	43.9		13.1

[x] All grass species were tested three weeks after mechanical inoculation with WSMV isolate Conrad-I for incidence (inc.) and relative absorbance (rel. abs.).
[y] Means followed by the same letter within each grass species and column are not significantly different at P = 0.05. Data were log transformed and analyzed by ANOVA with experiments designated as a random factor. Means comparisons were performed using a Fisher's LSD.
[z] SE, standard error.

Absorbance in grassy weeds relative to spring wheat was measured as a way to estimate the susceptibility of grassy weed species to WSMV. The relative absorbance varied by grass species ($P < 0.01$; Tables 3.7). Among the individual grass species, *A. fatua* (104.3%) was as susceptible to WSMV as spring wheat, followed by *A. cylindrica* (87.1%), *B. secalinus* (79.7%), and *S. cereale* (72.2%, Table 3.7). The relative absorbance of *B. japonicus* was 54.6% and *B. tectorum* was 48.0%. For the remaining

two summer annuals, *S. viridis* had 38.2% relative absorbance while *E. crus-galli* had 56.0% that of spring wheat. *E. canadensis* and *Lolium sp.* showed very low relative absorbance. *B. catharticus*, *B. inermis*, *E. cilianensis*, *S. halapense*, and wheatgrasses were not significantly different from the negative control (data not shown). Among the grass species with multiple state biotypes, *A. cylindrica* was only species which showed a significant difference between Montana and the other states ($P < 0.01$; Table 3.7). Differences in relative absorbance due to state of origin were not observed for other grass species ($P_{A.\ fatua} = 0.84$, $P_{A.\ repens} = 0.23$; $P_{B.\ tectorum} = 0.18$, $P_{S.\ viridis} = 0.58$ $P_{S.\ cereale} = 0.66$).

Evaluation of WSMV Transmission by WCM from Grassy Weeds to Wheat

Transmission of WSMV from grassy weeds to cereal crops is an important step in evaluating grassy weeds as a source of inoculum. Grass species varied significantly in their capacity to serve as transmission hosts for WSMV by the WCM ($P < 0.01$; Table 3.9). The highest transmission efficiency was observed from spring wheat (46.7%). *A. cylindrica, B. japonicus*, *B. secalinus*, and *B. tectorum* had approximately 20.0% transmission efficiency. *E. canadensis*, *E. crus-galli*, *Lolium sp.*, *S. cereale*, and *S. viridis* were poor virus sources with less than 10.0% transmission efficiency. No transmission was observed from *A. cristatum*, *A. fatua*, *A. repens*, or *B. inermis*.

Table 3.9. Comparison of *Wheat streak mosaic virus* (WSMV) transmissibility in percent incidence (%) by the wheat curl mite (WCM) from grassy weeds to spring wheat, and susceptibility to WCM and WSMV according to previous studies[wx].

	Susceptibility[y]		Transmission		
Scientific name	WCM	WSMV	Incidence (%)		(SE)[z]
Aegilops cylindricae	S	S	22.5	bc	7.5
Agropyron repens	S	I	0	d	0
Avena fatua	I	S	0	d	0
Bromus japonicus	S	S	23.3	b	6.7
Bromus secalinus	S	S	23.3	b	3.3
Bromus tectorum	S	S	20	bc	4.1
Echinochloa crus-galli	S	S	5	d	2.9
Elymus canadensis	S	S	6.7	d	3.3
Lolium sp.	S	S	6.7	d	3.3
Secale cereale	S	S	10	cd	0
Setaria viridis	S	S	10	cd	5.8
Triticum aestivum (positive control)	S	S	46.7	a	8.8
Triticum aestivum (negative control)	S	S	0		0

[w] WSMV source plants were ELISA-positive plants from relative susceptibility experiments. Wheat controls were spring wheat variety 'Choteau'.

[x] Percent incidence was calculated as the number of plants ELISA positive for WSMV / total number plants infested with viruliferous mites. This experiment was performed four times and analyzed by ANOVA where replications were designated as a random factor. Means followed by the same letter are not significantly different at P = 0.05. Means comparisons were performed with a least significant difference test.

[y] Data for WCM susceptibility are from Somsen and Sill, 1970; Townsend and Johnson, 1996; and Brey *et al* 1998 (S = susceptible; I = immune).

[z] SE, standard error

Discussion

This study represents the first quantification of the susceptibility of alternative hosts to WSMV, the first characterization of the susceptibility of weed biotypes from a large geographic area to WSMV, the first demonstration of mite transmission of WSMV

from grassy weeds to wheat, and the first report of TriMV infecting alternative hosts in the field.

Winter annuals from across the Great Plains included more species susceptible to WSMV in terms of incidence and relative absorbance than the summer annuals or perennials. In our field survey of Montana, winter annuals were more frequently identified and denser in population than summer annuals. This observation fits with crop management in the surveyed area, which produces primarily winter wheat. The summer annual, *A. fatua,* was as susceptible to WSMV as spring wheat. However, *A. fatua* does not support WCM and was found infected at very low numbers (<1%) in field surveys and a past study (Coutts *et al*, 2008). In addition, mites were not able to transmit WSMV from *A. fatua* to wheat. The life cycle of winter annuals closely matches winter wheat, which has been thought to limit their role in the 'green bridge' during the summer. However, winter annuals germinate in the fall may provide a very important overwintering reservoir for the virus and mite vector in northern climates. In addition, early maturity as compared to the cereal crop may encourage movement of the mite off a winter annual during the tillering or early reproductive stages of both winter and spring crops. This movement of the mite to reproductive hosts (summer annuals including crops) would then build the mite populations for infection of winter annuals in the fall. In contrast, summer annuals will not provide an overwintering reservoir of virus or mite and are less susceptible to WSMV. Other studies have favored summer annuals such as *E. crus-galli* and *S. viridis* as important sources of WSMV (Christian and Willis, 1993). However, it is unclear what the source of infection in that study was, and whether the

plant community could be a significant source of viral inoculum to the crop. It is likely that the importance of grass species as a source of WSMV and WCM varies according to geographic region and cropping practices. Components contributing to the importance of the inoculum source include the cropping system (population, composition and density of plant species), as well as the temporal dynamics of these species (Malmstrom *et al*, 2005).

In addition to testing grass species *per se*, we also investigated whether grass species from different states in the Great Plains that varied in the frequency of WSMV epidemics would also vary in their susceptibility to WSMV. We found many grassy weeds prevalent in the Great Plains region are susceptible to WSMV and can serve as sources of inoculum for transmission by the WCM. However, there was very little variation in the susceptibility to one strain of WSMV based on the geographic origin of the plant, indicating that variation in regional weed biotype is not a major contributor to epidemic frequency. However, regional variations in WSMV strains and mite biotypes may contribute to epidemic frequency. A study by McNeil *et al* (1996) indicated genetic variability of WSMV is high within a single crop field. Preliminary testing in the greenhouse and field indicates that the origin of WSMV, either different states or within the same state, will affect the susceptibility of the wheat variety (data not shown). For example, varieties of winter wheat from Texas are more susceptible to a strain of WSMV collected from Texas than the Conrad-I strain from Montana (data not shown). This indicates potential regional variability of viral strains.

In addition to viral strains, the genetic variation in mites and their host adaptation affect transmission efficiency and mite reproduction. Siriwetwiwat (2006) found WCM biotypes were as variable on a single head of wheat as in an entire field, and Seifers (2002) and Coutts (2008) found regional variation in the transmission of WMoV and WSMV, respectively, from different sources of WCM. Our laboratory (data not shown) and Siriwetwiwat (2006) have found that WCM reproduce more quickly on plants infected with WSMV collected in the same geographic area as the WCM. The capacity of WCM to reproduce is reduced on a host to which it is not adapted (Del Rosario and Sill, 1965; Orlob, 1966). This was seen in our laboratory, where WCM collected from many grass species would not feed and reproduce on wheat (data not shown), and with other eriophyid mites such as *Abacarus hystrix* (Skoracka and Kuczyński, 2006). Taken together, regional and host adaptation of virus and mite biotypes are likely critical to epidemic development.

Cultural practices are still the key to controlling WSM epidemics. The high prevalence of continuous wheat cropping in southern states (Padgitt *et al*, 2000) favors epidemics. Sites in our study with continuous wheat, such as sites 8 and 12 (Table 3.1), had relatively high viral incidences in the crop. The 'green bridge' in northern climates is increasingly favored by incorporation of 'stay-green' traits in spring wheat varieties (Blake *et al*. 2007), late maturity of cereal crops and early planting of winter wheat to increase yields and to ensure adequate growth before winter (Matz, 1991). In addition, an increase in the density of grassy weeds has been facilitated by widespread use of semi-dwarf crop varieties, late weed control, reduced or no tillage, and application of

broadcast nitrogen fertilizer (Blackshaw, 1994; Derksen *et al*, 2002). No-till practices associated with continuous cropping increase densities of grassy weeds such as *B. tectorum* (Anderson *et al*, 1998; Blackshaw, 1994). In addition, changes in climate (Anderson *et al*, 2004; Harvell *et al*, 2002; Jones, 2009) and invasive species composition (Malmstrom *et al*, 2005) may alter viral epidemiology in future years. These factors, and the identification of WMoV and TriMV throughout the Great Plains (Burrows *et al*, 2009a, 2009c), may increase the importance of cereal viruses in the future.

One problem encountered during this study was a high background absorbance level of healthy grassy weeds in the ELISA assay. Their absorbance values were lower than the value of healthy wheat; however when assaying alternative host species, researchers must be aware of this issue. Additionally, raw absorbance values of controls varied from plate to plate due to variations in incubation time and age and condition of host plant leaf tissue (D. Ito, *personal observation*). In this study, controls were included to account for this variation, but our method could be improved for future studies. Specifically, an improved ELISA method used in our laboratory includes 1) calculating a 99% confidence interval for each plant species to establish the negative threshold; 2) reducing background level and error rate (false negative) by optimizing blocking agents and antibody concentrations for each plant species; and 3) comparing relative absorbance using a more robust standardized scale, such as Z-scores (Z. Miller, *unpublished data*). The Z-score compares plate standard deviations in absorbance of the different host species to a plate-level mean absorbance. Preliminary results indicate the sensitivity of ELISA is increased by use of these modifications (Z.Miller, *personal communication*).

This study is the first to report TriMV infection of alternative hosts in the field. Studies here are consistent with results from Seifers *et al.* (2010) who identified differences in the host ranges of WSMV and TriMV. Although barley is resistant to mechanical inoculation by WSMV (Ito *et al*, 2010), it is susceptible to TriMV as found in our survey and results from Kansas (Seifers *et al*, 2010).

This study indicates susceptibility of the grassy weeds measurable by incidence and relative absorbance is one of the contributing factors for understanding WSMV epidemiology. Grassy weeds showing either high incidence or relative absorbance are likely WSMV reservoirs, but not always key hosts in the field. We must consider local distribution of the grassy weeds and WCM movements in the field, which differ in the geography for further understanding of frequency and severity of local WSMV epidemics.

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CONCLUSION

This field study of currently popular wheat varieties revealed that the tolerance to *Wheat streak mosaic virus* (WSMV) infection varies by cultivar. Level of symptom severity and viral incidence do not truly represent the effect of WSMV on yield. Our study indicates evaluation of the variety tolerance to WSMV must be strictly evaluated by yield loss due to WSMV infection, not by symptoms or incidence. Incidence and relative absorbance (representing relative viral titer) vary by the varieties and their state of origin in greenhouse studies. This does not support our assumptions that states with high frequency of WSMV epidemics may have more susceptible wheat varieties than the low frequency states. We believe differences in the frequency of WSMV epidemics in the region is likely due to: 1) different agricultural practices rather than overall variety measurable susceptibility to WSMV including incidence and symptom severity; and 2) strain variability of WSMV and wheat curl mite (WCM).

Prevalent grassy weeds in the Great Plains states were semi-quantitatively compared for the first time for WSMV. The viral incidence and relative absorbance vary by the grassy weeds species, and the difference was not observed in all regional biotypes of the grassy weeds. Summer annuals were not more distinctly susceptible to WSMV than winter annuals. Together with our field surveys, we postulate that summer annuals are not as important in local WSMV epidemiology as volunteer crops. Despite moderate susceptibility, *B. tectorum* was frequently identified from small grain fields in Montana, indicating viral movement between wheat and the grassy weeds is abundant near the WSMV infected field. We believe the relative importance of grassy weeds as a WSMV

reservoir depends on their local distributions often associated with geography and cultural practices, and additionally by virulifeous WCM movements.

This study can be a steppingstone for further studies useful for future breeding programs and for further evaluation of relative importance of grassy weeds in local WSMV epidemiology. Susceptibility of wheat varieties and grassy weeds are only one of the contributing factors, so distribution of grassy weeds, cultural practices, and WCM movements at local levels must be taken into account to understand wheat virus epidemics and insight into the efficient control of the wheat viruses.

APPENDICES

APPENDIX A

EVALUATION OF POPULAR WHEAT VARIETIES IN MONTANA TO WHEAT STREAK MOSAIC INFECTION, 2008

Table A.1. Disease severity scale (1-25) as measured by visual symptoms and incidence (%) of Montana winter wheat varieties mechanically inoculated with *Wheat streak mosaic virus* (WSMV) in 2008[wxyz]

	Disease severity scale (1-25)						Incidence (%)			
	Fall			Spring			Fall		Spring	
Variety			(SE)			(SE)		(SE)		(SE)
CDC Falcon	3.3	ab	0.8	17.5	ab	5	8.8	5.5	57.5	15.6
Genou	3.3	ab	0.8	7	c	3	10	2	65	6.5
Jagalene	4	a	0	16.5	a	3.3	12.5	6.6	75	6.1
Ledgar	1.8	bc	0.8	3.3	c	0.8	10	4.6	28.8	10.1
Morgan	1.8	bc	0.8	16.5	a	3.3	2.5	2.5	73.8	11.1
MTV0734	1	c	0	4.5	c	1.7	6.3	2.4	38.8	16.8
Neeley	1	c	0	4	c	0	6.3	4.7	55	9.1
Pryor	2.5	abc	0.9	5.3	c	1.3	10	7.1	38.8	7.5
Rampart	3	abc	2	7.5	c	3.3	6.3	3.8	43.8	12
Rocky	1	c	0	8.3	bc	2.8	3.8	2.4	50	15.4
Tiber	2.5	abc	0.9	4.5	c	1.7	16.3	5.5	37.5	1.4
Yellowstone	1.8	bc	0.8	7.5	c	3.3	7.5	6	55	10
Mean	2.2		0.3	8.5		1	8.3	1.3	51.6	3.4

[w] Hail damage at 22 July, 2008 prevented an accurate estimate of symptom severity, incidence, and agronomic variables.

[x] Symptom severity was rated as 1 = no symptoms to 5 = severe yellowing and streak. Disease severity scale was calculated as described in the materials and methods. Fall represents fall inoculation and spring represents spring inoculation. WSMV incidence was measured as the percent of ELISA- positive flag leaves (n = 20) in inoculated plots. Fall represents fall inoculation and spring represents spring inoculation.

[y] Means followed by the same letter are not significantly different at P = 0.10. Severity data were rank transformed and incidence data were log transformed. Both were analyzed by PROC GLM and compared using Fisher's LSD. Symptom severity varied by variety ($P_{fall} = 0.06$; $P_{spring} < 0.01$).

[z] SE, standard error.

Table A.2. Disease severity scale and incidence (%) of Montana spring wheat and barley varieties mechanically inoculated with *Wheat streak mosaic virus* (WSMV) in 2008[wxyz].

	Disease severity scale (1-25)			Incidence (%)	
Variety			(SE)		(SE)
Amidon	7.0	cd	2.5	40.0	17.4
Choteau	22.8	a	2.3	41.3	16.6
Conan	14.3	bc	1.8	35.0	14.9
Corbin	5.3	d	1.3	47.5	6.6
Ernest	18.8	ab	3.9	61.3	8.3
Fortuna	9.5	cd	2.5	17.5	4.3
Hank	17.0	abc	4.6	30.0	9.8
Mcneal	4.0	d	0.0	56.3	5.2
Reeder	14.3	bc	1.8	40.0	3.5
Scholar	12.5	bc	2.0	57.5	11.3
Haxby	N/A			N/A	
Metcalfe	N/A			N/A	
Mean	12.6		1.1	42.6	3.7

[w] Hail damage at 22 July, 2008 prevented an accurate estimate of symptom severity, incidence, and agronomic variables.

[x] Symptom severity was rated as 1 = no symptoms to 5 = severe yellowing and streak. Disease severity scale was calculated as described in the materials and methods. WSMV incidence was measured as the percent of ELISA- positive flag leaves (n = 20) in inoculated plots. Fall represents fall inoculation and spring represents spring inoculation.

[y] Means followed by the same letter are not significantly different at P = 0.10. Severity data were rank transformed and incidence data were log transformed. Both were analyzed by PROC GLM and compared using Fisher's LSD. Symptom severity varied by variety ($P < 0.01$).

[z] SE, standard error.

Table A.3. The effect of variety and time of *Wheat streak mosaic virus* (WSMV) mechanical inoculation on yield (kg/ha) of winter wheat in Bozeman, Montana in 2008[w] [xyz]

	Yield (kg/ha)		Yield compared to control (kg)					
Variety	Control	(SE)	Fall		(SE)	Spring		(SE)
CDC Falcon	2293	106	276	abc	140	576	a	244
Genou	3251	360	-384	bcd	327	-808	cde	190
Jagalene	2719	396	-271	bc	299	-280	bc	366
Ledger	2716	191	787	a	399	848	a	346
MTV0734	3619	414	-1128	bc	265	-1341	e	185
Morgan	2873	308	-210	d	201	-696	cde	288
Neeley	3017	184	-534	cd	277	-1284	e	267
Pryor	2820	239	400	ab	250	407	ab	323
Rampart	3395	184	-495	cd	207	-530	cde	268
Rocky	2090	26	-111	bc	355	-398	bc	262
Tiber	2925	365	-1102	d	280	-1195	de	398
Yellowstone	2994	259	-490	cd	358	-409	bcd	373
Mean	2893	93	-272		108	-426		128

[w] Hail damage at 22 July, 2008 prevented an accurate estimate of symptom severity, incidence, and agronomic variables.

[x] Control, uninoculated rows; Fall, fall inoculated rows; Spring, spring inoculated rows.

[y] Means within each column followed by same letter are not significantly different from each other at P = 0.10. Means were analyzed by ANOVA over variety after yield of control and the inoculated plots were paired (see materials and methods), and compared by Fisher's LSD. Yield loss varied by variety ($P_{fall} < 0.01$; $P_{spring} < 0.01$).

[z] SE, standard error.

Table A.4. Effect of variety and mechanical inoculation of *Wheat streak mosaic virus* (WSMV) on yield (kg/ha) in spring wheat in Bozeman, 2008[xyz]

	Yield (kg/ha)		Yield compared to control (kg)		
Variety	Control	(SE)	Spring		(SE)
Amidon	771	41	68	a	66
Choteau	1914	221	643	bc	147
Conan	1702	87	411	bc	132
Corbin	1641	166	296	ab	132
Ernest	1241	91	430	bc	97
Fortuna	1414	138	463	bc	167
Hank	2148	52	468	bc	136
McNeal	1718	71	685	c	159
Reeder	1189	72	271	ab	103
Scholar	1222	117	561	bc	144
Mean	1496	70	430		46

[x] Hail damage at 22 July, 2008 prevented an accurate estimate of symptom severity, incidence, and agronomic variables.

[y] Means within each column followed by same letter are not significantly different from each other at P = 0.10. Data were analyzed by ANOVA over variety after yield of control and the inoculated plots were paired (see materials and methods), and compared by Fisher's LSD. Yield loss varied by variety (P = 0.07).

[z] SE, standard error.

Table A.5. The effect of timing of mechanical inoculation of *Wheat streak mosaic virus* (WSMV) on seed quality in winter wheat and spring wheat varieties planted in Bozeman, Montana, 2008[wxyz].

	Protein (%)			Test weight (kg/hL)			1000 kernel weight (g)		
Inoculation			(SE)			(SE)			(SE)
Winter wheat									
Control	13.4	ab	0.2	78.2	a	0.3	25.1	ab	0.4
Fall	13.4	b	0.2	77.9	a	0.3	25.3	a	0.4
Spring	13.8	a	0.2	76.1	b	0.4	24.4	b	0.5
Spring wheat									
Control	17.6		0.2	77.0	a	0.4	29.9	a	0.9
Spring	17.6		0.1	75.1	b	0.6	27.5	b	0.8

[w] Hail damage at 22 July, 2008 prevented an accurate estimate of symptom severity, incidence, and agronomic variables.

[x] Control, uninoculated rows; Fall, fall inoculated rows; Spring, spring inoculated rows.

[y] Means followed by the same letter within each column are not significantly different at P=0.10. Data were analyzed by ANOVA and means separations calculated using Fisher's LSD. All variables in winter wheat were varied by inoculation (P < 0.01). Test weight and a thousand kernel weight in spring wheat varied by inoculation (P < 0.01).

[z] SE, standard error

Table A.6. Effect of variety and mechanical inoculation of *Wheat streak mosaic virus* (WSMV) on the agronomic variables in winter wheat, 2008[xyz]

	Protein content (%)			Test weight (g/hL)			Thousand kernel weight (g)				
Variety	Control	Fall	Spring	Control	Fall	Spring	Control	Fall		Spring	
CDC Falcon	13.5	0.1	-0.6	75.8	-1.0	3.6	21.2	-0.4	abc	1.2	cde
Genou	13.7	0.0	-0.4	78.7	0.1	2.5	22.2	-0.6	ab	1.3	cde
Jagalene	13.1	0.4	-0.2	80.1	-0.4	1.5	26.6	-0.6	ab	-0.7	ab
Ledger	12.9	0.4	0.3	78.5	0.4	-0.5	26.2	-1.2	a	-0.9	a
Morgan	12.8	-0.1	-0.5	78.6	0.9	2.9	25.4	-0.3	abc	0.8	abcde
MTV0734	12.7	-0.5	-0.9	78.3	1.3	0.4	27.9	0.7	bc	1.0	bcde
Neeley	14.5	0.2	-0.1	77.0	0.4	2.1	26.3	-0.8	ab	0.0	abcd
Pryor	13.0	0.4	0.2	79.7	0.7	1.4	24.3	-1.0	a	-0.3	abc
Rampart	15.1	-0.1	-0.3	77.2	1.4	-0.8	23.6	0.6	bc	1.1	bcde
Rocky	12.5	-0.3	-0.9	78.6	0.6	3.1	22.2	0.4	bc	1.4	de
Tiber	14.0	-0.2	-0.9	77.6	-0.1	2.5	25.6	1.0	c	2.2	e
Yellowstone	13.2	0.3	0.1	78.2	-1.1	4.0	28.5	-0.7	ab	0.2	abcd
Mean	13.4	0.0	-0.3	78.2	0.3	1.9	25.0	-0.2		0.6	

[x] Hail damage at 22 July, 2008 prevented an accurate estimate of symptom severity, incidence, and agronomic variables.

[y] Control, uninoculated rows; Fall, variables from fall inoculated rows compared to control; Spring, variables from spring inoculated rows compared to control.

[z] Means followed by the same letter within each column are not significantly different at P=0.10. Statistically significant differences were not observed in any variables without mean separation letters. Data were analyzed by ANOVA over variety after each variable of control and the inoculated plots were paired (see materials and methods) and means separations calculated using Fisher's LSD. Differences in a thousand kernel weight due to WSMV inoculation varied by variety ($P_{fall} = 0.06$; $P_{spring} = 0.04$).

Table A.7. Effect of variety and mechanical inoculation of *Wheat streak mosaic virus* (WSMV) on the agronomic variables in spring wheat and barley varieties planted in Montana, 2008[xyz].

	Protein content (%)		Test weight (g/hL)			Thousand kernel weight (g)			Height (cm)	
Variety	Ctrl	Inoc.	Ctrl	Inoc.		Ctrl	Inoc.		Ctrl	Inoc.
Amidon	18.4	-0.6	76.2	-2.7	bc	24.3	-0.9	a	69.6	-14.4
Choteau	17.7	0.1	77.6	-1.5	ab	28.4	-1.8	ab	59.6	-12.1
Conan	16.6	0.2	76.8	-0.9	ab	31.0	-1.1	ab	57.3	-7.8
Corbin	17.0	0.1	78.7	-2.3	bc	31.7	-2.1	ab	64.3	-8.1
Ernest	18.1	-0.1	77.8	-2.1	bc	26.8	-2.4	abc	68.1	-14.8
Fortuna	17.8	0.2	77.7	-2.5	bc	33.2	-4.4	cd	71.5	-10.9
Hank	16.8	-0.1	75.2	-1.3	ab	35.3	-2.3	abc	65.2	-10.1
Mcneal	18.4	0.6	75.4	-3.6	c	31.4	-5.0	d	60.6	-11.1
Reeder	17.5	0.1	76.6	0.0	a	27.8	-1.1	ab	64.2	-12.0
Scholar	17.8	-0.2	78.2	-1.9	abc	29.3	-3.0	bcd	63.7	-11.5
Mean	17.6	0.0	77.0	-1.9		29.9	-2.4		64.4	-11.3

[x] Hail damage at 22 July, 2008 prevented an accurate estimate of symptom severity, incidence, and agronomic variables.

[y] Ctrl, uninoculated rows; Inoc., variables from inoculated rows compared to control.

[z] Means followed by the same letter within each column are not significantly different at P=0.10. Statistically significant differences were not observed in any variables without mean separation letters. Data were analyzed by ANOVA over variety after each variable of control and the inoculated plots were paired (see materials and methods) and means separations calculated using Fisher's LSD. Differences due to WSMV inoculation varied by variety in test weight ($P = 0.05$) and a thousand kernel weight ($P < 0.01$).

Table A.8. Spearman correlation among disease severities and change in agronomic variables caused by mechanical inoculation of *Wheat streak mosaic virus* (WSMV) in fall inoculated winter wheat, 2008[xyz].

	Yield	Protein	TWT	TKW	Severity	Incidence
Yield (kg/ha)	1.00					
Protein (%)	-0.55 (< 0.01)	1.00				
Test weight (g/hL)	0.11 (0.45)	-0.17 (0.26)	1.00			
TKW (g)	0.49 (0.00)	-0.70 (< 0.01)	0.40 (0.01)	1.00		
Severity	-0.13 (0.37)	0.01 (0.93)	-0.21 (0.16)	0.04 (0.76)	1.00	
Incidence (%)	-0.10 (0.49)	0.21 (0.15)	-0.03 (0.82)	0.00 (0.99)	0.47 (< 0.01)	1.00

[x] Hail damage at 22 July, 2008 prevented an accurate estimate of symptom severity, incidence, and agronomic variables.

[y] Data were analyzed by PROC CORR (SAS v.9.2, Cary, NC) after each variable of control and the inoculated plots were paired as the calculation described in the materials and methods.

[z] P-value of each combination was shown in parenthesis.

Table A.9. Spearman correlation among disease severities and change in agronomic variables caused by mechanical inoculation of *Wheat streak mosaic virus* (WSMV) in spring inoculated winter wheat, 2008[xyz].

	Yield	Protein	TWT	TKW	Severity	Incidence
Yield (kg/ha)	1.00					
Protein (%)	-0.49 (0.00)	1.00				
Test weight (g/hL)	0.19 (0.19)	-0.29 (0.05)	1.00			
TKW (g)	0.37 (0.01)	-0.67 (< 0.01)	0.47 (0.00)	1.00		
Severity	-0.11 (0.44)	-0.07 (0.66)	0.41 (0.00)	0.10 (0.50)	1.00	
Incidence (%)	0.12 (0.43)	-0.14 (0.34)	0.38 (0.01)	0.24 (0.11)	0.48 (0.00)	1.00

[x] Hail damage at 22 July, 2008 prevented an accurate estimate of symptom severity, incidence, and agronomic variables.

[y] Data were analyzed by PROC CORR (SAS v.9.2, Cary, NC) after each variable of control and the inoculated plots were paired as the calculation described in the materials and methods.

[z] P-value of each combination was shown in parenthesis.

Table A.10. Spearman correlation among disease severities and change in agronomic variables caused by mechanical inoculation of *Wheat streak mosaic virus* (WSMV) in spring wheat, 2008[xyz].

	Yield	Protein	TWT	TKW	Height	Severity	Incidence
Yield (kg/ha)	1.00						
Protein (%)	-0.30 (0.06)	1.00					
Test weight (g/hL)	0.24 (0.13)	-0.14 (0.39)	1.00				
TKW (g)	0.44 (0.00)	-0.34 (0.03)	0.22 (0.18)	1.00			
Height (cm)	0.18 (0.27)	0.05 (0.77)	0.34 (0.03)	0.00 (0.99)	1.00		
Severity	0.08 (0.64)	0.43 (0.01)	-0.48 (0.00)	-0.32 (0.04)	0.04 (0.79)	1.00	
Incidence (%)	0.13 (0.41)	0.19 (0.24)	0.21 (0.18)	-0.09 (0.60)	0.29 (0.07)	0.09 (0.57)	1.00

[x] Hail damage at 22 July, 2008 prevented an accurate estimate of symptom severity, incidence, and agronomic variables.

[y] Data were analyzed by PROC CORR (SAS v.9.2, Cary, NC) after each variable of control and the inoculated plots were paired as the calculation described in the materials and methods.

[z] P-value of each combination was shown in parenthesis.

APPENDIX B

ADDITIONAL INFORMATION ON RESULTS FOR EVALUATION OF POPULAR WHEAT VARIETIES IN MONTANA TO WHEAT STREAK MOSAIC INFECTION, 2009

Table B.1. Effect of variety and mechanical inoculation of *Wheat streak mosaic virus* (WSMV) on the agronomic variables in winter wheat, 2009[yz]

	Protein content (%)			Test weight (g/hL)				Thousand kernel weight (g)		
Variety	Control	Fall	Spring	Control	Fall	Spring		Control	Fall	Spring
CDC Falcon	13.5	-0.1	0.4	79.8	-0.7	-3.2	ef	31.8	0.4	-2.2
Genou	13.8	0.1	0.2	79.3	0.8	-0.7	ab	32.0	0.4	-1.3
Jagalene	13.9	0.0	0.2	80.8	0.5	-1.6	abc	38.6	-0.3	-1.9
Ledger	13.4	-0.1	0.2	79.0	0.0	-0.4	a	37.1	-0.3	-2.1
Morgan	13.2	0.2	0.4	79.6	-0.6	-3.5	f	34.0	0.2	-2.7
MTV0734	14.1	0.2	0.3	78.1	-0.4	-1.2	abc	35.9	1.6	-0.2
Neeley	12.7	0.1	0.7	80.7	0.2	-2.9	def	39.7	0.1	-3.8
Pryor	12.0	0.5	0.6	81.0	-0.7	-3.9	f	32.3	0.6	-1.5
Rampart	14.6	0.1	0.1	78.6	0.1	-1.8	bcd	31.0	1.3	0.2
Rocky	13.7	0.2	0.3	80.1	-0.4	-1.6	bc	30.9	-0.6	-1.2
Tiber	13.8	-0.1	-0.2	80.3	0.3	-0.9	ab	36.7	-0.5	-1.2
Yellowstone	12.6	-0.2	0.2	79.7	-0.2	-2.1	cde	39.1	-0.1	-3.0
Mean	13.4	0.1	0.3	79.8	-0.1	-2.0		34.9	0.2	-1.7

[y] Control, uninoculated rows; Fall, variables from fall inoculated rows compared to control; Spring, variables from spring inoculated rows compared to control.

[z] Means followed by the same letter within each column are not significantly different at P=0.10. Statistically significant differences were not observed in any variables without mean separation letters. Data were analyzed by ANOVA over variety after each variable of control and the inoculated plots were paired (see materials and methods) and means separations calculated using Fisher's LSD. Differences in test weight varied by variety in spring-inoculated rows ($P_{spring} < 0.01$).

Table B.2. Effect of variety and mechanical inoculation of *Wheat streak mosaic virus* (WSMV) on the agronomic variables in spring wheat and barley varieties planted in Montana, 2009 [yz]

	Protein content (%)		Test weight (g/hL)			Thousand kernel weight (g)			Height (cm)		
Variety	Ctrl	Inoc.	Ctrl	Inoc.		Ctrl	Inoc.		Ctrl	Inoc.	
Amidon	14.8	0.4	78.1	-1.9	abc	33.6	-2.8	bcd	84.0	-8.7	bc
Choteau	15.0	0.2	79.0	-2.6	bc	33.6	-2.9	cd	66.6	-3.9	bc
Conan	14.7	0.2	78.5	-0.8	ab	37.3	-1.0	ab	68.9	-3.0	bc
Corbin	13.6	0.3	80.6	-2.2	ab	40.6	-2.7	bcd	69.6	-4.4	bc
Ernest	14.8	0.7	80.0	-2.4	bc	34.6	-2.1	bc	81.9	-11.6	c
Fortuna	14.6	0.6	79.8	-2.2	bc	42.1	-4.3	d	80.8	-4.7	bc
Hank	13.8	0.3	77.3	-1.2	ab	41.5	-1.8	abc	65.5	12.1	a
Mcneal	14.4	0.7	77.3	-3.3	c	34.8	-3.2	cd	72.0	-6.2	bc
Reeder	15.0	-0.1	78.8	-1.5	ab	33.8	-1.5	abc	74.6	-5.7	bc
Scholar	15.3	0.1	78.9	-1.8	ab	34.6	-2.5	bc	78.9	-7.1	bc
Haxby	12.1	0.0	67.3	-0.1	a	39.3	-2.4	a	63.7	-2.0	b
Metcalfe	13.1	0.1	68.2	0.0	a	40.8	-0.2	a	75.9	-1.7	b
$Mean_{spring\ wheat}$	14.6	0.4	78.8	-2.0		36.7	-2.5		74.3	-4.3	
$Mean_{barley}$	12.6	0.1	12.6	0.0		12.6	-1.3		12.6	-1.9	

[y] Ctrl, uninoculated rows; Inoc., variables from inoculated rows compared to control.

[z] Means followed by the same letter within each column are not significantly different at P=0.10. Statistically significant differences were not observed in any variables without mean separation letters. Data were analyzed by ANOVA over variety after each variable of control and the inoculated plots were paired (see materials and methods) and means separations calculated using Fisher's LSD. Differences due to WSMV inoculation varied by variety in test weight (P = 0.06), a thousand kernel weight (P = 0.09), and height (P = 0.06).

Table B.3. Spearman correlation among disease severities and change in agronomic variables caused by mechanical inoculation of *Wheat streak mosaic virus* (WSMV) in fall inoculated winter wheat, 2009[yz].

	Yield	Protein	TWT	TKW	Severity	Incidence
Yield (kg/ha)	1.00					
Protein (%)	-0.08 (0.59)	1.00				
Test weight (g/hL)	0.06 (0.68)	-0.01 (0.93)	1.00			
TKW (g)	0.17 (0.25)	0.17 (0.24)	-0.28 (0.06)	1.00		
Severity	0.00 (0.98)	-0.21 (0.15)	0.03 (0.82)	-0.05 (0.74)	1.00	
Incidence (%)	0.11 (0.44)	-0.17 (0.24)	0.07 (0.64)	-0.16 (0.28)	0.15 (0.31)	1.00

[y] Data were analyzed by PROC CORR (SAS v.9.2, Cary, NC) after each variable of control and the inoculated plots were paired as the calculation described in the materials and methods.
[z] P-value of each combination was shown in parenthesis.

Table B.4. Spearman correlation among disease severities and change in agronomic variables caused by mechanical inoculation of *Wheat streak mosaic virus* (WSMV) in spring inoculated winter wheat, 2009[yz].

	Yield	Protein	TWT	TKW	Severity	Incidence
Yield (kg/ha)	1.00					
Protein (%)	-0.30 (0.04)	1.00				
Test weight (g/hL)	0.36 (0.01)	-0.57 <.0001	1.00			
TKW (g)	0.44 (0.00)	-0.18 (0.23)	0.36 (0.01)	1.00		
Severity	-0.05 (0.74)	-0.01 (0.94)	0.10 (0.48)	-0.14 (0.36)	1.00	
Incidence (%)	0.06 (0.71)	-0.12 (0.41)	0.22 (0.13)	-0.15 (0.30)	0.24 (0.10)	1.00

[y] Data were analyzed by PROC CORR (SAS v.9.2, Cary, NC) after each variable of control and the inoculated plots were paired as the calculation described in the materials and methods.
[z] P-value of each combination was shown in parenthesis.

Table B.5. Spearman correlation among disease severities and change in agronomic variables caused by mechanical inoculation of *Wheat streak mosaic virus* (WSMV) in spring wheat, 2009[yz].

	Yield	Protein	TWT	TKW	Height	Severity	Incidence
Yield (kg)	1.00						
Protein (%)	-0.03 (0.83)	1.00					
Test weight (g/hL)	0.33 (0.04)	-0.28 (0.08)	1.00				
TKW (g)	0.08 (0.61)	0.16 (0.31)	0.47 (0.00)	1.00			
Height (cm)	0.31 (0.05)	-0.05 (0.78)	0.23 (0.16)	0.23 (0.15)	1.00		
Severity	0.17 (0.30)	0.00 (1.00)	0.15 (0.36)	0.02 (0.88)	0.24 (0.13)	1.00	
Incidence (%)	0.13 (0.42)	-0.04 (0.82)	0.29 (0.07)	0.28 (0.08)	0.35 (0.03)	-0.13 (0.42)	1.00

[y] Data were analyzed by PROC CORR (SAS v.9.2, Cary, NC) after each variable of control and the inoculated plots were paired as the calculation described in the materials and methods.

[z] P-value of each combination was shown in parenthesis.