



Epidemiology of wheat curl mite (*Aceria to.*) and wheat streak mosaic virus on feral grass species and effect of glyphosate on wheat curl mite dispersal
by Christopher William Brey

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in
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Abstract:

Wheat streak mosaic virus (WSM) is a devastating virus disease found throughout the Great Plains of the United States and Canada. The wheat curl mite (WCM) *Aceria tosichella* Keifer, is the only known vector of wheat streak mosaic virus (WSMV). The primary overwintering host for the mite and virus is volunteer wheat. However, in some cases where volunteer wheat was low or absent outbreaks have occurred suggesting feral grasses may be serving as alternate hosts for the mite and virus.

The goal of this project was to examine the role of feral grass species in WCM and WSMV epidemiology. Annual and perennial grasses from a major winter wheat producing area in north central Montana were surveyed for *A. tosichella*, and assayed by protein antibody sandwich enzyme linked immunosorbent assay (PAS-ELISA) for WSMV during the summers of 1995, 1996, and 1997. *Aceria tosichella* was collected from *Bromus inermis* Leys, *Poa interior* Rydberg, *Poa pratensis* L., and *Triticum aestivum* L. Three of the annual grasses, *Bromus japonicus* Thunb., *Bromus tectorum* L., and *T. aestivum*, and five species of perennial grasses, *Agropyron cristatum* (L.) Gaertn., *B. inermis*, *P. pratensis*, *Stipa comata* Trin. & Rupr., and *Stipa viridula* Trin., tested positive for the WSMV. None of the grass plants that tested positive had WSM-like symptoms except *T. aestivum*. The results of the pathogenicity tests indicated that only *Aegilops cylindrica* Host, and *Aven fatua* L. were susceptible to both the WCM and WSMV. More importantly, WCMs that acquire the WSMV from these two grass species can transmit the virus to healthy wheat. *Bromus inermis*, *P. pratensis* and *S. comata* were shown to be suitable hosts for WCMs, but in all tests were immune to WSMV. The results of the molecular analysis indicated that WSMV was easily detected by the two serological and two nucleic acid based assays for infected *T. aestivum*, *A. cylindrica* and *A. fatua* grown in the greenhouse and symptomatic *T. aestivum* plants collected in the field. Although, serological tests indicated *P. pratensis* exposed to viruliferous WCMs in the laboratory as negative for WSMV infection, the presence of WSMV was detected in immunocapture reverse transcription polymerase chain reaction (IC-RT-PCR) test. However, the PCR signal was faint suggesting WSMV detection in WCMs and not the plant host. These findings suggest, with the exception of *A. fatua* and *A. cylindrica*, that feral grasses play a limited role in WSMV and WCM epidemiology and that volunteer wheat is still the primary host for the mite and virus.

In addition, the effect of glyphosate, a broad spectrum herbicide, was assessed to determine the dispersal response of WCMs on wheat seedlings exposed to field rates of 420 and 840 ml/ha inside a laboratory wind tunnel. The major conclusion of this study was that the recommended rate (840 ml/ha) of glyphosate did increase WCM dispersal within 42 hours of initial application.

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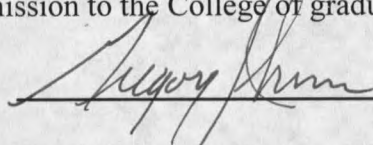
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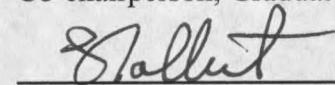
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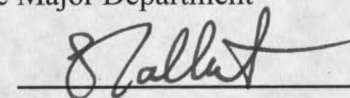


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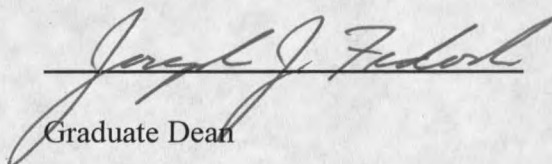


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I dedicate this thesis to my parents William and Bernadine Brey and in memory of
James Gabor, research entomologist.

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ABSTRACT

Wheat streak mosaic virus (WSMV) is a devastating virus disease found throughout the Great Plains of the United States and Canada. The wheat curl mite (WCM) *Aceria tosichella* Keifer, is the only known vector of wheat streak mosaic virus (WSMV). The primary overwintering host for the mite and virus is volunteer wheat. However, in some cases where volunteer wheat was low or absent outbreaks have occurred suggesting feral grasses may be serving as alternate hosts for the mite and virus.

The goal of this project was to examine the role of feral grass species in WCM and WSMV epidemiology. Annual and perennial grasses from a major winter wheat producing area in north central Montana were surveyed for *A. tosichella*, and assayed by protein antibody sandwich enzyme linked immunosorbent assay (PAS-ELISA) for WSMV during the summers of 1995, 1996, and 1997. *Aceria tosichella* was collected from *Bromus inermis* Leys, *Poa interior* Rydberg, *Poa pratensis* L., and *Triticum aestivum* L. Three of the annual grasses, *Bromus japonicus* Thunb., *Bromus tectorum* L., and *T. aestivum*, and five species of perennial grasses, *Agropyron cristatum* (L.) Gaertn., *B. inermis*, *P. pratensis*, *Stipa comata* Trin. & Rupr., and *Stipa viridula* Trin., tested positive for the WSMV. None of the grass plants that tested positive had WSM-like symptoms except *T. aestivum*. The results of the pathogenicity tests indicated that only *Aegilops cylindrica* Host. and *Avena fatua* L. were susceptible to both the WCM and WSMV. More importantly, WCMs that acquire the WSMV from these two grass species can transmit the virus to healthy wheat. *Bromus inermis*, *P. pratensis* and *S. comata* were shown to be suitable hosts for WCMs, but in all tests were immune to WSMV. The results of the molecular analysis indicated that WSMV was easily detected by the two serological and two nucleic acid based assays for infected *T. aestivum*, *A. cylindrica* and *A. fatua* grown in the greenhouse and symptomatic *T. aestivum* plants collected in the field. Although, serological tests indicated *P. pratensis* exposed to viruliferous WCMs in the laboratory as negative for WSMV infection, the presence of WSMV was detected in immunocapture reverse transcription polymerase chain reaction (IC-RT-PCR) test. However, the PCR signal was faint suggesting WSMV detection in WCMs and not the plant host. These findings suggest, with the exception of *A. fatua* and *A. cylindrica*, that feral grasses play a limited role in WSMV and WCM epidemiology and that volunteer wheat is still the primary host for the mite and virus.

In addition, the effect of glyphosate, a broad spectrum herbicide, was assessed to determine the dispersal response of WCMs on wheat seedlings exposed to field rates of 420 and 840 ml/ha inside a laboratory wind tunnel. The major conclusion of this study was that the recommended rate (840 ml/ha) of glyphosate did increase WCM dispersal within 42 hours of initial application.

CHAPTER 1

LITERATURE REVIEW

Distribution of Wheat Streak Mosaic Disease

Wheat streak mosaic (WSM) is one of the most important and widely distributed virus diseases of wheat (*Triticum aestivum* L.). First observed in Nebraska in 1922 by Peltier (1), it is now recognized throughout the Great Plains of North America, eastern Europe, and regions of the Ukraine (2). It is most prevalent in the central and western parts of North America, where severe damage to winter and spring wheat crops occurs annually. Since 1949, WSM has caused heaviest wheat yield losses in Kansas (3,4,5) and southern Alberta, Canada (6,7) and from time to time has caused serious economic losses in Nebraska (8), South Dakota (9), North Dakota (10), Colorado (11), Wyoming (12), and Montana (13, 14). Although less severe, WSM has been reported in Idaho (15), Washington (16), California (17), Oklahoma (18), and Texas (19). Outside North

America, WSM has been identified in Rumania, Jordan, Yugoslavia, Russia and Turkey (20, 21).

Economic Importance

In the Great Plains, WSM is estimated to reduce annual wheat yields by 2% each year. However, localized losses of up to 100% are not uncommon in some endemic regions (22). Greatest losses over the past 20 years have occurred in Kansas. From 1976 to 1987, the state averaged 2.5% wheat yield loss annually, and in 1988 estimated statewide losses were 13%, or 42 million bushels (23). From 1988 to 1991, losses in the state have averaged 15.5 million bushels per year (3). One of the most severe WSM epidemics in the northern Great Plains occurred in North Dakota in 1988. Statewide wheat yield losses that year were approximately \$40.1 million (10). Since 1954, when the first case of wheat streak mosaic was observed in Montana, major outbreaks have occurred in 1964, 1981, 1993 and 1994 (13). The most recent (1993-1994) wheat streak epidemic in Montana caused severe crop losses valued at \$50 million (J. Riesselman, Department of Plant Pathology, Montana State University, personal communication).

Disease Symptomology

The first symptoms of WSM consist of a faint green mottling of the leaves, followed by the development of light green and yellow blotches or streaks running

parallel to the leave veins. As the disease progresses, a general stunting of the plant can be detected (8). In addition, plants are prostrate and produce an abnormally large number of tillers. At a distance, severely diseased wheat appears lighter in color and by casual examination may be misinterpreted as nitrogen deficiency (8, 24). Severely infected plants tend to be less upright and do not produce heads; less seriously infected plants may produce sterile heads containing shriveled kernels. Symptoms may vary depending on virus strain, variety of wheat, plant health, time of infection, soil fertility, co-infection with another virus (i.e., High Plains virus) and temperature (8, 14, 24).

Etiological Agent: Wheat Streak Mosaic Virus

Wheat streak mosaic virus (WSMV), *Marmor virgatum* McK., is a member of the genus *Rymovirus* (25) in the family Potyviridae. A recent submission to reclassify WSMV based on its coat protein and 3' non-coding region into a separate genus, *Whestervirus*, has been proposed (26). The virus is a long (700 nm X 13 nm) flexous, rod shaped viron containing a single-stranded RNA genome of approximately 8,500 base pairs. The nucleotide sequence of the 3' poly (A) terminal region encoding the capsid gene protein is known, but the majority of the genome has not yet been characterized (27). At least two characteristics distinguish WSMV from other potyviruses: 1) the virus is vectored solely by the wheat curl mite (WCM), *Aceria tosichella* Keifer (28), and 2) the capsid protein is larger than the 32 to 36 Kda coat protein reported for most potyviruses. However, WSMV has also been known to be transmitted in the field

between infected and healthy wheat plants through abrasive leaf action during high winds (29). Despite these differences WSMV is currently classified as a member of the mite transmitted subgroup 3 potyvirus (30) because, similar to other potyviruses, it induces the production of pinwheeled shaped cytoplasmic inclusion bodies composed of a 66 Kda protein (31). Under field conditions an individual infected wheat plant may be co-infected with High Plains virus (HPV) (32), Wheat spot mosaic virus (WspMV) (33) and agropyron mosaic virus (AgMV) (34).

Description and Biology of the Wheat Curl Mite

Aceria tosichella, a member of the family Eriophyoidea, is the only known vector of WSMV. Originally the WCM was designated *Aceria tulipae* (Keifers), described from tulips, however since 1970 the mite occurring on wheat has been renamed *Aceria tosichella* Keifer (35, 36). Adult mites are (length: ca. 250 μ m) wingless, white, cigar-shaped arthropods with two pairs of legs near their anterior cephalic region (8). They prefer to feed near the edges of wheat leaves causing the leaves to curl and bend inward producing a protective microhabitat. The mites reproduce by laying eggs. Females are capable of laying 8 to 12 eggs during their lifetime (8). The shortest life cycle from egg to egg was reported to be seven days at 25°C (37). The normal life cycle from egg to egg occurs between 10 to 12 days (8). Like many other eriophyids, *A. tosichella* has two nymphal instars. The virus is not transmitted transovarially, but by both adult and nymphal stages (37). Wheat streak mosaic virus is transmitted by *A. tosichella* in a

persistent manner, that is mites removed from a virus source are still able to transmit the virus after 7 days (38). Approximately 15 minutes are required for the mite to acquire the virus, and it may remain infective for 7-9 days through molts without additional acquisitions (37). The primary mode of mite dispersal from plant to plant is by wind. Other insects such as aphids and leaf hoppers have been reported to transport mites from plant to plant (39, 40). In addition to WSMV, *A. tosicHELLa* has transmitted High Plains virus (32, 40), WspMV (33) and brome streak mosaic virus (42).

Methodologies for Detection of WSMV in Native and Cereal Grasses

Since WSMV was first recognized in Nebraska (1), researchers have used a variety of methods for detecting the virus in Gramineae species. Techniques for the diagnosis of WSMV infection in feral and cultivated grasses have ranged from the use of indicator plants to the molecular technique of reverse transcription polymerase chain reaction (RT-PCR). Detection tests for WSMV include indicator plants, immunological specific electron microscopy (ISEM), enzyme linked immunosorbent assays (ELISA), dot blots, and RT-PCR are described below.

Indicator Plants

McKinney (43) first described the carborundum method for determining monocotyledonous plants infected with WSMV. This procedure involves the mechanical inoculation of sap from the suspected source plant to an indicator plant, (i.e., a host plant

that expresses disease symptoms when infected). Since then, researchers have used this method for distinguishing WSMV infection in feral grasses (44, 45) and for the separation of virus strains based on symptom expression in cereals (46, 47). Indicator plants such as, *Agropyron intermedium* L., *Hordeum jubatum* L. and *Avena sativa* L. have been used to aid in the differentiation of WSMV, AgMV, and an unrecognized hordeum mosaic virus found in Canada, respectively (48).

Immunological Specific Electron Microscopy (ISEM)

Immunological specific electron microscopy, more commonly known as leaf-dip serology, first reported by Ball and Brake (49) has become a fast, efficient, and popular method for virus identification (50). Carroll et al. (47) employed this technique to distinguish WSMV infection from barley yellow dwarf and nitrogen deficiency in diseased spring and winter wheat plants collected from commercial fields in Montana. Leaf-dip serology was used to aid in WSMV identification in several wheat cultivars in Colorado (46). Langenberg (51) discovered antigen reaction to WSMV antiserum in leaf-dips depended on the age of the wheat leaf. He found that the young fully expanded leaves reacted completely or partially with antiserum, whereas virons from older leaves did not react. He also observed that histologically fixed WSM virons in ultrathin sections could be immunolabelled in leaves of all ages. He attributes this negative antiserum reaction in the leaf-dip test to the degradation of virons in the older leaves during the extraction step.

Enzyme Linked Immunosorbent Assay (ELISA)

Clarke and Adams (52) first reported the use of ELISA as a very effective detection assay for plant viruses. Many variations of this basic procedure have been used in an attempt to optimize the test for a specific purpose. The basic method is very economical and sensitive for detecting virus concentrations as low as 1-10 nanograms per ml (53).

Direct double antibody sandwich ELISA (52), involves a solid phase (i.e., polystyrene microtiter plate) which is first coated with virus antibody in the immunoglobulin (IgG) form. Next, the IgG traps the virus antigen. The trapped virus is then revealed by an enzyme-labeled specific antibody that reacts when a substrate is added to give rise to a visible product which can be measured in a spectrophotometer. Two major disadvantages of this procedure are: 1) it is highly strain specific and 2) requires a different enzyme-conjugated antibody for each virus that is tested.

Indirect double antibody sandwich ELISA involves the final detection step to be conjugated to an antiglobulin IgG (54) or protein A (55). In this procedure, antiviral IgG is prepared in two animal species; one to trap (raised in a rabbit) and one to detect (chicken antirabbit globulin) the virus with enzyme conjugate specific for the latter. Thus, one conjugated IgG can be used to assay a range of related viruses.

Various modified ELISA procedures have been used by many researchers for WSMV detection in feral grasses (56, 57) and cereals such as spring and winter wheat cultivars, maize, barley, and sorghum crops (58, 59, 60, 61, 62). Christian and Willis (57) used an indirect ELISA assay as described by Lommel et al. (63) to determine which

grass species were infected with WSMV in Kansas. Montana et al. (56) employed a direct antigen plating ELISA to detect agropyron mosaic virus and WSMV in grasses near the periphery of wheat fields in Oklahoma. A case reported by Hunger and Sherwood (60) showed that a polyclonal antibody to WSMV cross-reacted with healthy wheat. To remedy this situation they substituted the polyclonal antiserum with a monoclonal antibody and reaction to healthy wheat was eliminated.

Dot Blot and Leaf Squash Assays

Both the dot blot (review: 64, 65) and leaf squash (review: 66) methods are simpler, faster, and cheaper assays than ELISA, but they allow quantification of virus only when bound antibody is monitored by radioactivity (64). Bottacin and Nassuth (64) used these procedures to detect levels of WSMV infection in mechanically inoculated wheat, barley, maize, and oats grown under laboratory conditions in Canada. They found that both assays revealed a positive correlation between symptom severity and amount of virus that accumulated in the four cereals. Also, they found grinding a small amount of young leaf tissue in a mortar and pestle in buffer and then blotting this material onto nitrocellulose paper was sufficient to transfer enough WSMV antigen to be detected by a WSMV antibody. An earlier comparison studied by Sherwood (67) also concluded that filter paper immunobinding assay (similar to dot blot assay) was easier, faster, and cheaper to use than both western blotting and ELISA when detecting WSMV, but ELISA was the best assay for virus quantification. Makkouk and Jarikji (62) reported ELISA to be

1000X more sensitive than double immunodiffusion (similar to dot blot assay) in detecting WSMV in purified and wheat plant extracts.

Reverse Transcription Polymerase Chain Reaction

Reverse transcription polymerase chain reaction (review: 68, 69) has become an important molecular technique for detecting low titer RNA viruses and generally has been found to be more sensitive than any of the other protein, nucleic acid, and serological based assays (70). A comparative study conducted by Mathews et al. (71) showed that RT-PCR was more reliable than ELISA at detecting citrus tristeza virus in trees in the field during months of nonoptimal titer. They reported RT-PCR detected citrus tristeza virus when ELISA could not, and that ambiguous ELISA readings could be retested with RT-PCR to resolve the erroneous ELISA results. There has been a concerted effort to develop RT-PCR assays for many of the pathogenic plant RNA viruses. RT-PCR assays have been developed for apple chlorotic leaf spot and apple stem grooving virus (70), soybean mosaic virus (72), tomato spotted wilt tospovirus (73), cucumber mosaic virus (74) zucchini yellow mosaic virus (75), barely yellow dwarf virus (76) and WSMV (77, 78).

Dependent on the choice of primers, RT-PCR facilitates the detection of a broad group of related or specific virus strains. For instance, differentiation of strains of cucumber mosaic virus (74) and citrus tristeza virus (71) have been enhanced by primers derived from the variable region of their genomes and then differentiated by further

analysis of amplified products by the use of restriction endonuclease and restriction fragment length polymorphism (RFLPs). A similar study by McNeil et al. (77) used RT-PCR and RFLP technique to characterize genomic differences in WSMV isolates collected from within and among wheat fields in six Nebraska wheat counties. They found three main and twenty nine minor lineages of WSMV co-circulating in the region over two years. This was one of the first studies to report virus variation from plant to plant in nature.

Cereals as Hosts for the Wheat Curl Mite and Wheat Streak Mosaic Virus

Wheat streak mosaic virus and WCM have a broad host range encompassing many cereal crops. They employ all varieties of wheat, rye, barley, and oats as hosts (79). Several varieties of maize (80, 81), sorghum (61), rice (82) and millet (39) are also susceptible to both the mite and virus. However, winter wheat is the primary overwintering host harboring both the WCM and WSMV, and volunteer wheat germinating before or after harvest is the most important oversummering host for the mite and virus (8, 83).

Annual Grasses as Hosts for the Wheat Curl Mite
and Wheat Streak Mosaic Virus

Many native annual grass species have been reported susceptible to manual inoculation with WSMV and/or viruliferous WCMs (refer to Table 1.1 for host summary). In order to be important in WSM epidemiology, it is not only necessary that annual grasses provide a source of virus, but also be a suitable host for the mite. Several annual grasses such as *Aegilops cylindrica* (83), *Digitaria sanguinalis* (84), and *Setaria viridis* (37) have been documented to support both the WCM and WSMV naturally in the field, apparently posing a potential threat to wheat (Table 1.2).

McKinney and Fellows (85) isolated WSMV from *A. cylindrica* growing along roadways in western Kansas, while Connin (86) collected WCMs from this species in several central Kansas counties. Greenhouse studies indicate *A. cylindrica* to be a fair to good host for WCM survival and reproduction (86). Although actual mite numbers were not reported, he did observe the characteristic rolling and trapping of leaves in this species. He also found that WCMs from *A. cylindrica* could transmit WSMV to wheat. Similar results were obtained in a greenhouse study by Burns et al. (14). *Aegilops cylindrica* is genetically related to wheat (87); it is a winter annual with a life-cycle that parallels winter wheat. Many have speculated that it is probably not an important host in WSM epidemiology (8, 84, 85). However, in some cases before winter wheat matures, *A. cylindrica* may have the potential to be an important early summer host (green period May to June) (45).

Table 1.1. Susceptibility of annual grass species to wheat curl mite (WCM) infestation and wheat streak mosaic virus (WSMV) infection under laboratory conditions.

Scientific name	Common Name	Increase of WCM	WSMV Transmission		Authority
			Manual Inoculation	WCM Infestation	
<i>Aegilops crassa</i> Boiss.			Susceptible		(3, 4)
<i>Aegilops cylindrica</i> Host.	Jointed goatgrass	Fair-Good	Susceptible	Immune, Susceptible	(2, 3, 4, 11, 12)
<i>Aegilops ovata</i> L.			Susceptible		(3, 4)
<i>Aegilops triuncialis</i> L.	Barb goatgrass		Susceptible		(3, 4)
<i>Aegilops ventricosa</i> Tausch.			Susceptible		(3, 4)
<i>Aristida adscensionis</i> L.	Sixweek threeawn	Resistant			(2)
<i>Aristida oligantha</i> Mich.	Prarie threeawn		Immune	Immune	(7)
<i>Avena fatua</i> L.	Wild oats	None	Susceptible	Susceptible	(1, 2, 4, 11)
<i>Beckmannia syzigachne</i> (Steud.) Fernald	American sloughgrass	Susceptible	Immune		(2)
<i>Bromus japonicus</i> Thumb.	Japanese brome	None	Susceptible	Susceptible	(1, 2, 3, 4, 12)
<i>Bromus tectorum</i> L.	Cheatgrass brome	None, Susceptible	Susceptible	Immune	(1, 2, 11, 12)
<i>Carex</i> L.	Sedge	Susceptible	Immune		(2)
<i>Cenchrus pauciflorus</i> Benth.	Sandbur	Good	Susceptible	Susceptible	(2, 4, 7, 11, 12)
<i>Coix lacryma-jobi</i> Toun.	Job's tears		Immune		(1, 4)
<i>Digitaria ischaemum</i> (Schreb)	Smooth crabgrass	Fair-good	Susceptible	Susceptible	(3, 4, 11, 12)
<i>Digitaria sanguinalis</i> (L.) Scop.	Crabgrass	None, Susceptible Very-poor	Susceptible	Susceptible, Immune	(1, 2, 4, 7, 11, 12)
<i>Echinochloa crusgalli</i> (L.) Beauv.	Barnyard grass	Poor	Susceptible	Susceptible	(1, 2, 4, 7, 11, 12)
<i>Eleusine indica</i> (L.) Gaertn.	Goosegrass	None	Immune	Immune	(2, 4, 11, 12)
<i>Eragrostis cilianensis</i> (All.)	Stinkgrass	Poor, Susceptible	Susceptible, Immune	Susceptible	(1, 2, 4, 11, 12)
		Poor, None	Susceptible	Susceptible, Immune	
<i>Eragrostis pilosa</i> (L.) Beauv.	Indian lovegrass	Resistant			(2)

Table 1.1. (continued)

Scientific name	Common Name	Increase of WCM	WSMV Transmission		Authority
			Mannual Inoculation	WCM Infestation	
<i>Euchlaena mexicana</i> Schrad.	Teosinte	Poor, Resistant	Immune	Immune	(2, 11, 12)
<i>Eriochloa contracta</i> Hitch.	Praire cupgrass			Susceptible	(7)
<i>Haynaldia villosa</i> Schur.			Susceptible		(3, 4)
<i>Hordeum gussonianum</i> Parl.	Mediterranean barley		Susceptible		(3, 4)
<i>Hordeum jubatum</i> L.	Wild barley		Immune		(4)
<i>Hordeum murinum</i> Huds		None, Susceptible	Susceptible		(3, 4)
<i>Panicum capillare</i> L.	Witchgrass		Susceptible, Immune	Susceptible	(1, 2, 4, 8, 11, 12)
<i>Panicum dichotomiflorum</i> Mchx.	Fall panicum			Immune	(7)
<i>Phalaris paradoxa</i>			Susceptible		(1, 3, 4)
<i>Phleum pratense</i> L.	Ttimothy	Poor	Susceptible		(2)
<i>Poa annua</i> L.	Annual bluegrass	Poor	Susceptible		(2)
<i>Schedonnardus</i>		None			
<i>paniculatus</i> (Nutt.) Trel.	Tumblegrass			Immune	(7)
<i>Setaria faber</i> Herrm.	Giant foxtail			Susceptible	(7)
<i>Setaria lutescens</i>					
(Weigel) Hubb.	Yellow foxtail		Immune	Immune	(1, 4, 9, 12)
<i>Setaria verticillata</i> Beauv.	Bristly foxtail	Poor	Susceptible	Susceptible	(1, 2, 4, 12)
<i>Setaria viridis</i> (L.) Beauv.		None, Poor			
Hermgreen	Foxtail		Susceptible	Susceptible	(1, 3, 4, 7, 9, 11, 12)

1: Slykhuis 1955

2: Somsen & Sill 1970

3: McKinney & Fellows 1951

4: Sill & Connin 1953

5: Gibson 1957

6: Anderson 1971

7: Christian 1993

8: Brake et al. 1990

9: Staples & Allington 1956

10: Gibson & Painter 1957

11: Connin 1956

12: Wehing 1956

Table 1.2. Annual grasses reported to be naturally infected with the wheat streak mosaic virus (WSMV) and/or infested with wheat curl mites (WCM).

Scientific name	Common name	WSMV	WCM	Authority
<i>Aegilops cylindrica</i> Host	Jointed goatgrass	Susceptible	Fair-good	(1, 2, 3)
<i>Avena fatua</i> L.	Wild oats	Susceptible	None	(1)
<i>Bromus japonicus</i> Thunb.	Japanese brome	Susceptible	Fair	(1, 2, 3)
<i>Bromus secalinus</i> L.	Cheatgrass	Susceptible	Poor	(2)
<i>Bromus tectorum</i> L.	Downy brome	Susceptible	Poor	(1, 3, 4)
<i>Cenchrus pauciflorus</i> Benth.	Sandbur	Susceptible	Poor	(1, 2, 5, 6)
<i>Digitaria sanguinalis</i> (L.) Scop.	Crabgrass	Susceptible	Fair-good	(2, 5, 6)
<i>Echinochloa crusgalli</i> (L.) Beauv.	Barnyard grass	Susceptible	Poor	(1, 2, 5, 6, 7)
<i>Eragrostis cilianensis</i> (All.) Lutati.	Stinkgrass	Susceptible	Fair-poor	(1, 2, 5, 6, 9)
<i>Eriochloa contracta</i> Hitchc.	Prairie cupgrass	Susceptible	No data	(7)
<i>Panicum capillare</i> L.	Common witchgrass	Susceptible	Fair-poor	(1, 2, 5, 6, 7, 9)
<i>Panicum dichotomiflorum</i> Michx.	Fall panicum	Susceptible	No data	(8)
<i>Setaria faberi</i> Herrm.	Giant foxtail	Susceptible	No data	(7)
<i>Setaria viridis</i> (L.) P. Beauv.	Green foxtail	Susceptible	Fair-good	(1, 2, 4, 5, 6, 7)
<i>Setaria verticillata</i> L.	Bristly foxtail	Susceptible	Poor	(6)

1: Slykhuis 1955

2: Somsen & Sill 1970

3: McKinney & Fellows 1951

4: Sill & Connin 1953

5: Gibson 1957

6: Anderson 1971

7: Christian 1993

8: Brake et al. 1990

9: Staples & Allington 1956

Digitaria sanguinalis has been found to be frequently infected with WSMV in nature and easily infected with the virus in the greenhouse (45). Gibson (84) reported thriving populations of *A. tulipae* (the nomenclature has since been changed to *A. tosicHELLa*) on several *D. sanguinalis* plants collected in a shaded basement well at the Kansas State University Agriculture Experiment Station in Colby, Kansas. McKinney and Fellows (85) found *D. sanguinalis* to give mosaic symptoms by manual inoculation. Sill and Connin (45) reported WCM reproduction to be very poor on *D. sanguinalis*, whereas Somson and Sill (83) and Connin (86) found this species to be an acceptable WCM host. Interestingly, Connin (81) discovered the few mites that survived on *D. sanguinalis* when transferred to wheat were still able to transmit the virus. *D. sanguinalis* is a warm weather troublesome grass species found in temperate and subtropical areas of North America. In Montana, it is known to be common throughout the state (88). It reaches maturity in late summer before senescing in early fall. As the plant dies in early fall, mites are forced to leave the plant in search of a more succulent host. Dispersed by wind currents, they may land in adjacent wheat fields initiating a WSM outbreak (85).

Setaria viridis is probably the most frequent WSMV virus infected grass (83). A recent grass survey in Kansas serologically confirmed this species to be infected with WSMV in the field (57). McKinney and Fellows (85) reported *S. viridis* as susceptible to mechanical inoculation, and Slykhuis (37) found this species to be successfully inoculated with virus both manually and with mites. Connin (86) and Slykhuis (28) were unable to establish a WCM population on this species in the greenhouse, while del

Rosario and Sill (89) reported fair colonies on 85% of the 40 *S. viridis* plants infested. They postulated that different biotypes of *A. tosichella* exist which may account for their successful colonization of WCMs on *S. viridis* (previous attempts had failed). *S. viridis* has been found to be naturally infested with WCMs in Kansas (84, 85) while researchers in Nebraska have found only one or two mites on many sampled *S. viridis* (24, 45). However, there have been a few isolated outbreaks of WSM in Nebraska traced to *S. viridis* (90). Since *S. viridis* is cool season grass that grows from July through September, it may provide a suitable intermediate host for the mite and virus during the most critical period between the harvest and planting of winter wheat (83).

The general opinion in the scientific community is that the majority of annual grasses are probably not important in WSM epidemiology (8, 24). Although many grasses can be infected with WSMV in the greenhouse by WCMs and/or mechanical inoculation, the combination of poor mite hosts and plant host life-cycle (i.e., *A. cylindrica* and *D. sanguinalis*) are not conducive to contributing to a WSM epidemic. However, it is possible that under the right circumstances, a few viruliferous mites from annual grasses (i.e., *S. viridis*) might establish a foci in volunteer wheat or in cultivated wheat and cause a local outbreak.

Perennial Grasses as Hosts for the Wheat Curl Mite
and Wheat Streak Mosaic Virus

Numerous species of perennial grasses have been reported susceptible to WSMV and WCM in the greenhouse (refer to Table 1.3 for host summary). A few perennial grasses have been shown to be natural hosts for the mite or virus (Table 1.4), but only *Elymus canadensis*, *Elymus virginicus*, *Bouteloua hirsuta*, *Lolium perenne*, and *Sitaton hystrix* have been reported as hosts for both. Although, *Pascopyrum smithii* is immune to the virus, it is believed to be the most important perennial grass host for the wheat curl mite (83).

Elymus canadensis is an important range and pasture species often infected with the virus (45, 85). This species rarely carries large populations of mites, but occasionally some heavily infested plants are found (83). A grass survey in western Nebraska found *E. canadensis* to have flourishing WCM populations on only two young plants sampled (8). Slykhuis (37) was able to achieve mite virus infection in *E. canadensis* despite failure to establish a WCM colony on this grass species. He ascertained that a strain difference in the mite may account for this vector-host barrier. Connin (86) however was able to obtain fair mite infestations on *E. canadensis*, but was unable to obtain virus infection by mites. McKinney and Fellows (80) reported *E. canadensis* as being either a symptomless carrier, or exhibiting local lesions or mosaic symptoms when mechanically inoculated with the virus. *Elymus canadensis* is a hardy perennial grass that may serve as

Table 1.3. Susceptibility of perennial grass species to wheat curl mite (WCM) infestation and wheat streak mosaic virus (WSMV) infection under laboratory conditions.

Scientific name	Common Name	Increase of WCM	WSMV Transmission		Authority
			Manual Inoculation	WCM Infestation	
<i>Agropyron cristatum</i> (L.) Gaertn	Fairway wheatgrass	Susceptible	Immune		(10)
<i>Agropyron dasystachyum</i> (Hook.) Scribn.	Thickspike wheatgrass		Immune		(10)
<i>Agropyron elongatum</i> Beauv.	Tall wheatgrass	None	Immune	Immune	(10, 12)
<i>Agropyron lasianthum</i> Boiss.			Susceptible		(10)
<i>Agropyron repens</i> (L.) Beauv.	Quackgrass	Susceptible	Immune		(10)
<i>Agropyron rigidum</i> Beauv.			Immune		(10)
<i>Agropyron trachycaulum</i> (Link) Malte	Slender wheatgrass	Susceptible	Immune		(10)
<i>Agropyron trichophorum</i> (Link) Richt.	Stiffhair wheatgrass		Immune		(10)
<i>Alopercurus pratensis</i> L.	Meadow foxtail	None	Immune	Immune	(12)
<i>Andropogon gerardii</i> Vitm.	Big bluestem	Susceptible	Immune	Immune	(10)
<i>Andropogon scoparius</i> Michx.	Little bluestem	None	Immune	Immune	(10)
<i>Arrhenatherum elatius</i> L. Beauv.	Tall oatgrass	Poor	Immune	Immune	(12)
<i>Bouteloua hirsuta</i> Lag.	Grama	Good	Symptomless Immune	Susceptible, Immune	(10)
<i>Bromis inermis</i> Leyss.	Smooth brome	Very poor	Immune	Immune	(10, 12)
<i>Bromis tectorum</i> L.	Downy brome	Susceptible	Susceptible		(2)
<i>Buchloe dactyloides</i> (Nutt.) Engelm.	Buffalograss	None, susceptible	Immune	Immune	(2, 7, 12)
<i>Cynodon dactyon</i> (L.) Pers.	Bermudagrass	Susceptible	Susceptible	Immune	(2, 7)
<i>Dactylis glomerata</i> L.	Orchard grass	None	Immune	Immune	(11, 12)
<i>Distichlis stricta</i> (Torr.) Rydb.	Inland saltgrass	Susceptible	Susceptible		(2)
<i>Elymus canadensis</i> L.	Canada wildrye	Fair	Susceptible	Immune, Susceptible	(2, 3, 4, 11, 12)
<i>Elymus condensatus</i> Presl.	Giant wildrye		Susceptible		(3, 4)

Table1.3. (continued)

Scientific name	Common Name	Increase of WCM	WSMV Transmission		Authority
			Manual Inoculation	WCM Infestation	
<i>Eragrostis sessilispica</i> Buckl.	Tumble lovegrass		Immune	Immune	(7)
<i>Eragrostis trichodes</i> (Nutt.) Wood	Sand lovegrass		Susceptible		(2, 3, 4)
<i>Festuca</i> L.	Fescue	Susceptible		Immune	(3)
<i>Festuca rubra</i> L.	Red fescue		Immune		(4)
<i>Hordeum pusillum</i> Nutt.	Little barley	Susceptible	Immune		(2)
<i>Koeleria cristata</i> (L.) Pers.	Prairie junegrass	Susceptible			(2)
<i>Lolium perenne</i> L.	Perennial ryegrass	Susceptible			(2)
<i>Muhlenbergia mexicana</i> (L.) Trin.	Bearded wirestem mugly	Susceptible			(2)
<i>Munroa squarrosa</i> (Nutt.) Torr.	False buffalograss	Susceptible			(2)
<i>Oryzopsis hymenoides</i> (Roem. & Schult) Ricker	Indian ricegrass	Poor	Symptomless	Susceptible	(3, 4, 12)
<i>Panicum virgatum</i> L.	Switchgrass	None, Susceptible	Immune	Immune	(2, 3, 4, 11, 12)
<i>Phalaris arundinacea</i> L.	Reed canary grass	None	Immune	Immune	(4, 11, 12)
<i>Pascopyrum smithii</i> Rybd.	Western wheatgrass	Poor-Fair	Immune	Immune	(10, 12)
<i>Phleum pratense</i> L.	Timothy	Susceptible	Immune		(2, 4)
<i>Poa annua</i> L.	Annual bluegrass		Susceptible		
<i>Poa bulbosa</i> L.	Bulbous bluegrass		Susceptible		(3, 4)
<i>Poa compressa</i> L.	Canada bluegrass		Susceptible		(3,4)
<i>Poa pratensis</i> L.	Kentucky bluegrass	Susceptible	Immune		(2, 4)
<i>Poa stenantha</i> Trin.			Symptomless		(3, 4)
<i>Puccinellia airoides</i> (Nutt.)	Nuttall alkaligrass	Resistant			(2)
<i>Schedonnardus paniculatus</i> (Nutt.) Trel.	Tumblegrass	None			(2)
<i>Setaria pumila</i> (Poir.) Roem. & Schult.	Yellow foxtail	Susceptible	Susceptible, Immune		(2, 7)
<i>Sitanion hystrix</i> (Nutt.) J.G. Smith	Bottlebursh	None	Susceptible		(2)
<i>Elymus giganteus</i> Vahl.			Susceptible		(3, 4)

Table 1.3. (continued)

Scientific name	Common Name	Increase of WCM	WSMV Transmission		Authority
			Manual Inoculation	WCM Infestation	
<i>Elymus virginicus</i> L.	Virginia wildrye	Susceptible	Susceptible		(2, 3, 4)
<i>Sorghastrum nutans</i> (L.) Nash	Indian grass	None	Immune	Immune	(2, 3, 4, 7, 12)
<i>Sorghum halepense</i> L.	Johnson grass	Good, None	Immune	Immune	(2, 4, 11, 12)
<i>Sporobolus asper</i> (Michx.) Kunth.	Tall dropseed		Immune	Immune	(7)
<i>Sporobolus cryptandrus</i> (Torr.) A. Gray	Sand dropseed		Immune	Immune	(7)
<i>Sporobolus neglectus</i> Nash	Puffsheath dropseed	Susceptible	Susceptible		(2)
<i>Stipa comata</i> Trin. & Rupr.	Needle & thread	Susceptible			(2)
<i>Stipa robusta</i> (Vasey) Scribn.	Sleepygrass		Susceptible		(3, 4)
<i>Stipa viridula</i> Trin.	Green needlegrass	Susceptible			(2)
<i>Tripsacum dactyloides</i> (L.) L.	Eastern gamagrass	Resistant	Immune		(2)

1: Slykhuis 1955

2: Somsen & Sill 1970

3: McKinney & Fellows 1951

4: Sill & Connin 1953

5: Gibson 1957

6: Anderson 1971

7: Christian 1993

8: Brake et al. 1990

9: Staples & Allington 1956

10: Gibson & Painter 1957

11: Connin 1956

12: Wehing 1956

Table 1.4. Perennial grasses reported to be naturally infected with the wheat streak mosaic virus (WSMV) and/or infested with wheat curl mites (WCM).

Scientific name	Common name	WSMV	WCM	Authority
<i>Agropyron repens</i> (L.) Beauv.	Quackgrass	Immune	Fair-good	(2)
<i>Bouteloua hirsuta</i> Lag.	Hairy grama	Susceptible	Fair	(6, 8)
<i>Buchloe dactyloides</i> (Nutt.) Englem.	Buffalograss	Immune	Fair	(2)
<i>Cynodon dactylon</i> (L.) Pers.	Bermudagrass	Immune	Fair	(2)
<i>Elymus canadensis</i> L.	Canada wildrye	Susceptible	Fair	(2, 4, 6, 7)
<i>Elymus virginicus</i> L.	Virginia wildrye	Susceptible	Fair	(1, 2, 3, 4, 5, 6)
<i>Hordeum pusillum</i> Nutt.	Little barley	Immune	Fair	(2)
<i>Lolium perenne</i> L.	Ryegrass	Susceptible	Fair	(9)
<i>Pascopyrum smithii</i> Rydb.	Western wheatgrass	Immune	Fair-good	(2, 5, 7)
<i>Poa nervosa</i> Hook	Wheeler's bluegrass	Immune	Fair	(7)
<i>Oryzopsis hymenoides</i> R. & S	Indian ricegrass	Immune	Fair	(3, 7)
<i>Setaria lutescens</i> (Weigel) Hubb.	Yellow foxtail	Immune	Fair	(5, 7)
<i>Sitanon hystrix</i> Nutt.	Bottlebrush squirrtail	Susceptible	Fair	(1)
<i>Stipa comata</i> Trin. & Rupr.	Needle-&-threadgrass	Immune	Poor	(7)

1: Slykhuis 1955

2: Somsen & Sill 1970

3: McKinney & Fellows 1951

4: Sill & Connin 1953

5: Gibson 1957

6: Anderson 1971

7: Staples & Allington 1956

8: Gibson & Painter 1957

9: Connin 1956

alternate for the virus and mite during adverse periods (78). This species is widely distributed in Montana (83).

Elymus virginicus, a less common perennial, has been shown to be naturally infected with WSMV in Kansas (45). McKinney and Fellows (85) found this species to display mosaic symptoms when subject to mechanical inoculation with WSMV. However, *E. virginicus* seldom harbors large populations of WCMs in the field (83). The importance of this grass host to other perennials may diminish based on its inability to support populations of mites and its relatively low abundance in certain locations.

Bouteloua hirsuta may be a very important host because WCMs reproduce well on it, and it is susceptible to WSMV (86). Connin (86) found this species to be susceptible to virus through mite transmission. He stated that of the 14 perennial grasses tested, mites transmitted WSMV only to an undetermined *Bouteloua* species. Similar to previous perennial grass hosts for the mite and virus, *Bouteloua* is capable of serving as an intermediate host during adverse periods (83).

Lolium perenne and *Sitaton hystrix*, may be important hosts in WSM epidemiology. Although reports of these two species as naturally infected with WSMV in the field, there has been no mention whether WCMs were found on these grasses (45, 86).

Pascopyrum smithii is widely distributed, green, and succulent during most of the summer and is common along the borders of wheat fields. This is the most widely studied perennial grass as a host for the WCM (83, 91). This grass species is important in maintaining mite populations, but because it is immune to the virus, it is not capable of

producing viruliferous WCMs for infecting wheat. However, because it has been reported to harbor significant mite populations from May through September, it is considered a serious host in WSM epidemiology (86). A host response study by del Rosario and Sill Jr. (89) found that when WCMs from *P. smithii* were placed on wheat the mites adapted poorly to wheat. In addition to harboring WCMs, other eriophyoids, *Aceria agropyronis* (Keifer), *A. slykhuis* (Hall) and *Aculus mckenzie* (Keifer) have been reported to inhabit this grass species (92).

Because perennial grasses provide green host plant material year round, they are more prone than annual grasses to serve as reservoirs for the WCM and WSMV. As yet no perennial grass species that harbors the mite and virus has been shown to be a serious source of infection for wheat in the field (93). However, under the right climatic and growing conditions, viruliferous mites may be carried from a perennial to an adjacent cultivated wheat field or volunteer wheat patch. Thus, WSMV spread from perennial grasses need not be a frequent occurrence to be significant in virus epidemiology. Also, WSMV infection depends on whether viruliferous mites that land directly onto wheat can transmit the virus.

Control of Wheat Streak Mosaic Disease

Control of WSM depends primarily on disrupting the life cycle of the WCM (33, 84, 94). Because the vector can only survive for 4-6 days without a green host, any disruption of host succession (2-3 wks) will cause a reduction in the incidence of WSM

(95). Prevention of infestation is paramount to any successful management program, because once cultivated wheat fields are infested nothing can be done to salvage them

(90). Over the last half century there have been a series of successful management strategies used to combat WSM outbreaks. These strategies include: 1) scouting potential wheat and grass reservoirs for WCM and WSMV, 2) implementation of resistant wheat cultivars, 3) delay in planting date, 4) complete destruction of volunteer wheat and other potential grass and weed sources for the mite and virus. The use of miticides and insecticides have been unsuccessful in controlling the wheat curl mite (96, 97, 98).

Randomly collecting wheat plants from cultivated wheat fields in the fall and spring has facilitated forecasting potential WSM outbreaks. Somsen and Sill (83) found randomly surveying fields in Kansas for WCMs was a very good tool for predicting potential epidemics. Early predictive schemes involved monitoring wheat plants in the greenhouse transplanted from the field for development of WSMV symptoms (99). Recently, ELISA assays have been used for measuring WSMV titers in wheat varieties grown in South Dakota (100). Another method for predicting disease outbreak is the examination of individual wheat plants (cultivated and volunteer) for the presence of WCMs.

Plant resistance to the WCM is a primary tactic used in an integrated WSM management program (101). Wheat lines resistant to the WCM have been developed primarily from chromosome substitutions from *Agropyron* (3) and *Secale* (6) grass

species. Resistant wheat varieties can not withstand a WSM disease epidemic, but moderate resistance or field tolerance is valuable in reducing wheat losses (3). Recently TAM 107, a winter wheat variety that previously showed promising WCM resistance, has been reported to be susceptible to WCM populations in the field and laboratory (101).

The importance of planting date on the severity of WSM has been documented. Willis (102) summarized a 10 year study conducted by W. S. Gardner in South Dakota, who concluded that WSM was most severe in early planted winter wheat, and that late planting decreased the disease dramatically. Fellows and Sill (99) determined that winter wheat must be infected early in the fall when plants are young before serious yield losses occur and that spring infection in winter wheat causes little or no winter wheat loss. Thus, wheat plants infected early in the fall stage have a greater risk of becoming infected with the disease than plants infected in the spring. The later winter wheat is planted, the less it is exposed to viruliferous WCMs in the fall. However, the effectiveness of late planting is predicated on the extent of warm weather and moisture in the fall. Because of variable growing seasons, the recommended planting date is different for each wheat producing state or region. However, the general rule for planting winter wheat is two weeks following the harvest of spring wheat and/or the destruction of volunteer wheat (94). Thus, there is no danger of serious mosaic infection in winter wheat regardless of planting date if there are no susceptible hosts for the vector and virus nearby (103).

Destruction of volunteer wheat or other potential mite and virus reservoir grass

hosts before planting effectively controls WSMV (83). Volunteer wheat plants are controlled by conventional tillage or herbicide applications. Slykhuis (103) reported that the use of mouldboard plow was more than sufficient to destroy volunteer wheat plants and eliminate any possibility of WSMV infection in adjacent wheat fields. A contact herbicide, such as glyphosate, has been used to control volunteer wheat in north central Montana.

Summary

Wheat streak mosaic is a serious virus disease of winter and spring wheat. It can occur anywhere wheat is grown. Wheat yield losses attributed to WSM have ranged from partial to complete in some regions. Wheat streak mosaic is caused by the wheat streak mosaic virus (WSMV), *Marmor virgatum* McK. which is vectored solely by the wheat curl mite (WCM), *Aceria tosichella* Keifer. Although numerous grass species have been implicated as hosts for both the mite and virus, the primary oversummering (volunteer) and overwintering (winter) host is winter wheat. The mite and virus can only survive for 4 to 6 days off of a green host. The main WSM control tactic is destroying potential reservoir hosts before planting the next wheat crop.

The absence of volunteer wheat after a WSM outbreak introduces two important questions which have not been thoroughly addressed. First, do resident grasses serve as oversummering reservoirs for the mite and virus ? Second, if host grasses can support WCMs and WSMVs can they serve as an intermediary bridge host to wheat ? Many

studies have tested WCM and WSMV susceptibility and examined mite virus transmission to a range of grass species. However, nobody has examined the WCM and WSMV epidemiology through a combination of field surveys, laboratory transmission and acquisition tests, and sensitive serological and molecular assays for WSMV detection in field and laboratory grass samples. The following chapters represent an accumulation of field and laboratory data that addresses these questions for 13 potential grass species relative to their importance in WSMV and WCM epidemiology.

Chapter 2 and 3 describe an extensive WCM and WSMV grass survey over three summers (1995 to 1997) in a selected area in north central Montana that has had a recent history of significant WSM outbreaks. Chapter 4 expands on these grasses by attempting to develop infection by mechanical and viruliferous WCM transmission using a Montana WSMV and WCM source. Chapter 5 presents serological and nucleic acid based assays on field and laboratory grasses for WSMV detection, and Chapter 6 describes a wind tunnel test on WCM to determine what effect glyphosate has on mite dispersal.

In conclusion, understanding the host range of WSMV and WCM is essential for further development of pest and pathogen management control strategies. Wheat streak mosaic epiphytotics are not a yearly occurrence in Montana. However, acquiring knowledge of where the mite and virus are residing during non-outbreak years when limited volunteer wheat is available is very important in understanding the WSM disease cycle.

Objectives

1. Determine which species of grasses are infested with WCMs and infected with WSMV in areas bordering wheat fields, if infected grass hosts could maintain virus infection and support WCM populations from year to year, and whether early detection of commonly infected grasses may facilitate predicting a WSM outbreak in Montana.
2. Evaluate the susceptibility of 13 common grass species to a Montana WCM and WSMV, and if susceptible can viruliferous WCMs from grasses transmit WSMV to wheat.
3. Compare the sensitivity of serological and nucleic acid based assays for the detection of WSMV in field collected and laboratory infected Gramineae species.
4. Ascertain the dispersal response of WCMs on wheat seedlings exposed to glyphosate a broad spectrum herbicide.

References

1. Haskell, R. J., and J. I. Wood. 1923. Diseases of cereal and forage crops in the United States in 1922. U.S.D.A. Bur. Plant Indus. Plant Disease Bull. Sup. 27: 164-226.
2. Wiese, M. V. 1991. Wheat streak mosaic. pp. 80-81 in: Compendium of wheat diseases 2nd ed. American Phytopathological Society. St. Paul, MN. 112 pp.
3. Harvey, T. L., T. J. Martin and D. L. Seifers. 1994. Importance of resistance to insect and mite vectors in controlling virus diseases of plants: resistance to the wheat curl mite (Acari: Eriophyidae). J. Agric. Entomol. 11: 271-277.
4. Sim, T. IV, W. G. Willis and M. G. Eversmeyer. 1988. Kansas plant disease survey. Plant Disease. 72: 832-836.
5. Niblett, C. L., E. G. Hyne, C. L. King and R. W. Livers. 1974. Controlling wheat streak mosaic. Kans. Agric. Exp. AES-7: 3.
6. Conner, R. L., J. B. Thomas and E. D. P. Whelan. 1991. Comparison of mite resistance for control of wheat streak mosaic. Crop Sci. 31: 315-318.
7. Atkinson, T. G., and M. N. Grant. 1967. An evaluation of streak mosaic losses in winter wheat. Phytopathology. 57: 188-192.
8. Staples, R., and W. B. Allington. 1956. Streak mosaic of wheat in Nebraska and its control. Univ. Nebraska. Agr. Exp. Stn. Res. Bull. 178: 41 pp.
9. Slykhuis, J. T. 1952. Virus diseases of cereal crops in South Dakota. S. Dak. A & M. Exp. Stn. Tech. Bull. 11, 29 pp.
10. McMullen, M. P. and D. R. Nelson. 1989. Wheat streak mosaic severe in 1988. North Dakota State Univ. Ext. Pub. 46: 14-16.
11. Atkinson, R. E. 1949. Western wheat mosaic in Colorado and its transmission by the grain aphid *Toxoptera gramineum*. Phytopathology. 39: 2.
12. Walters, H. J. 1954. Plant Disease Reporter. 38: 836-837.
13. Bamford, M., J. Riesselman and S. Blodgett. 1996. Wheat streak mosaic. Montana St. Univ. MT. MontGuide. Ext. Serv. MT9606. pp. 1-4.

14. Burns, E. E., T. W. Carroll and B. C. Moehling. 1975. Wheat streak mosaic virus disease. *Plant Diseases*. 1162: 1-6.
15. Finley, A. M. 1957. Wheat streak mosaic, a disease of sweet corn in Idaho. *Plant Disease Reporter*. 41: 589-591.
16. Bruehl, G. W. and H. H. Keifer. 1958. Observations on wheat streak mosaic in Washington, 1955-1957. *Plant Disease Reporter*. 42: 32-35.
17. Houston, B. R. and J. W. Oswald. 1952. A mosaic disease of wheat, barley, and oats new to California. *Phytopathology*. 42: 12.
18. Wadsworth, D. F. 1949. *Plant Disease Reporter*. 33: 482.
19. Ashworth, L. J. and M. C. Futrell. 1961. Sources, transmission, symptomatology, and distribution of wheat streak mosaic virus in Texas. *Plant Disease Reporter*. 45: 220-224.
20. Brakke, M. K. 1971. Wheat streak mosaic virus. C.M.I./A.A.B. in *Description of Plant Viruses*. 48: 4 pp.
21. Bremer, K. 1971. Wheat streak mosaic virus in Turkey. *Phytopathology Medit*. 10: 280-282.
22. Appel, J. A., R. L. Bowden and W. G. Willis. 1991. Kansas wheat disease loss estimate. Kansas State Univ. Manhattan.
23. Sim, T., and Willis, W. G. 1988. Kansas wheat disease loss estimate. *Plant Disease Survey Rep. State Board of Agriculture*.
24. Wehing, J. L. 1956. Wheat streak mosaic: Its cause and control in Nebraska. *Nebr. Agric. Exp. Stn*. 180: 1-8.
25. Gao, J. G. and A. Nassuth. 1993. Alteration of major cellular organelles in wheat leaf tissue infected with wheat streak mosaic *Rymovirus* (Potyviridae). *Phytopathology*. 83: 206-213.
26. Salm, S. N., M. E. C. Rey and E. P. Rybicki. 1996. Phylogenetic justification for splitting the *Rymovirus* genus of the taxonomic family Potyviridae. *Arch. Virol*. 141: 2237-2242.

27. Niblett, C. L., K. R. Zagula, L. A. Calvert, T. L. Kendall, D. M. Stark, C. E. Smith, R. N. Beachy and S. A. Lommel. 1991. cDNA cloning and nucleotide sequence of the wheat streak virus capsid protein. *J. Gen. Virol.* 72: 499-504.
28. Slykhuis, J. T. 1953. Wheat streak mosaic in Alberta and factors related to its spread. *Can. J. Agri. Sci.* 33: 195-197.
29. Sill, W. J. Jr. 1953. Wind and blowing soil factors in the spread of wheat streak mosaic virus. *Phytopathology.* 43: 484.
30. Hollings, M. J. and A. A. Brunt. 1981. Potyvirus group. CMI/AAB Descriptions of Plant Viruses. no. 245.
31. Brake, M. K., R. N. Skoop and L. C. Lane. 1990. Degradation of wheat streak mosaic virus capsid protein during leaf senescence. *Phytopathology* 80: 1401-1405.
32. Mahmood, T., G. L. Hein and S. G. Jensen. 1998. Mixed infections of hard red winter wheat with High Plains virus and wheat streak mosaic virus from wheat curl mites. *Plant Disease.* 82: 311-315.
33. Slykhuis, J. T. 1956. Wheat spot mosaic caused by a mite transmitted virus associated with wheat streak mosaic. *Phytopathology.* 46: 682-687.
34. Seifers, D. L. 1992. Partial characterization of Colorado isolate of agropyron mosaic virus. *Plant Disease.* 76: 564-569.
35. Shevchenko, V. G., A. P. De-Millo, G. M. Razvyazkina and E. A. Kapkova. 1970. Taxonomic separation of similar species of eriophyid mites, *Aceria tulipae* Keif. and *A. tritici* sp. n. (Acarina, Eriophyoidea) - vectors of viruses of onion and wheat. *Internat. J. Acarol.* 22: 149-160.
36. Keifer, H. H. 1969. Eriophyid Studies C-3. Agr. Res. Svc., USDA. 24 pp.
37. Slykhuis, J. T. 1955. *Aceria tulipae* Keifer (Acarina: Eriophyidae) in relation to the spread of wheat streak mosaic. *Phytopathology.* 45: 116-128.
38. Paliwal, Y. C. and J. T. Slykhuis. 1967. Localization of wheat streak mosaic virus in the alimentary canal of its vector *Aceria tulipae* Keifer. *Virology.* 32: 344-353.
39. Maramorosch, K. 1962. Biological transmission of disease agents. Academic Press. p.42.

40. Gibson, W. W. and R. H. Painter. 1957. Transportation by aphids of the wheat curl mite, *Aceria tulipae* (K.), A vector of the wheat streak mosaic virus. J. Kan. Entomol. Soc. 30: 147.
41. Seifers, D. L., T. L. Harvey, J. T. Martin and S. G. Jensen. 1997. Identification of the wheat curl mite as the vector of the High Plains virus of corn and wheat. Plant Disease. 81: 1161-1166.
42. Salm, S. N., M. E. C. Rey, N. L. Robertson, R. French, F. Rabenstein and J. Schubert. 1996. Molecular cloning and nucleotide sequencing of the partial genomes of Agropyron and Hordeum mosaic viruses, two members of the Rymovirus genus in the taxonomic family Potyviridae. Arch. Virol. 141: 2115-2127.
43. McKinney, H. H. 1949. Virus isolates from mosaic wheat in the hard red winter wheat area. Plant Disease Reporter. 33: 346-349.
44. Sill, W. H. Jr. and P. C. Agusiobo. 1955. Host range studies of the wheat streak mosaic virus. Plant Disease Reporter. 39: 633-642.
45. Sill, W. H. Jr. and R. V. Connin. 1953. Summary of the known host range of the wheat streak-mosaic virus. Trans. Kan. Acad. Sci. 56: 411-417.
46. Shahwan, I. M. and J. P. Hill. 1984. Identification and occurrence of wheat streak mosaic virus in winter wheat in Colorado and its effects on several cultivars. Plant Disease. 68: 579-581.
47. Carroll, T. W., S. K. Zaske and R. H. Brlansky. 1982. Separation of Montana isolates of wheat streak mosaic virus on Michigan amber wheat. Plant Disease. 66: 916-918.
48. Slykhuis, J. T. and W. Bell. 1966. Differentiation of Agropyron mosaic, wheat streak mosaic, and a hithro unrecognized Hordeum mosaic virus in Canada. Can. J. Botany. 44: 1191-1208.
49. Ball, E. M. and M. K. Brakke. 1968. Leaf-dip serology for electron microscopic identification of plant viruses. Virology. 36: 152-155.
50. Langenberg, W. G. 1986. Deterioration of several rod-shaped wheat viruses following antibody decoration. Phytopathology. 76: 339-341.
51. Langenberg, W. H. 1989. Rapid antigenic modification of wheat streak mosaic virus in vitro is prevented in gluteraldehyde fixed tissue. J. Gen. Virol. 70: 969-973.

52. Clark, M. F. and A. N. Adams. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *J. Gen. Virol.* 34: 474-483.
53. Mathews, R. E. F. 1991. *Plant Virology*. 3rd ed. Academic Press. New York. NY 835 pp.
54. Koenig, R. 1981. Indirect ELISA methods for the broad specificity detection of plant viruses. *J. Gen. Virol.* 55: 53-62.
55. Edwards, M. L. and J. I. Cooper. 1985. Plant virus detection using a new form of indirect ELISA. *J. Virol. Methods.* 11: 309-319.
56. Montana, J. R., J. L. Jacobs, R. M. Hunger and J. L. Sherwood. 1994. First reports of *Agropyron* mosaic virus in wheat and mixed infections with wheat streak mosaic virus in Oklahoma. *Disease Notes.* 78: 432.
57. Christian, M. L. and W. G. Willis. 1993. Survival of wheat streak mosaic virus in hosts in Kansas from wheat harvest to fall wheat emergence. *Plant Disease.* 77: 239-242.
58. Macron, A., S. M. Kaeppler and S. G. Jensen. 1997. Genetic variability among maize inbred lines for resistance to the high plains virus-wheat streak mosaic virus complex. *Plant Disease* 81: 195-198.
59. Seifers, D. L., T. J. Martin, T. L. Harvey and B. S. Gill. 1995. Temperature sensitivity and efficacy of wheat streak mosaic virus resistance derived from *Agropyron intermedium*. *Plant Disease.* 79: 1104-1106.
60. Hunger, R. M., J. L. Sherwood, C. K. Evans and J. R. Montana. 1992. Effects of planting date and inoculation date on severity of wheat streak mosaic in hard red winter cultivars. *Plant Disease.* 76: 1056-1060.
61. Harvey, T. L. and D. L. Seifers. 1991. Transmission of wheat streak mosaic virus to sorghum by the wheat curl mite (Acari: Eriophyidae). *J. Kan. Entomol. Soc.* 64: 18-22.
62. Makkouk, K. M. and O. A. Jarikji. 1983. Detection of sap-transmissible viruses infecting cereals in Jordan, Lebanon and Syria. *J. Plant Diseases and Protection.* 90: 12-17.

63. Lommel, S. A., A. H. McCain and T. J. Morris. 1982. Evaluation of indirect enzyme-linked immunosorbent assay for the detection of plant viruses. *Phytopathology*. 72: 1018-1022.
64. Bottacin, A. and A. Nassuth. 1990. Evaluation of Ontario-grown cereals for susceptibility to wheat streak mosaic virus. *Can. J. Plant Pathology*. 12: 267-272.
65. Haber, S. and H. Knapen. 1989. Filter paper sero-assay (FiPSA): A rapid, sensitive technique for sero-diagnosis of plant viruses. *Can. J. Plant Pathology*. 11: 109-113.
66. Navot, N. R. and H. Czosnek. 1989. Rapid detection of tomato yellow leaf curl virus in squashes of plant and insect vectors. *Phytopathology*. 79: 562-568.
67. Sherwood, J. L. 1987. Comparison of a filter paper immunobinding assay, western blotting and an enzyme linked immunosorbent assay for the detection of wheat streak mosaic virus. *Phytopathology*. 118: 68-75.
68. Cane, P. A. and C. R. Pringle. 1995. Molecular epidemiology of respiratory syncytial virus: A review of the use of reverse transcription-polymerase chain reaction in the analysis of genetic variability. *Electrophoresis*. 16: 329-333.
69. Henson, J. M. and R. French. 1993. The polymerase chain reaction and plant disease diagnosis. *Ann. Rev. Phytopathology*. 31: 81-109.
70. Kinard, G. R., S.W. Scott and O.W. Barnett. 1996. Detection of Apple chlorotic leaf spot and apple stem grooving viruses using RT-PCR. *Plant Disease*. 80: 616-621.
71. Mathews, D. M., K. Riley and J. A. Dodd. 1997. Comparison of detection methods for citrus tristeza virus in field trees during months of nonoptimal titer. *Plant Disease*. 81: 525-529.
72. Omunyin, M. E., J. H. Hill and W. A. Miller. 1996. Use of unique RNA sequence-specific oligonucleotide primers for RT-PCR to detect and differentiate soybean mosaic virus strains. *Plant Disease*. 80: 1170-1174.
73. Weekes, R., I. Barker and K. R. Wood. 1996. An RT-PCR test for the detection of tomato spotted wilt tospovirus incorporating immunocapture and colorimetric estimation. *J. Phytopathology*. 144: 575-580.
74. Rizos, H., L. V. Gunn, R. D. Parés and M. R. Gillings. 1992. Differentiation of cucumber mosaic virus isolates using polymerase chain reaction. *J. Gen. Virol.* 73: 2009-2013.

75. Thomson, K. G., R. G. Dietzgen, A. J. Gibbs, Y. C. Tang, W. Liesack, D.S. Teakle, and E. Stackbrandt. 1995. Identification of zucchini yellow mosaic potyvirus by RT-PCR and analysis of sequence variability. *J. Virol. Methods.* 55: 83-96.
76. Robertson, N. L., R. French and S. M. Gary. 1991. Use of group specific primers and the polymerase chain reaction for the detection and identification of luteoviruses. *J. Gen. Virol.* 72: 1473-1477.
77. McNeil, J. E., R. French, G. L. Hein, P. S. Baenziger and K. M. Eskridge. 1996. Characterization of genetic variability among natural populations of wheat streak mosaic virus. *Phytopathology.* 86: 1222-1227.
78. French, R. and N. L. Robertson. 1994. Simplified sample preparation for detection of wheat streak mosaic virus and barley yellow dwarf virus by PCR. *J. Virol. Methods.* 49: 93-100.
79. Sill, W. H. Jr., R. C. Bellingham and H. Fellows. 1964. Reactions of wheat crosses, barley, rye, and oats to wheat streak mosaic virus. *Tech. Bull. Kan. Agric. Exp. Stn.* 132: 3-15.
80. Tunac, J. B. and C. M. Nagel. 1969. Reaction of dent corn inbreds and hybrids to *Aceria tulipae* (K.) and wheat streak mosaic virus. *Plant Disease Reporter.* 53: 662-664.
81. Finley, A. M. 1957. Wheat streak mosaic, a disease of sweet corn in Idaho. *Plant Disease Reporter.* 41: 589-592.
82. Berger, P. H., J. A. Percich and J. K. Ransom. 1981. Wheat streak mosaic virus in wild rice. *Plant Disease.* 65: 695-696.
83. Somsen, H. W. and W. H. Jr. Sill. 1970. The wheat curl mite, *Aceria tulipae* Keifer, in relation to epidemiology and control of wheat streak mosaic. *Tech. Bull. Kan. Agric. Exp. Stn.* 162: 1-15.
84. Gibson, W. W. 1957. Biological and ecological studies of the wheat curl mite, *Aceria tulipae* (K), on winter wheat in Kansas. Ph.D. Dissertation. Kansas State Univer. Manhattan, 148 pp.
85. McKinney, H. H. and H. Fellows. 1951. Wild and forage grasses found to be susceptible to the wheat streak-mosaic virus. *Plant Disease Reporter.* 35: 441-442.
86. Connin, R. V. 1956. The host range of the wheat curl mite, vector of the wheat streak-mosaic. *J. Econ. Entomol.* 49: 1-4.

87. Lyon, D. J. Jointed goatgrass biology. Regional Jointed goatgrass conference. Billings, MT, 30 January 1997. 34 pp.
88. Booth, W. E. and J. H. Rumely. 1989. Grasses of Montana. Dept. of Biology. Montana State University. 74 pp.
89. del Rosario, M. S. and W. H. Jr. Sill. 1965. Physiological strains of *Aceria tulipae* and their relationships to the transmission of wheat streak mosaic virus. *Phytopathology*. 55: 1168-1175.
90. Watkins, J. E., R. C. French, J. B. Campbell, E. D. Kerr, and R. N. Klein. 1989. Wheat streak mosaic disease. Nebraska Coop. Ext. Serv. EC 89-1871.
91. Painter, R. H. and J. Schesser. 1954. Western wheatgrass, an overwintering host of *Aceria tulipae* (Keifer), vector of streak mosaic of wheat. *J. Kansas Entomol. Soc.* 27: 118-119.
92. Daniels, N. E. 1963. Eriophyid mite collection in the Texas panhandle. *Annals Ent. Soc. America*. 56: 879.
93. Slykhuis, J. T. 1965. Mite transmission of plant viruses. *Advances in Virus Research*. 11: 97-137
94. McMullen, M. P. 1991. Wheat streak mosaic. North Dakota St. Univ. Extension Service. PP-646 (Revised). pp.1-4.
95. Chubbs, D. E. 1981. A review of wheat streak mosaic. M.S. thesis. Kansas State University. 50 pp.
96. Harvey, T. L. D. L. Seifers, T. J. Martin. 1997. Effect of imidacloprid seed treatment on infestations of wheat curl mite (Acari: Eriophyidae) and the incidence of wheat streak mosaic virus. *J. Agric. Entomol.* 15: 75-81.
97. Harvey, T. L., T. J. Martin and C. A. Thompson. 1979. Controlling wheat curl mite and wheat streak mosaic virus with systemic insecticide. *J. Econ. Entomol.* 72: 854-855.
98. Kantack, E. J. and H. Knutson. 1958. Chemical control studies of wheat curl mite. *J. Econ. Entomol.* 51: 68-72.
99. Fellows, H. and W. H. Sill Jr. 1955. Predicting wheat streak mosaic epiphytotics in winter wheat. *Plant Disease Reporter*. 39: 291-295.

100. Wu, Z., M. A. C. Langham and S. D. Haley. 1996. Relationship between wheat streak mosaic virus titer, symptom expression, and vegetative growth among eight winter wheat genotypes. Abstract. *Phytopathology*. 86: 72.
101. Harvey, T. L., T. J. Martin, D. L. Seifers and P. E. Sloderbeck. 1995. Adaptation of wheat curl mite (Acari: Eriophyidae) to resistant wheat in Kan. *J. Agric. Entomol.* 12: 119-125.
102. Willis, W. G. 1984. Wheat diseases. Kansas State Univ. Coop. Ext. Serv. Bull. S-23.
103. Slykhuis, J. T., Andrews J. E. and U. J. Pittman. 1957. Relation of date of seeding winter wheat in southern Alberta to losses from wheat streak mosaic, root rot, and rust. *Can. J. Plant Sci.* 37: 113-127.

CHAPTER 2

SURVEY OF MONTANA GRASSES FOR WHEAT CURL MITE (ACARI: ERIOPHYIDAE), THE VECTOR OF WHEAT STREAK MOSAIC VIRUS

Introduction

Wheat streak mosaic virus (WSMV) is solely vectored by the wheat curl mite (WCM), *Aceria tosichella* Keifer (1). Wheat curl mites acquire the virus by feeding on infected hosts and later transmit it to healthy plants as they feed on them. Under favorable environmental and cultural conditions, mite populations can build up to enormous numbers. Geske and Riesselman (2) reported 150,000 mites per volunteer wheat plant in the field. The mites are small (average adult length = 250 μ m) and can be easily dispersed by wind from plant to plant (1). Thus, susceptible wheat fields planted near diseased plants can become infested. Warm, dry, windy conditions in the spring,

and, especially, in the fall are favorable for mite population increases and spread into wheat fields (3).

High incidence of WSM is associated with early planting of winter wheat, use of susceptible cultivars (only when WSMV is present), hailstorms, and unusually warm, dry, fall and spring weather. Willis (4) reported that WSM is most severe in early fall planted wheat, and that late planting dramatically reduces disease incidence. Wheat cultivars such as 'TAM 107' have been shown to be resistant to the mite, although Harvey et al. (5) reported that WCMs in Kansas have adapted to this variety. Volunteer wheat, caused by grain shattering and sprouting as a result of hailstorms, usually serves as the principal reservoir host for the mite over the summer (6). Because the mites can only survive 4 to 6 days in the absence of a green host (1), destroying all volunteer wheat, either with herbicides or tillage, 2 wk before planting, dramatically reduces the likelihood of a WSM outbreak (7).

In 1992, a combination of early season drought, disease-stressed crops, shattered wheat heads, and late-season moisture contributed to excessive volunteer wheat production in north central Montana. These conditions were thought to contribute to the WSM epidemic in 1993-1994. Since 1995, weather conditions and cultural control practices by producers have greatly reduced the incidence of volunteer wheat in this region. It has been suggested because of the expansive host range of the vector that the mite may reside in non-cultivated host grasses. Previous studies have shown several

annual and perennial grasses as potential hosts for the WCM under field conditions (1, 8, 9, 10).

My objectives were to determine which grass species in Montana were infested with WCMs in grass areas bordering wheat fields; if infested grass hosts can support mite populations from year to year; and whether early detection of commonly infested grasses can facilitate predicting a WSM outbreak in Montana. I also report on the occurrence of other eriophyid species collected from grasses during this study.

Material and Methods

Field Survey

Annual and perennial grasses growing naturally in grassy roadside areas and fields bordering winter wheat crops were examined for the presence of *A. tosichella*. The four collection sites were located within a 8 km radius in the northwest section of Chouteau County, MT (latitude 48° 2' - 8' N; longitude 111° 16' - 23' W) at a mean elevation of 3,500 m. In 1995, grass samples were collected twice a month beginning 5 May and ending 25 August. In 1996 and 1997, samples were collected on 13 July and 16 August, and 23 May, 19 June, 26 July, and 26 August, respectively. Grasses with inflorescence were identified to species and collected in randomly selected plots (0.5 m diameter) throughout each site. Depending on the time of the year, the number of plots at each site varied from 4 to 8. Immature grass plants (without inflorescence) were transported back to the laboratory after collection and identified with a Gramineae taxonomic key (11). In

each plot, 10 to 25 whole plants of a single Gramineae species were collected, stored on ice, and taken to the laboratory, where they were preserved at 4°C for no more than 5 days until examined for mites. In addition, 25 to 50 wheat plants were randomly collected from fields adjacent (1-5 m) to the grass collection sites and processed similarly as the grass samples. Grass composition at each site was determined by using the Daubenmire frame technique (12).

Eriophyid Mite Detection

Grass and wheat plants from each site were examined for the presence of eriophyid mites by using a dissecting microscope (16X). Initially, only the ligule area was examined, but it was determined early in the investigation that the majority of the eriophyid mites were found over the entire abaxial surface of the leaves (all leaves for each plant sample were inspected). Grass and wheat plants that harbored eriophyid mites were stored at -20°C for later mite species classification. Two to 30 eriophyid mites from each grass and wheat plant were mounted on microscope slides for species identification. Mites were cleared in sorbitol: chloral hydrate (1:2), and mounted on slides in gum arabic (13). Representative specimens were sent to J. W. Amrine Jr. (Division of Plant and Soil Sciences, West Virginia University, Morgantown) for verification of identifications. Voucher specimens are deposited in the Montana Entomology Collection, Montana State University, Bozeman.

Results

1995 Survey

Population densities of grass species differed among sites. Site 1 had, *Pascopyrum smithii*, *A. trachycaulum*, *P. pratensis*, and *S. viridula* comprising 85% of the grass composition (Figure 2.1). *Pascopyrum smithii*, *B. inermis*, and *P. pratensis* were the predominate perennials at Site 2 and comprise 80% of the species (Figure 2.2). At Site 3, (Figure 2.3) *A. cristatum*, *B. inermis*, and *S. viridula* comprised 50% of the species, and at Site 4 (Figure 2.4), 65% of the species composition was made up of *P. smithii* and *S. viridula*.



Figure 2.1. Site 1: A roadside grass area adjacent to a wheat field, 25 mi. east of Brady, MT (photo: July 1995).



Figure 2.2 Site 2: An undisturbed (7 yr.) grass field surrounded by cultivated wheat fields 25 mi. east of Brady, MT (photo: July 1995).



Figure 2.3. Site 3: A grass strip adjacent to cultivated wheat fields 25 mi. east of Brady, MT (photo: July 1995).



Figure 2.4. Site 4: A grass area dividing two wheat fields 25 mi. east of Brady, MT (photo: August 1995).

Fifteen grass species totaling 1,780 plants were individually inspected for the presence of *A. tosichella*. Wheat curl mites were identified on *B. inermis* and *P. pratensis* (Table 2.1). Five mites were found on 175 *B. inermis* plants and 130 were found on 120 *P. pratensis* plants. The number of WCMs on *P. pratensis* was less than half the total number of mites observed; an *Aceria* n. sp., that was morphologically similar to the WCM, was present on 50% of the plants examined. In addition, the eriophyid species *Aculodes dubius* Nalepa was identified on *P. smithii*. Also, *Aceria* n. sp. was discovered on collections of *P. smithii*, *A. trachycaulum*, *S. viridula*; *Aculodes* n. sp. was found on each of *P. pratensis*, *Stipa comata* Trinius & Ruprecht., and *S. viridula* (Table 2.2).

Table 2.1. Wheat curl mite (WCM) infestation on annual (A) and perennial (P) grasses collected in Chouteau County, MT, 1995-1997.

	1995			1996			1997		
	WCM			WCM			WCM		
	No. Plants	% Infest.	Mean $\pm$ SD ^a	No. Plants	% Infest.	Mean $\pm$ SD ^a	No. Exam	% Infest	Mean $\pm$ SD ^a
<i>P. interior</i> (P)	----	----	----	10	50	8 $\pm$ 7 (12) ^b	----	----	----
<i>P. pratensis</i> (P)	120	41	3 $\pm$ 2 (18)	200	35	4 $\pm$ 6 (56)	1150	8	6 $\pm$ 8 (85)
<i>B. inermis</i> (P)	175	1	1	150	5	1 $\pm$ 2 (2)	475	5	1 $\pm$ 2 (2)
<i>T. aestivium</i> (A) (Cultivated wheat)	320	0	0	85	0	0	550	1	1
<i>T. aestivium</i> (A) (Volunteer wheat)	70	0	0	25	1	1	350	1	1

^a Mean ($\pm$ SD) number of WCMs per total number of infested plants examined.

^b Number in parentheses equals the maximum number of eriophyid mites found on a single plant for that particular year.

Table 2.2. Eriophyid species infestation on annual (A) and perennial (P) grasses collected in Chouteau County, MT, 1995-1997.

Grass host	1995		1996		1997		Eriophyid Species
	No. Plants	% Infest.	No. Plants	% Infest.	No. Plants	% Infest	
<i>A. cristatum</i> (P)	120	< 1	20	0	75	0	Unidentified
<i>A. trachycaulum</i> (P)	315	14	110	10	25	40	<i>Aceria</i> n.sp.
<i>A. fatua</i> (A)	35	0	20	0	50	0	
<i>B. gracilis</i> (P)	10	0	--- ^a	---	---	---	
<i>B. japonicus</i> (P)	30	0	---	---	---	---	
<i>B. tectorum</i> (A)	45	0	---	---	125	0	
<i>E. canadensis</i> (P)	20	0	---	---	---	---	
<i>H. jubatum</i> (A)	35	0	20	80	50	0	<i>Aceria</i> n.sp., <i>A. dubuis</i>
<i>P. secunda</i> (P)	20	0	---	---	---	---	
<i>P. smithii</i> (P)	115	44	40	25	50	0	<i>Aceria</i> n.sp., <i>A. dubuis</i>
<i>S. comata</i> (P)	30	45	---	---	---	---	<i>Aculodes</i> n.sp.
<i>S. viridula</i> (P)	240	29	135	75	50	0	<i>Aceria</i> n.sp., <i>Aculodes</i> n.sp.

^a No plants were collected.

Eriophyid mites were not observed on *A. fatua*, *B. gracilis*, *B. japonicus*, *B. tectorum*, *E. canadensis*, *H. jubatum*, *P. secunda*, or wheat plants. All grass and wheat plants examined were asymptomatic for WSMV.

1996 Survey

Two additional grass species, *P. interior* and volunteer wheat, along with *B. inermis* and *P. pratensis*, were found to harbor WCMs in 1996. *Poa interior* supported a small population of *A. tosichella* mites on 50% of the plants inspected. Eight WCMs were observed on 150 *B. inermis* plants, whereas 140 WCMs were found on 200 *P. pratensis* plants. The composition of other eriophyid species identified on *P. pratensis* included *Aculodes* n. sp. (50%) and *Aceria* n. sp. (15%). In addition, *A. dubuis* and an *Aceria* n. sp. were identified on *H. jubatum*. All wheat and grass plants examined were asymptomatic for WSMV.

1997 Survey

Because 1995 and 1996 results indicated that *B. inermis* and *P. pratensis* were the most suitable hosts for WCMs, survey and sampling efforts in 1997 focused on these species. *Bromus inermis* and *P. pratensis* comprised 79% of all of the grass individuals examined. Although five times (approximately 1150 plants) as many *P. pratensis* plants were examined compared to the previous year, only 357 eriophyoid mites (30% *A. tosichella*, 40% *Aceria* n. sp., and 30% *Aculodes* n. sp.) were observed on this species.

All grass plants examined were asymptomatic for wheat streak mosaic virus.

The first symptomatic volunteer wheat plants were collected from Site 4 on 19 June. These plants showed distinct WSMV-like symptoms of greenish-yellow dashes and streaks parallel to the axis of their leaves. However, only a few WCMs were found on 50 volunteer wheat plants that were examined from this site. As the season progressed, WSMV symptoms were also observed at sites 1 and 3 in the cultivated winter wheat fields. Winter wheat plants collected on 26 July were selected based on WSM leaf symptoms. Winter wheat plants that displayed symptoms were dispersed randomly within the cultivated field. Only three WCMs were found on 150 winter symptomatic wheat plants collected from sites 1, 3, 4 on this date.

Discussion

Six of the grass species sampled in my study have been reported to be naturally infested with WCMs; all grass species except *P. secunda* have been reported to be susceptible to the mite in the greenhouse (1, 9, 10). *Aceria tosichella* was recovered from *P. pratensis*, *P. interior* and *B. inermis*. This is the first report of *A. tosichella* collected from *P. interior*. Because of the low number of *P. interior* samples collected, it is unknown whether this species can support large populations of WCMs from year to year. My results suggest that *P. pratensis* is a suitable host for the WCM. Slykhuis (14) reported WCMs can become abundant on *P. pratensis* in the field.

Based on the data collected it is unknown whether *P. pratensis* can maintain

sufficient populations of WCMs to be a serious epidemiological source of viruliferous mites in the field. However, *P. pratensis* is a common perennial that remains relatively green from May to September (11), and the natural folding of its leaf blade provides a protective microhabitat for eriophyid mites. Also, the low number of *A. tosichella* found on many *P. pratensis* plants could be associated with interspecific competition between *A. tosichella*, *Aceria* n. sp., and *Aculodes* n. sp. The number of n. spp. of eriophyid mites collected on several of the grass species is not unusual since an estimated 70% of the species in the subfamily Eriophyidae have yet to be identified (15). Several greenhouse studies have shown *P. pratensis* to be susceptible to WCMs, but resistant to WSMV (10, 16, 17, 18).

Bromus inermis harbored WCMs all 3 yr. However, the extremely low number of WCMs found on *B. inermis* supports other work that suggests this species is a poor host for the mite. Laboratory reports by Connin (8) found *B. inermis* to be a poor host for the mite and immune to the virus. McMullen and Nelson (19) found this species to be positive for the virus in the laboratory but did not indicate whether WCMs could survive on this species.

Because of the limited number of *A. fatua*, *B. gracilis*, *B. japonicus*, *E. canadensis*, and *Stipa comata* collected, I am unable to draw conclusions about the relative importance of these species as alternate hosts for the WCM. However, it is interesting to compare my results with other studies in the northern Great Plains of the United States and Canada. In Nebraska a large, thriving population of WCMs was found on a few *E.*

canadensis plants, but only six were found on *S. comata* plants, while none was discovered on *B. gracilis* (9). In southern Alberta, Slykhuis (1) reported *B. japonicus* and *A. fatua* to be naturally infected with WSMV but did not indicate whether WCMs were found on these species.

Sources of WSMV infection in wheat fields in 1997 were unknown. Although a few mites were found on the 880 wheat plants that were examined, the spread of the disease from these plants to adjacent wheat plants was not indicative of mite transmission. Rather the patches of symptomatic wheat plants suggests virus transmission through leaf rub. Transmission has been reported between infected and healthy wheat plants through leaf abrasions during high winds (20). My observations suggest an early spring infestation of seedlings (2-3 leaf stage) by viruliferous WCMs. However, for unknown reasons these mites were unable to develop a significant population on these plants. The absence of large WCM numbers on volunteer wheat suggests that weather conditions such as high temperatures, low relative humidity, and extended dry periods may have contributed to the suppression of the WCM population. Possible infestation sources other than grasses were mites blown in from other regions or early spring volunteer wheat plants that survive near susceptible wheat fields. Slykhuis (1) captured WCMs as far as 3.2 km from the nearest wheat field. According to Shahwan and Hill (21) there is always a low level of WCMs in wheat fields every year.

In conclusion, my survey indicates that WCMs originating from *P. pratensis* and *B. inermis* do not develop to epidemic levels during non-outbreak years. Rather they may

provide the background WCM populations found in wheat fields every year. On the basis of this study, annual and perennial grasses are of limited importance as alternate hosts for WCMs in Montana.

References

1. Slykhuis, J. T. 1955. *Aceria tulipae* Keifer (Acarina: Eriophyidae) in relation to the spread of wheat streak mosaic. *Phytopathology*. 45: 116-128.
2. Geske, S. and J. Riesselman. 1988. Barley yellow dwarf and wheat streak mosaic virus in small grains. Montguide MT 8802. Montana State Univ. Ext. Serv. Bozeman.
3. McMullen, M. P. 1991. Wheat streak mosaic. North Dakota State Univ. Ext. Pub. PP646.
4. Willis, W. G. 1984. Wheat diseases. Kansas State Univ. Coop. Ext. Serv. Bull. S-23.
5. Harvey, T. L., T. J. Martin, D. L. Seifers and P. E. Sloderbeck. 1995. Adaptation of wheat curl mite (Acari: Eriophyidae) to resistant wheat in Kansas. *J. Agric. Entomol.* 12: 119-125.
6. Slykhuis, J. T. 1953. Wheat streak mosaic in Alberta and factors related to its spread. *Can. J. Agric. Sci.* 33: 195-197.
7. Watkins, J. E., R. C. French, J. B. Campbell, E. D. Kerr, and R. N. Klein. 1989. Wheat streak mosaic disease. *Nebr. Coop. Ext. Serv.* EC 89-1871.
8. Connin, R. V. 1956. The host range of the wheat curl mite, vector of wheat streak-mosaic. *J. Econ. Entomol.* 49: 1-4.
9. Staples, R. and W. B. Allington. 1956. Streak mosaic of wheat in Nebraska and its control, Research bulletin (Univ. of Nebraska, Lincoln campus), Agricultural Experiment Station 178: 40 pp.
10. Somsen, H. W. and W. H. Sill. 1970. The wheat curl mite, *Aceria tulipae* Keifer, in relation to epidemiology and control of wheat streak mosaic. Kansas Agricultural Experiment Station Tech. Bull., Kansas State Univ. 162: 1-24.
11. Hitchcock, A. S. 1950. Manual of the grasses of the United States, 2nd ed. United States Government Printing Office, Washington D. C., 1051 pp.
12. Daubenmire, R. 1959. A canopy-coverage method of vegetational analysis. *Northwest Sci.* 33: 43-63.

13. Jepson, L. R., H. H. Keifer and E. W. Baker. 1975. Eriophyoidea Nalepa, pp. 390-392. *In* Mites injurious to economic plants. Univ. California Press, Berkeley, 614 pp.
14. Slykhuis, J. T. 1963. Mite transmission of plant viruses. *Advances in Acarology*. 1: 326-340.
15. Amrine, J. W., Jr. and T. A. Stasny. 1994. Catalog of the Eriophyoidea (Acarina: Prostigmata) of the world. Indira Publishing Co., W. Bloomfield, MI. 798 pp.
16. Bremer, K. 1971. Wheat streak mosaic virus in Turkey. *Phytopathology Medit.* 10: 280-282.
17. Sill, W. J. Jr. and R. V. Connin. 1953. Summary of the known host range of the wheat streak mosaic virus. *Trans. Kan. Acad. Sci.* 56: 411-417.
18. Slykhuis, J. T. 1965. Mite transmission of plant viruses. *Advances in Virus Research*. 11: 97-137.
19. McMullen, M. P. and D. R. Nelson. 1989. Wheat streak mosaic severe in 1988. *North Dakota State Univ. Ext. Pub.*, 46: 14-16.
20. Sill, W. J. Jr. 1953. Wind and blowing soil factors in the spread of wheat streak mosaic virus. *Phytopathology*. 43: 484.
21. Shahwan, I. M. and J. P. Hill. 1984. Identifications and occurrence of wheat streak mosaic virus in winter wheat in Colorado and its effects on several cultivars. *Plant Disease Reporter*. 68: 579-581.

CHAPTER 3

INCIDENCE OF WHEAT STREAK MOSAIC VIRUS IN MONTANA GRASSES

Introduction

Winter wheat is the second most important crop in Montana, accounting for about 2,000,000 ha annually. It is sown from mid-September to mid-October, overwinters as young tillered plants, and is harvested in July. Wheat streak mosaic virus epiphytotics have occurred sporadically in winter wheat fields throughout Montana since it was first positively identified in the state in 1953 (1). However, the epidemiology of WSMV in Montana has not been closely studied (2).

Outbreaks of WSM are strongly associated with the presence of volunteer wheat, which serve as the principle oversummering host for the virus and its only known vector, the WCM (3). However, there have been instances where WSM damage has occurred in winter wheat fields where no volunteer wheat plants were present (4). The occurrence of WSM outbreaks in areas where volunteer wheat plants were scarce suggest that other grass

species might serve as hosts for the WSMV.

In 1992, a combination of early season drought, diseased crops, shattered wheat heads, and late season moisture contributed to excessive volunteer wheat production in north central Montana. These conditions were thought to be the catalyst for a WSM epidemic in 1993-1994. Since 1995, weather conditions and cultural control practices by producers have greatly reduced the incidence of volunteer wheat in this region, suggesting that the virus may be residing in the grasses. Previous studies have shown several annual and perennial grasses as potential hosts for the WSMV (4, 5, 6, 7, 8, 9). Many of these studies were conducted in the greenhouse and limited to symptomatic observations and mechanical inoculations in an attempt to determine if the grass species implicated were susceptible to WSMV.

I report here on the survey for the prevalence of WSMV in grass species commonly occurring in fields and grassy roadside areas near winter wheat fields in north central Montana. In addition, the incidence of WSMV in cultivated and fallow wheat fields also were monitored.

Material and Methods

Grass Population Size for Percent WSMV Prediction

Population size for percent WSMV grass infection was determined using the normal binomial distribution equation (10). In this equation, N is the predicted number

of individual plants needed to be collected for a specified α , β , q , and p values, where p

$$N = \left(\frac{z - \beta\sqrt{p(1-p)} + z - \alpha\sqrt{q(1-q)}}{q - p} \right)^2$$

is the percent infection value, q is fixed at 0, and α , β , are the adjustable type I and II errors, respectively (Table 3.1).

Table 3.1. Population size estimation for predetermined WSMV percent infection.

α	β	q	p	N
0.01	0.05	0	0.001	2,719
			0.01	269
			0.02	133
0.01	0.01	0	0.001	1636
			0.01	162
			0.02	80
0.01	0.20	0	0.001	721
			0.01	71
			0.02	35

Field Survey

Four collection sites were located in the north west section of Chouteau County, Montana (latitude $48^{\circ} 2' - 8' N$; longitude $111^{\circ} 16' - 23' W$) at a mean elevation 3,500 meters. In 1995, grass samples were collected twice a month beginning 5 May and ending 25 August. In 1996 and 1997 samples were collected on 13 July and 16 August, and 23 May, 19 June, 26 July and 26 August, respectively. Wheat plants from cultivated

and fallow fields were also collected, identified, and assayed for the detection of WSMV. Grass specimens were collected from 0.5 m² plots randomly located throughout each site. In each plot, 100 to 200 whole plant specimens of the same species were collected (estimation of a 1 % infection were based on α and $\beta = 0.01$ level Table 3.1) and stored on ice and returned to the laboratory where they were preserved at 4°C for no more than 5 days until tested for WSMV.

Wheat Streak Mosaic Virus Assay

The presence of WSMV in grass samples was detected serologically by a modified protein-A sandwich enzyme-linked immunosorbent assay (PAS-ELISA), as described by Edwards and Cooper (11). A polyclonal antiserum to WSMV at 1:2500 dilution in PBS, pH 7.4, containing 0.05% Tween-20 (PVAS-701, from American Type Culture Collection (ATTC), Rockville, MD) was used in 1995 for both the trapping and detecting antibody layers. A pooled grass sample was composed of plant extracts (1.0 - 2.0 gm leaf tissue) prepared from approximately 20 - 25 plants of the same species by macerating leaf tissues with a mortar and pestle in PBS-Tween 0.5% (1:10 wt/vol). Because plant age differed throughout the season, green leaf tissue was selected whenever possible. Pooled samples were triplicated on each microtiter plate and incubated overnight at 20 - 22°C. During all subsequent incubation steps, plates were covered and put into plastic bags containing a moist paper towel and incubated at 20 - 22°C for 2 h. Reactions were recorded in a Minireader II photometer (Dynatech Laboratories, Chantilly, VA) at

A_{405nm} . All readings in the same plate greater than four times that for the negative wheat control were considered positive.

A second antiserum (WSMV #260, provided by R. Gergerich, Univ. of Arkansas, Fayetteville) was used in 1996 and 1997 because PVAS-701 was no longer available. Liquid nitrogen was used to enhance tissue pulverization during the grinding step, and a low speed centrifugation step was performed before aliquoting samples to plate wells. Reactions in 1997 were recorded in an automated Kinetic Microplate reader (Molecular Devices Corp., Sunnyvale, CA) at A_{405nm} .

Control Plants

Rambo spring wheat was grown in sterilized soil at 25°C. Selected plants were inoculated with WSMV (the original virus source was from wheat plants collected from a diseased field in 1995 near Conrad, MT) by mechanical inoculation (11) approximately 14 days after emergence. Infected and non-infected leaves were harvested 2-3 weeks after inoculation and used as a positive PAS-ELISA control on that day or stored at -20° C for future tests. Uninfected plants served as the negative control.

Results

1995 Survey

A total of 4,652 grass plants, representing 13 perennial and four annual grass

species, were tested for WSMV infection. Ninety three percent of these pooled plant samples assayed negative for the virus (Table 3.2). Three of the annual grasses, *B. japonicus*, *B. tectorum*., and *T. aestivum*, and five species of perennial grasses, *A. cristatum*, *B. inermis*, *P. pratensis*, *S. comata* and *S. viridula* tested positive for WSMV. None of the grass plants that tested positive had WSMV symptoms.

Positive PAS-ELISA values of eight to 12 times healthy wheat controls were obtained from *P. pratensis* pooled samples collected in June, July and August. The first positive *P. pratensis* sample was found on 13 June at Site 2. Sites 1 and 2 where the two sites were *P. pratensis* was positive on more than two separate sampling dates. Site 1 positive samples were collected on 15 July and 25 August, samples collected in August at Sites 2 and 3 were more than 10 times the healthy wheat controls.

By contrast, PAS-ELISA values for *B. inermis* pooled samples tended to range from a low of 4 to a high of 12 times healthy wheat controls. The first positive pooled sample was collected at site 1 on the 5 May. PAS-ELISA value for 5 May pooled sample was only 4 times the healthy wheat control, whereas PAS-ELISA values for *B. inermis* pooled samples collected in August at Sites 2 and 3 were more than 10 times the healthy wheat control.

Other species that tested PAS-ELISA positive for WSMV at least once during the season were *A. cristatum*, *B. japonicus*, *B. tectorum*, *S. comata*, *S. viridula*, and *T. aestivum*. An absorbance reading of 26 times healthy wheat control for *S. comata* collected at Site 2 on 30 June was the highest of the positive grass samples tested during

Table 3.2. Annual (A) and perennial (P) grass species protein-A sandwich enzyme linked immunosorbent assay (PAS-ELISA) absorbance readings, 1995-1997.

Scientific Name	1995		1996		1997	
	Infected / pool sampled ^a	PAS-ELISA A ₄₀₅	Infected / pool sampled ^a	PAS-ELISA A ₄₀₅	Infected / pool sampled ^a	PAS-ELISA A ₄₀₅
<i>A. cristatum</i> (P)	2/16	0.055 ± 0.045 (0.128 ± 0.003) ^b	0/2	0.080 ± 0.023	0/2	0.051 ± 0.011
<i>A. dasystachyum</i> (P)	0/4	0.035 ± 0.010	---- ^c	----	----	----
<i>A. trachycaulum</i> (P)	0/34	0.027 ± 0.020	0/8	0.063 ± 0.051	0/4	0.036 ± 0.011
<i>A. fatua</i> (A)	0/4	0.013 ± 0.008	0/2	0.046 ± 0.033	0/2	0.061 ± 0.006
<i>B. gracilis</i> (P)	0/2	0.012 ± 0.010	----	----	----	----
<i>B. japonicus</i> (P)	1/4	0.104 ± 0.052 (0.215)	----	----	----	----
<i>B. inermis</i> (P)	5/17	0.169 ± 0.113 (0.247 ± 0.054)	6/8	0.235 ± 0.233 (0.258 ± 0.071)	5/25	0.278 ± 0.386 (1.207 ± 0.569)
<i>B. tectorum</i> (A)	1/3	0.096 ± 0.031 (0.130)	----	----	0/10	0.036 ± 0.015
<i>E. canadensis</i> (P)	0/2	0.041 ± 0.008	----	----	----	----
<i>H. jubatum</i> (A)	0/4	0.027 ± 0.007	0/2	0.065 ± 0.024	----	----
<i>K. nitida</i> (P)	0/7	0.026 ± 0.023	----	----	----	----
<i>P. interior</i> (P)	----	----	0/1	0.046 ± 0.013	----	----

Table 3.2. (continued)

Scientific Name	1995		1996		1997	
	Infected / pool sampled ^a	PAS-ELISA A ₄₀₅	Infected / pool sampled ^a	PAS-ELISA A ₄₀₅	Infected / pool sampled ^a	PAS-ELISA A ₄₀₅
<i>P. pratensis</i> (P)	5/26	0.216 ± 0.144 (0.344 ± 0.067)	5/21	0.119 ± 0.086 (0.263 ± 0.072)	6/68	0.105 ± 0.022 (0.111 ± 0.039)
<i>P. secunda</i> (P)	0/1	0.031	----	----	----	----
<i>P. smithii</i> (P)	0/18	0.028 ± 0.030	0/6	0.017 ± 0.009	0/2	0.066 ± 0.023
<i>S. comata</i> (P)	2/8	0.130 ± 0.160 (0.503 ± 0.012)	1/2	0.119 ± 0.066 (0.174)	----	----
<i>S. viridula</i> (P)	1/40	0.058 ± 0.029 (0.295)	1/12	0.045 ± 0.043 (0.142)	0/12	0.027 ± 0.012
<i>T. aestivum</i> (A) (Cultivated)	3/35	0.066 ± 0.083 (0.390 ± 0.067)	2/8	0.112 ± 0.073 (0.193 ± 0.063)	12/31	0.118 ± 0.149 (0.448 ± 0.174)
<i>T. aestivum</i> (A) (Volunteer)	0/5	0.042 ± 0.013	1/2	0.205 ± 0.234 (0.341)	7/17	0.144 ± 0.116 (0.257 ± 0.135)
Negative Control (Healthy						
'Rambo' wheat variety)	n = 10 ^d	0.034 ± 0.023	n = 4	0.040 ± 0.035	n = 7	0.036 ± 0.013

^a A pooled sample is equivalent to 20-25 plants of the same grass species (1:10 wt/vol).

^b PAS-ELISA A₄₀₅ positive sample mean ± STD.

^c No samples collected.

^d Total number of PAS-ELISA assays (1:10 wt/ vol).

the year. Over all sites and dates, infection rates of 2.5 % and 12.5% for *S. viridula* and *A. cristatum* respectively were the lowest of the pooled grass species that assayed positive for the virus. Of the 790 wheat plants tested for WSMV, 7.5% of the pooled samples were positive for virus. The low number of positive pooled samples found in the survey precluded statistical comparison among sites and sampling dates.

1996 Survey

Thirty percent of the pooled samples from the five grass species that were PAS-ELISA positive in 1995 also tested positive in 1996 (Table 3.2). Similar to 1995, none of the grass or wheat pooled samples that tested positive in 1996 was symptomatic for WSMV. The incidence of WSMV in grass pooled samples collected at all sites were 30.2 % in July. *Bromus inermis* and *P. pratensis* pooled samples collected in July were 23.8% and 75% infected with the virus, respectively. The first positive PAS-ELISA value for volunteer wheat was from pooled samples collected in August at Site 4. The overall number of virus infections in both grass and wheat field pooled samples remained relatively the same, even though fewer samples were collected and assayed in 1996 when compared to the previous year.

1997 Survey

Because 1995 and 1996 results indicated *B. inermis* and *P. pratensis* were the most suitable hosts for WSMV, survey and sampling efforts focused on these two species. In

1997, *B. inermis* and *P. pratensis* comprised 79% of all of the grass species assayed for the virus. Although five times (approximately 1150 plants) as many *P. pratensis* plants were tested, the incidence of infection in both *P. pratensis* and *B. inermis* in 1997 was found to be 10% of all pooled samples evaluated.

The first symptomatic wheat plants were collected from site 4 on 19 June. The plants showed distinct WSMV-like symptoms of greenish-yellow dashes and streaks parallel to the axis of their leaves. As the season progressed, virus symptoms were also observed at Sites 1 and 3 in the cultivated wheat. Wheat collected from these sites tested positive for the virus. Wheat plants collected on 26 July were selected based on leaf symptoms. Wheat plants that displayed WSMV symptoms were dispersed randomly within the cultivated field.

Discussion

This is the first study to report feral *A. cristatum*, *P. pratensis*, *S. comata* and *S. viridula* as hosts for WSMV, and confirms that *B. inermis*, *B. japonicus* and *B. tectorum*, can be infected with the virus in their natural environment. However, this survey shows a low viral infection level (8% of pooled samples) in *A. cristatum*, *B. japonicus*, *B. tectorum*, *S. comata*, and *S. viridula*. This suggests that these five grass species may be unsuitable hosts for WSMV. Staples and Allington (7) indicated grass hosts may serve as a reservoir for WSMV but are deficient epidemiologically since they are poor hosts for the vector.

For grasses to function as an efficient host for WSMV in quantities sufficient to cause an epidemic they must: 1) exist in sufficient density near or adjacent to wheat fields, 2) be susceptible to virus infection and multiplication, 3) support a mite population, 4) have a life-cycle that supports the virus and mite when primary wheat host is absent, and 5) be able to serve as a virus reservoir that through vector transmission can infect wheat (4). This study indicated *P. pratensis* to be a host suitable for WSMV. This common perennial grass species is green from May to September providing a overwintering reservoir for the virus. However, it is unknown whether *P. pratensis* can maintain sufficient populations of WCMs to be a serious epidemiological source of viruliferous mites in the field. Of the two grasses that tested positive for virus, only *P. pratensis* fits the above criteria for a potential epidemic host. *Poa pratensis* in the laboratory was reported to be immune to the virus (6, 12, 13, 14).

Bromus inermis tested positive for WSMV each year. Positive *B. inermis* samples could be explained by an early infestation of WCMs that were able to transmit the virus but unable to survive on this host grass. Laboratory reports by Connin (15) found *B. inermis* to be a poor host for the mite and immune to the virus, while McMullen and Nelson (16) found this species to be positive for the virus in the laboratory. Field samples of *B. inermis* assayed by Christian and Willis (4) were all negative for WSMV.

Sources of WSMV infection in 1997 were unknown. In surveyed wheat fields, infected plants were widely spaced and randomly distributed. These plants showed classic WSM symptoms of green and yellow streaks along the axis of their leaves. These

observations suggests an early spring infection of seedlings (2-3 leaf stage) by viruliferous WCMs. Weihing (17) suggested that winter wheat which becomes infected in the spring develops some mild WSM-like symptoms of the leaves but yield loss will be low if it occurs at all.

There is always a low level of WSMV in wheat fields every year (18). Though I found asymptomatic wheat plants that tested positive for the virus in 1995 and 1996, they were assumed to be the background infection level that is found yearly in fields. In 1997, there was an increase in the level of virus infection in wheat, the incidence of WSMV infection in *P. pratensis* was lower than the previous two years. This may indicate possible virus transmission from *P. pratensis* to wheat by WCMs. Another possible explanation is that volunteer wheat, which was prevalent in 1997, could have provided a more favorable host for the mite and virus. However, factors such as climatic conditions, early mite infestations, and alternate host grasses collectively may be required for a wheat streak mosaic outbreak to occur.

In summary, this survey shows that WSMV originating in perennial and annual grasses do not develop to epidemic proportions. Rather, they might provide the non-epidemic background level of virus in wheat fields year to year. However, *P. pratensis* may serve a role in the development of local WSM outbreaks, but environmental and cultural conditions have to coincide for a major epidemic to occur. Whether *P. pratensis* is important in WSM epidemiology depends on viruliferous WCM movement between wheat crops and *P. pratensis*.

References

1. McNeal, F. H. and Dubbs, A. L. 1956. Influence of wheat streak mosaic on winter wheat in area of Montana in 1955. *Plant Disease Reporter*. 40: 517-519.
2. Burns, E. E., T. W. Carroll and B.C. Moehling. 1975. Wheat streak mosaic virus disease. *Plant Diseases*. 1162: 1-6.
3. Slykhuis, J. T. 1953. Wheat streak mosaic in Alberta and factors related to its spread. *Can. J. Agric. Sci.* 33: 195-197.
4. Christian, M. L and W. G. Willis. 1993. Survival of wheat streak mosaic virus in grass hosts in Kansas from wheat harvest to fall wheat emergence. *Plant Disease*. 77: 239-242.
5. Anderson, L. W. 1971. Control of the wheat streak mosaic with chemicals in relation to epidemiology of the disease, 48-58: Ph.D. thesis. Univ. Nebraska, Lincoln.
6. Somsen, H. W. and W. H. Sill. 1970. The wheat curl mite, *Aceria tulipae* Keifer, in relation to epidemiology and control of wheat streak mosaic. *Agric. Exp. Stn. Kansas St. Univ.* 162: 1-24.
7. Staples, R. and W. B. Allington. 1956. Streak mosaic of wheat in Nebraska and its control, Univ. Nebraska, Lincoln, *Agric. Exp. Stn.*, 178: 41 pp.
8. Slykhuis, J. T. 1955. *Aceria tulipae* Keifer (Acarina: Eriophyidae) in relation to the spread of wheat streak mosaic. *Phytopathology*. 45: 116-128.
9. McKinney, H. H. and H. Fellows. 1951. Wild and forage grasses found to be susceptible to the wheat streak-mosaic virus. *Plant Disease Reporter* 35: 441-442.
10. Dixon, W. J. and Massey, F. J. 1983. Introduction to statistical. 4th ed. McGraw Hill Book Company, NY.
11. Edwards, M. L. and J. I. Cooper, 1985. Plant virus detection using a new form of indirect ELISA. *L. Virol. Methods*. 11: 309-319.
12. Bremer, K. 1971. Wheat streak mosaic virus in Turkey. *Phytopathology Medit.* 10: 280-282.

13. Sill, W. H. Jr. and R. V. Connin. 1953. Summary of the known host range of the wheat streak-mosaic virus. *Trans. Kan. Acad. Sci.* 56: 411-417.
14. Slykhuis, J. T. 1965. Mite transmission of plant viruses. *Advances in Virus Research.* 11: 97-137.
15. Connin, R. V. 1956. The host range of the wheat curl mite, vector of wheat streak-mosaic. *J. Econ. Entom.* 49: 1-4.
16. McMullen, M. P. & D. R. Nelson. 1989. Wheat streak mosaic severe in 1988. *North Dakota State Univ. Ext. Pub.* 46: 14-16.
17. Weihing, J. L. 1956. Wheat streak mosaic: Its cause and control. *Nebr. Agr. Exp. Stn.* 180: 1-8.
18. Shahwan, I. M. and J. P. Hill. 1984. Identification and occurrence of wheat streak mosaic virus in winter wheat in Colorado and its effects on several cultivars. *Plant Disease Reporter.* 68: 579-581.

CHAPTER 4

**EVALUATION OF THIRTEEN GRASS SPECIES FOR
SUSCEPTIBILITY TO WHEAT CURL MITE AND
WHEAT STREAK MOSAIC VIRUS**

Introduction

Wheat streak mosaic virus has been reported to infect 116 species of 34 genera of the family Gramineae (1). The host range of *A. tosichella* Keifer, the only known vector of WSMV (2), is even more expansive than the virus because many grass species that are immune to the virus are hosts for the mite. There have been conflicting reports in the literature on the susceptibility of some grass species to *A. tosichella* from wheat, and the susceptibility of wheat to *A. tosichella* from grasses (3, 4, 5). Different isolates of WSMV have been studied in wheat (6), but no studies have reported if different isolates exist in grasses. The majority of the grass species that are susceptible to WSMV show no symptoms of the disease (2).

The occurrence of different biotypes within aphid species that differ physiologically but not morphologically has been documented (7,8), but little is known about physiological differences in WCMs (3). Several researchers (3, 4, 5) have suggested that different strains of the WCM exist. Gibson (4) found that *Aceria tulipae* Keifer, since renamed *Aceria tosichella* Keifer (9), occurring naturally on *Pascopyrum smithii* colonized wheat with difficulty or not at all. In contrast, a study conducted by del Rosari and Sill (3) reported a small population of WCMs on *P. smithii* can adapt to wheat, although somewhat slowly. They indicated those mites that do adapt when transplanted to wheat are not only able to survive and reproduce on wheat but are efficient vectors of WSMV (3). Slykhuis (5) found that WCMs collected from the field on *Hordeum jubatum*, *Elymus canadensis* and *P. smithii* did not survive when placed on wheat plants and conversely mites collected from wheat when placed on these same grass species did not survive. Depending on the grass species, a wide difference in vector adaptation occurs when transfers are attempted from other host grass plants to wheat (10).

Most grass host susceptibility studies were conducted in Kansas, Nebraska and South Dakota using WCM strains and WSMV isolates collected from their respective states prior to 1970. The most recent WCM and WSMV grass susceptibility study conducted in Montana was in 1975 (11). Thus, with the lack of current WCM and WSMV grass host information for Montana, and knowledge from other states (i.e., Kansas, Nebraska, and South Dakota) that the mite and virus may have since adapted to previously immune grasses a series of grass host susceptibility tests were conducted using

a recently collected Montana WCM and WSMV.

The objectives of this study were to evaluate the suitability and susceptibility of 13 common grass species to *A. tosichella* and WSMV that were collected from a Montana wheat field in 1995. Additionally, transmission of WSMV by viruliferous WCMs from recipient host grass plants to wheat was investigated.

Material and Methods

Plant Material

Fourteen grass species including wheat representing nine different genera were evaluated for host status to WCMs and WSMV collected from Montana. Grass species selected were *A. cylindrica*, *A. cristatum*, *A. dasystachyum*, *A. fatua*, *A. trachycaulum*, *B. inermis*, *B. tectorum*, *E. canadensis*, *K. cristata*, *P. pratensis*, *P. smithii*, *S. comata*, *S. viridula* and *T. aestivum*. Seeds for *A. cristatum*, *A. dasystachyum*, *B. inermis*, *E. canadensis*, *K. cristata*, *S. comata*, and *S. viridula* were supplied by Townsend Seeds Co., Townsend, MT. Seeds for *A. trachycaulum*, *P. pratensis* and *P. smithii*, were purchased from Agribasics Seed Co., Belgrade, MT. *Aegilops cylindrica*, *A. fatua*, *B. tectorum* and *T. aestivum* seeds were obtained from colleagues in the Department of Plant Science, Montana State University, Bozeman.

Wheat Curl Mite and Wheat Streak Mosaic Virus Sources

Wheat curl mites and WSMV used for mechanical and transmission inoculation

tests were obtained from a winter wheat field, Teton County, Montana, August 1995. Colonies for viruliferous WCMs were started from a diseased infested leaf clipping overlaid on Rambo spring wheat seedlings in a growth chamber (Convion, Winnipeg, Manitoba, Can.) maintained at 22 to 25°C photoperiod 16h L/8h D ($400 \mu\text{mol s}^{-1}\text{m}^{-2}$) and relative humidity 40 to 70%. Sources for WSMV originated from symptomatic leaves that were manually inoculated on Rambo wheat plants in 2- to 3-leaf stage. Mite and virus sources were maintained and propagated on Rambo wheat throughout the study.

Mechanical Inoculation of Grass Plants

Individual plants were grown in Cone-tainersTM (3.75 cm diameter x 23.5 cm length) (Ray Leach Co., Canby, OR) in a walk-in growth chamber (Convion, Winnipeg, Manitoba, Canada) maintained at 22 to 25°C, photoperiod 16 h L/8h D ($600 \mu\text{mol s}^{-1}\text{m}^{-2}$), and relative humidity 10 to 20 %. Seedlings at the 2- to 3-leaf stage were sap inoculated with a WSMV suspension from symptomatic Rambo wheat leaves. Rambo leaves with mosaic symptoms were ground to a powder with a mortar and pestle in liquid nitrogen and mixed with 0.1 M, pH 7.0, sodium, sodium phosphate buffer (1:10 wt/vol). Inoculum was filtered through cheese cloth and transferred to a 50 ml plastic centrifuge tube on ice. Grass seedlings were dusted with carborundum (320 mesh) before each inoculation. Inoculum was hand rubbed with a cotton swab to each leaf on each seedling. Rambo, a known susceptible wheat variety, was used as a positive control. Three to four plants from each grass species were inoculated with buffer only and served

as negative controls. Plant symptoms and virus detection were conducted at 14, 28 and 42 days post-inoculation. The conditions for the serological assay for virus detection was previously described in Chapter 3, plant tissue (leaf sections from 10-12 grass plants of the same species) for protein antibody sandwich ELISA was weighed and ground in a mortar and pestle in a 1:20 wt/vol dilution of PBS Tween-20. Equivalent healthy grass plant controls were prepared similarly. Absorbance was measured at 405 nm using a Minireader II photometer (Dynatech Laboratories, Chantilly, VA). Protein antibody sandwich-ELISA absorbance values for each pooled grass species were considered positive if it was at least three times greater than its paired healthy control.

Viruliferous Wheat Curl Mite Tests

Test 1 was conducted to determine whether viruliferous WCMs could establish a population and infect four host grasses. The purpose of this test was to determine if a known number of WCMs could develop infection on four grass species. Grass species used in the study were *A. trachycaulum*, *P. pratensis*, *P. smithii* and *S. viridula*. *Triticum aestivum* (var. Rambo) was included to serve as a positive control. Ten plants of each grass species were germinated in 10 cm square pots. Plants at the 2- to 3-leaf stage were individually infested with 20 WCMs for a total of 200 mites per pot. Pots were caged in plastic cylinders (20 cm diameter, 50 cm height) after infestation and transferred to growth chamber maintained at 22 to 25°C, photoperiod (400 $\mu\text{mol s}^{-1} \text{m}^{-2}$) 16h L/8h D, relative humidity 20 to 40% and watered daily with a dilute fertilizer, 15:30:15 (N: P: K).

At the end of 28 days, plants were excised from each pot and 2.5 cm leaf sections were cut from the newly emerged leaf and pooled for a protein antibody-ELISA for WSMV detection test as previously described in Chapter 3. The remaining plant tissues were thoroughly dissected and number of *A. tosichella* per plant was recorded.

Test 2 was performed to determine if a WCM population and rate of WSMV infection could be increased by increasing the number of mites on each plant. In this test, leaf sections (ca. 2.5 cm) of a heavily infested Rambo wheat plant (estimated 50 to 100 mites per section) were excised and placed within the leaf axial of the basal leaf of each test plant (5 plants per species per 10 cm sq pot). To secure the leaf, the basal leaf and plant stem were tied together with string. Grasses used in this experiment were, *A. trachycaulum*, *P. pratensis*, *P. smithii* and *S. viridula*. *Triticum aestivum* (var. Rambo) was included as a positive control. After infestation, pots were placed in plastic cylinders for isolation and held in growth chambers as described in test 1. At the end of 28 days, plants were tested and inspected for WSMV and WCMs respectively as described in test 1.

Test 3 was designed to determine if WCM populations and WSMV infection could be developed on four grass species that were continuously exposed to a viruliferous WCM source for 28 days followed by 10 days of isolation. This test was conducted based on the failure of mites in the previous two tests to develop and infect manually infested grasses. Test grasses used in this experiment were *A. trachycaulum*, *B. inermis*, *P. pratensis* and *P. smithii*. *Triticum aestivum* (var. Rambo) was included as a positive

control. In a growth chamber (conditions similar to test 1) twenty plants of the same grass species were grown to seedling stage in 10 cm square pots. An electric fan was directed to continuously blow (2.5 ms^{-1}) onto the wheat infested plant source (ca. 1 m from grass test plants) in the direction of the grass plants. At the end of seven days, 10 random grass plants from each species were processed for WSMV detection and WCM presence as described in test 1. At 28 days, plants species were isolated (plastic cylinder cage) for another 10 days to determine if the mites could survive on the plants without exposure to a WCM source. After isolation, plants were processed for WSMV detection and WCM inspection as described in test 1.

Test 4 was designed to determine if intermixing individual grass plants in Cone-tainerTM might influence WSMV and WCM susceptibility. Sixteen seedlings of each grass species were grown in plastic Cone-tainersTM (one seedling per cone). Grass species used in this experiment were *A. cylindrica*, *A. trachycaulum*, *B. inermis*, *E. canadensis*, *K. cristata*, *P. pratensis*, *P. smithii*, *S. comata*, *S. viridula*, and *T. aestivum*. The Cone-tainersTM were randomized in a 0.5 X 1.0 m plastic rack holder. The rack of plants was placed in a growth chamber (same conditions as in test 1) and exposed to a continuous viruliferous WCM source for 28 days. At the end of 14 and 28 days, four plants were randomly chosen from each species and processed for WSMV detection and a second set of four plants were inspected for WCMs as previously described in test 1.

Test 5 was performed to verify results from the previous tests that *A. tosichella* was a host of *A. cylindrica*, *B. inermis*, *P. pratensis*, and *S. comata*. Also, *A. fatua* was

included because a previous report indicated this species to be susceptible to several WSMV isolates (12). In addition, mite numbers and virus titer were compared to Rambo spring and winter Tiber wheat varieties. Based on previous tests, this experiment was set up similarly to test 3 where grass species were grown in 10 cm sq. pots and continuously exposed to a viruliferous WCM source. Samples for virus detection and mite numbers were taken at 14, 28 and 35 days post exposure as previously described in test 1. To aid in determining mite numbers, each of 5 leaves for each species at each time period were placed on a black piece of paper with double sided sticky tape and left to dry overnight, the next day mites that were trapped in the tape were estimated.

Wheat Curl Mite Vector Competency

The vector competency test was designed to determine if WCMs from grasses could transmit WSMV to healthy wheat. Eight different species of grasses were used in this study. Fifteen plants of *A. cristatum*, *A. dasytachyum*, *A. trachycalaum*, *B. inermis*, *E. canadensis*, *P. pratensis*, *S. comata*, *S. viridula* and *T. aestivum* (varieties Rambo and Tiber) were grown in Cone-tainers™. Three blocks of five plants of each species were randomized in rack holders. A wheat infested viruliferous WCM source was stationed 10 cm from the grasses. An electric fan that generated a wind current (2.5 ms^{-1}) was positioned 20 cm behind the mite infested wheat source. Mites that dispersed from the wheat source were directed to blow over the grass seedlings. At the opposite end of the bench, a healthy set of wheat plants was placed.

2.5 m behind the grass seedlings. At the end of seven days of WCM exposure, the wheat plants at the opposite end of the bench were removed and isolated in a plastic cage (after 14 days, caged wheat plants were tested for WSMV and inspected for WCMs). In addition, the WCM source was also removed. After seven days with no wheat source (14 days post-infestation), six randomly chosen plants from each grass species were removed and tested for WSMV and examined thoroughly for WCMs. The next day (15 days post-infestation), a second set of healthy wheat plants was placed at the far end of the bench and, a fan was allowed to blow onto the grass seedling in the direction of the healthy wheat plants. The purpose was to determine if WCMs on the grasses could transmit WSMV to healthy wheat. At the end of seven days (21 days post-infestation) wheat plants were removed, tested and inspected for WCMs as previously stated above.

Field Collected Eriophyid Mite Grass Preference Test

Preference test conducted in July 1996, involved collecting mites from two feral grass species *P. pratensis* and *S. viridula*. Collection sites were 25 miles east of Brady, Montana within grass strips bordering wheat fields. A special attempt was made to collect immature plants because they were most likely to harbor eriophyid mites. Field collected eriophyid mites were transferred to laboratory grown grasses of the same species. To prevent eriophyid populations from mixing, each manually infested grass species treatment was placed in separate growth chamber and maintained at 22 to 25°C, photoperiod 16 h L/ 8h D ($400 \mu\text{mol s}^{-1} \text{m}^{-2}$), relative humidity 20 to 40 % and watered

daily with a dilute fertilizer, 15:30:15 (N: P: K). A rearing host was designated as a grass species that was manually infested with eriophyid mites and a test host was a grass species that was uninfested. A treatment consisted of a rearing host plus five test grass species and one spring wheat (Rambo, served as a positive control) test host species. A rearing host was comprised of sixteen host seedlings planted in plastic Cone-tainers™ infested with 10 eriophyid mites each. Mites were carefully picked up with a hair glued to a dowel and transferred to the youngest leaf of the rearing host seedling. Mite transfers were aided by a stereo microscope (16X). A total of two treatments (mites from *P. pratensis* and *S. viridula*) were conducted throughout the study (test hosts = *A. trachycaulum*, *P. pratensis*, *P. smithii*, *S. viridula*, and *T. aestivum*). Sixteen seedlings from each test host were randomly placed in the rack for a total of 96 test hosts per treatment. To facilitate the movement of mites, an electric fan was set up in each of the grass treatments and directed to blow against the area of the rack where the rearing hosts were nearest. At 14 and 28 days, four plants from each test host were randomly chosen and virus detection was performed as described in test 1. Additionally, 4 plants from each test host were randomly chosen at 14 and 28 days and inspected for WCMs.

Statistical Analysis

A. tosichella numbers were subject to analysis of variance and means of mites for each grass species were separated by Tukey's Studentized Range (HSD) test (13).

Results

In the mechanical inoculation test, *A. cylindrica* and *T. aestivum* were the only grass species to display WSM symptoms and test PAS-ELISA positive for WSMV at all three time periods (Table 4.1). Percentage of *A. cylindrica* plants that exhibited mosaic symptoms at 14, 28, and 42 days were 12 %, 30 %, and 40 % respectively. By 42 days, all *T. aestivum* plants were symptomatic for WSM disease. *Elymus canadensis* was the single graminaceous species to test PAS-ELISA positive for WSMV in the study. However, this species which tested positive at 28 days showed no WSM symptoms and when tested at 42 days was negative for WSMV.

Table 4.1. Detection of wheat streak mosaic virus (WSMV) from mechanically inoculated annual (A) and perennial (P) grass species.

Species	n	14 Days Post-Inoculation		28 Days Post-Inoculation		42 Days Post-Inoculation	
		WSMV ^a	Healthy Control ^b	WSMV ^a	Healthy Control ^b	WSMV ^a	Healthy Control ^b
<i>A. cristatum</i> (P)	11	0.015 ± 0.004	0.040 ± 0.008	0.098 ± 0.011	0.049 ± 0.011	0.095 ± 0.010	0.062 ± 0.012
<i>A. cylindrica</i> (A)	12	0.168 ± 0.017	0.043 ± 0.003	0.157 ± 0.022	0.048 ± 0.006	0.154 ± 0.012	0.064 ± 0.012
<i>A. dasystachyum</i> (P)	12	0.018 ± 0.010	0.017 ± 0.007	0.090 ± 0.007	0.060 ± 0.025	0.054 ± 0.006	0.074 ± 0.018
<i>A. fatua</i> (A)	12	0.068 ± 0.023	0.041 ± 0.012	0.064 ± 0.018	0.045 ± 0.017	0.056 ± 0.009	0.091 ± 0.014
<i>A. trachycaulum</i> (P)	10	0.018 ± 0.010	0.044 ± 0.003	0.077 ± 0.008	0.065 ± 0.012	0.075 ± 0.006	0.132 ± 0.005
<i>B. inermis</i> (P)	12	0.014 ± 0.005	0.052 ± 0.013	0.043 ± 0.023	0.062 ± 0.012	0.065 ± 0.016	0.022 ± 0.010
<i>B. tectorum</i> (P)	12	0.024 ± 0.015	0.032 ± 0.016	0.059 ± 0.010	0.071 ± 0.002	0.080 ± 0.006	0.027 ± 0.023
<i>E. canadensis</i> (P)	12	0.021 ± 0.005	0.019 ± 0.006	0.155 ± 0.014	0.045 ± 0.009	0.082 ± 0.003	0.092 ± 0.021
<i>K. cristata</i> (P)	12	0.067 ± 0.004	0.055 ± 0.011	0.129 ± 0.018	0.102 ± 0.008	0.099 ± 0.003	0.142 ± 0.007
<i>P. pratensis</i> (P)	12	0.022 ± 0.006	0.051 ± 0.009	0.061 ± 0.006	0.066 ± 0.014	0.012 ± 0.008	0.025 ± 0.018
<i>P. smithii</i> (P)	12	0.006 ± 0.010	0.037 ± 0.005	0.047 ± 0.013	0.043 ± 0.019	0.035 ± 0.006	0.072 ± 0.007
<i>S. comata</i> (P)	12	0.032 ± 0.007	0.047 ± 0.005	0.118 ± 0.013	0.115 ± 0.006	0.090 ± 0.005	0.078 ± 0.021
<i>S. viridula</i> (P)	12	0.011 ± 0.004	0.007 ± 0.003	0.036 ± 0.004	0.022 ± 0.005	0.045 ± 0.004	0.018 ± 0.005
<i>T. aestivum</i> (A)	12	0.291 ± 0.012	0.032 ± 0.005	0.456 ± 0.008	0.053 ± 0.007	0.634 ± 0.013	0.064 ± 0.008

^a Mean ± (STD) absorbance 405 nm of three PAS-ELISA wells of ten to twelve pooled grass plants of the same species.

^b Mean ± (STD) absorbance 405 nm of three PAS-ELISA wells of four pooled healthy plants of the same species.

Positive samples above 0.10 and greater than 3X healthy control were considered positive for wheat streak mosaic virus.

Wheat curl mites, transferred individually or on infested wheat leaf sections to four native graminaceous species and one wheat cultivar, flourished only on the wheat cultivar species (Table 4.2).

Table 4.2. Comparison of five graminaceous species susceptibility to viruliferous wheat curl mite (WCM) infestation for 28 days by: Test 1 individual transfer of 20 WCMs per plant and Test 2 placement of infested wheat leaf section (approximately 50 to 100 mites) per plant.

Species	28 Days Post-Infestation			
	Test 1		Test 2	
	WCM ^a	WSMV ^b	WCM ^a	WSMV ^b
<i>A. trachycaulum</i>	0.6 a	0.01 ± 0.01	1.2 a	0.02 ± 0.01
<i>P. pratensis</i>	0.0 a	0.00	1.0 a	0.01 ± 0.01
<i>P. smithii</i>	0.2 a	0.01 ± 0.01	1.2 a	0.00
<i>S. viridula</i>	1.0 a	0.02 ± 0.01	2.1 a	0.02 ± 0.01
<i>T. aestivum</i>	146 b	0.43 ± 0.07	172 b	0.53 ± 0.01

^a Mean number of WCM per 10 plants grown in 10 cm square plastic pots; Means within same column followed by the same letter are not significantly different at the 0.05 level of probability (Tukey's Studentized Range test).

^b Mean (± STD) absorbance values 4X negative wheat control (Test 1 = 0.02 ± 0.01 and Test 2 = 0.03 ± 0.01) were considered positive for WSMV infection.

There was a slight increase in WCM numbers in test 2 (infested leaf sections) when compared to test 1 (individually placed WCM), but these differences are not statistically significant. In both tests, all four grass species tested negative for WSMV.

Grass plants grown in 10 cm square pots and subject to continuous WCM exposure

for 7 days varied in degree of susceptibility to wheat (Table 4.3).

Table 4.3. Test 3: Susceptibility of five grass species grown in separate 10 cm square pots exposed to viruliferous WCM infested wheat source for 28 days followed by 10 days of isolation.

Species	Mean ^a Number of WCM per Plant		Mean ^b Optical Density	
	7 d ^c	38 d ^d	7 d ^c	38 d ^d
<i>A. trachycaulum</i>	12.7 c	3.1 a	0.00	0.03 ± 0.01
<i>B. inermis</i>	66.9 b	3.3 a	0.02 ± 0.01	0.01 ± 0.01
<i>P. pratensis</i>	3.0 a	3.9 a	0.01 ± 0.01	0.00
<i>P. smithii</i>	----	3.6 a	0.02 ± 0.02	0.00
<i>T. aestivum</i>	556 d	689 b	0.43 ± 0.07	0.56 ± 0.09

^a Mean number of WCM per 10 plants; Means within same column followed by the same letter are not significantly different at the 0.05 level of probability (Tukey's Studentized Range test).

^b Mean (± STD) absorbance values 4X negative wheat control (0.02 ± 0.01) were considered positive for WSMV infection.

^c Grass plants were exposed continuously to a viruliferous WCM colony for 7 days.

^d Grass plants exposed for 28 days to a viruliferous WCM colony followed by 10 days of isolation in a WCM and WSMV free growth chamber.

For example, *B. inermis* had significantly more WCMs than two of the grass entrants, but significantly less than the wheat cultivar. There was no relationship to mite numbers and WSMV infection in any of the four grass species at seven days post-infestation. By day 38 (after 28 days continuous WCM exposure followed by 10 days of isolation) mite populations in *B. inermis* and *S. viridula* decreased significantly while

mite numbers on *P. pratensis* increased slightly. After 28 days of constant WCM selection pressure and 10 days of isolation, WSMV infection or symptoms were not detected in the four feral grass species.

In the Cone-tainer™ study (Table 4.4), none of the grass species, with the exception of *A. cylindrica*, were able to establish significant WCM populations.

Table 4.4. Test 4: Exposure of ten grass species grown in separate Cone-tainers™ to viruliferous WCM infested wheat source for 28 days.

Species	Mean ^a Number of WCM per Plant		Mean ^b Optical Density	
	14 d	28 d	14 d	28 d
<i>A. cylindrica</i>	44.3 c	----	0.24 ± 0.04	----
<i>A. trachycaulum</i>	5.0 b	2.1 a	0.02 ± 0.01	0.00
<i>B. inermis</i>	3.2 a	2.4 a	0.03 ± 0.01	0.02 ± 0.01
<i>E. canadensis</i>	2.2 a	----	0.00	----
<i>K. cristata</i>	1.1 a	----	0.00	----
<i>P. pratensis</i>	1.2 a	2.6 a	0.02 ± 0.01	0.01 ± 0.01
<i>P. smithii</i>	2.0 a	3.5 a	0.03 ± 0.02	0.05 ± 0.02
<i>S. comata</i>	1.0 a	3.4 a	0.03 ± 0.01	0.02 ± 0.01
<i>S. viridula</i>	3.5 a	3.1 a	0.00	----
<i>T. aestivum</i>	100.3 d	20.5 b	0.46 ± 0.07	0.58 ± 0.09

^a Mean number of WCM per 4 plants; means within same column followed by the same letter are not significantly different at the 0.05 level of probability (Tukey's Studentized Range test).

^b Mean (± STD) absorbance value of three ELISA wells of 4 pooled grass plants of the same species. Absorbance values 4X negative wheat control (14 days post-infestation = 0.02 ± 0.01 and 28 days post-infestation = 0.03 ± 0.02) were considered positive for WSMV infection.

In addition, none of the grass species except *A. cylindrica* and *T. aestivum* tested positive for WSMV infection. Wheat curl mites from *A. cylindrica* plants when transferred to healthy wheat seedlings were able to infect the wheat plants after 14 days (data not shown: wheat plants exhibited mosaic symptoms and tested PAS-ELISA positive). This indicates WCMs that survive on *A. cylindrica* are able to transmit WSMV to wheat.

Over 35 days of constant WCM exposure there were significant differences in the mean number of WCMs found on the seven grass species that were grown in plastic pots (Table 4.5).

Table 4.5. Test 5: Comparison of WCM susceptibility of seven grass species that are under continuous viruliferous WCM exposure for 35 days.

Species	Mean ^a Number of WCM Per Plant		
	14 days	28 days	35 days
<i>A. cylindrica</i>	178 b	2258 b	8666 a
<i>A. fatua</i>	40 c	291 c	270 b
<i>B. inermis</i>	3 d	95 d	34 c
<i>P. pratensis</i>	35 c	42 e	15 c
<i>S. comata</i>	23 c	19 e	25 c
<i>T. aestivum</i> var. (winter wheat)	325 a	4500 a	12166 a
<i>T. aestivum</i> var. (spring wheat)	300 a	5833 a	----

^a Mean number of WCM per 5 plants; means within same column followed by the same letter are not significantly different at the 0.05 level of probability (Tukey's Studentized Range test).

By 14 days post-infestation the two wheat varieties had 100-fold more mites than *B. inermis*, five-fold more than *A. fatua*, *P. pratensis*, *S. comata* and two-fold more than *A. cylindrica*. Wheat curl mite eggs were observed on all of the grass species except *B. inermis* at 14 days post-infestation. This indicates that WCMs can survive and reproduce on these species.

By 28 days post-infestation mite numbers increased dramatically on the two wheat varieties, *A. cylindrica*, *A. fatua*, *B. inermis* while staying relatively constant for *P. pratensis* and *S. comata*. By 35 days, there were significant differences in WCM populations between winter wheat, *A. fatua*, and *A. cylindrica* compared to *B. inermis*, *P. pratensis* and *S. comata*. No WCM population differences were observed between *B. inermis*, *P. pratensis* and *S. comata*. *Aegilops cylindrica*, *A. fatua* and *T. aestivum* tested PAS-ELISA positive for WSMV at all three time periods. *Stipa comata* was the only feral grass to test positive for WSMV in the study (Table 4.6). Wheat streak mosaic symptoms were observed in all of the grass samples that tested positive for virus, except *S. comata*.

The vector competency test corroborated the results obtained in the previous tests, indicating that these grass species are non-hosts for the Montana WCM and WSMV (Table 4.7). This transmission test showed that mites landing on grass species are unable to survive. This is indicated by only finding one mite on *S. viridula* grass throughout the whole study.

The eriophyid mites collected from field *P. pratensis* and *S. viridula* were unable

to develop populations on five grass species including a known susceptible wheat cultivar (Table 4.8). Eriophyid mites collected in the field were probably a different eriophyid biotype or strain or if they were *A. tosichella*, they were present in very low numbers. *Poa pratensis* and *S. viridula* were the only grass species in the field that harbored sufficient numbers of eriophyid mites to conduct laboratory preference tests.

Table 4.6. Wheat streak mosaic susceptibility of seven grass species grown in separate square pots (10 cm) to 35 days of continuous viruliferous WCM exposure.

Host Grass	14 Days Post-Inoculation		28 Days Post-Inoculation		35 Days Post-Inoculation	
	WSMV ^a	Healthy Control ^b	WSMV ^a	Healthy Control ^b	WSMV ^a	Healthy Control ^b
<i>A. cylindrica</i>	1.263 ± 0.203	0.090 ± 0.034	0.432 ± 0.086	0.089 ± 0.057	0.261 ± 0.073	0.029 ± 0.045
<i>A. fatua</i>	0.367 ± 0.173	0.035 ± 0.027	0.446 ± 0.146	0.175 ± 0.028	0.204 ± 0.056	0.019 ± 0.010
<i>B. inermis</i>	0.042 ± 0.033	0.026 ± 0.013	0.117 ± 0.019	0.094 ± 0.051	0.028 ± 0.017	0.038 ± 0.012
<i>P. pratensis</i>	0.024 ± 0.012	0.006 ± 0.003	0.130 ± 0.009	0.124 ± 0.014	0.012 ± 0.005	0.019 ± 0.006
<i>S. comata</i>	0.015 ± 0.016	0.032 ± 0.012	0.129 ± 0.025	0.017 ± 0.015	0.041 ± 0.013	0.023 ± 0.015
<i>T. aestivum</i> (winter wheat)	1.081 ± 0.114	0.036 ± 0.015	0.505 ± 0.212	0.192 ± 0.026	0.422 ± 0.025	0.045 ± 0.023
<i>T. aestivum</i> (spring wheat)	1.042 ± 0.202	0.063 ± 0.034	0.696 ± 0.119	0.109 ± 0.029	----	----

^a Mean (± STD) absorbance 405 nm of three PAS-ELISA wells of four pooled grass plants of the same species.

^b Mean (± STD) absorbance 405 nm of three PAS-ELISA wells of four pooled healthy plants of the same species.
Postive sample above 0.10 and greater than 3X healthy control were considered positive for wheat streak mosaic virus.

Table 4.7. Transmission of WSMV via viruliferous WCM from infested wheat to grasses followed by transmission of WSMV from WCM infested grasses back to healthy wheat.

Grass Host	7 Days PI		14 Days PI		21 Days PI	
	WSMV	WCM	WSMV ^a	WCM ^b	WSMV	WCM
<i>A. cristatum</i>			0.036 ± 0.012	0		
<i>A. dasytachum</i>			0.029 ± 0.012	0		
<i>A. trachycalaum</i>			0.058 ± 0.004	0		
<i>B. inermis</i>			0.037 ± 0.010	0		
<i>E. canadensis</i>			0.027 ± 0.014	0		
<i>P. pratensis</i>			0.024 ± 0.012	0		
<i>S. viridula</i>			0.014 ± 0.005	1		
<i>S. comata</i>			0.064 ± 0.006	0		
<i>T. aestivum</i> ^c (spring wheat)	0.585 ± 0.0212	56 ± 12			0.052 ± 0.012	0
<i>T. aestivum</i> ^d (winter wheat)	0.314 ± 0.056	100 ± 24			0.057 ± 0.022	0

^a Mean ± (STD) of three PAS-ELISA wells of 4 pooled grass plants (1 inch leaf section per plant) of the same grass species; Negative wheat control = 0.034 ± 0.024 and positive wheat control = 0.326 ± 0.041.

^b Mean ± (STD) of WCM on 4 plants of the same grass species.

^c Mean ± (STD) of three PAS-ELISA wells of 10 pooled wheat plants (1 inch leaf section per plant); after 7 days wheat plants were removed and held for 14 days in plastic cages before mite numbers and ELISA tests were performed. The same protocol was conducted at 21 days for the second group of wheat plants.

^d Mean ± (STD) of three PAS-ELISA wells of 10 pooled wheat plants (1 inch leaf section per plant); after 7 days wheat plants were removed and held for 14 days in plastic cages before mite numbers and ELISA tests were performed. The same protocol was conducted at 21 days for the second group of wheat plants.

Table 4.8. Susceptibility of six grass species to exposure to field collected eriophyid mites from *P. pratensis* and *S. viridula* for 28 days.

Rearing Host	Test Host	Mean ^a Optical Density Per Test Host		Mean ^b Number of WCM Per Plant
		14 d	28 d	28 d
<i>P. pratensis</i>	<i>A. trachycaulum</i>	0.01 ± 0.01	0.03 ± 0.02	0 a
	<i>B. inermis</i>	0.02 ± 0.01	0.01 ± 0.01	1.0 a
	<i>P. pratensis</i>	0.00	0.03 ± 0.02	0 a
	<i>P. smithii</i>	0.03 ± 0.01	0.02 ± 0.01	0 a
	<i>S. viridula</i>	0.03 ± 0.01	0.02 ± 0.01	0 a
	<i>T. aestivum</i>	0.02 ± 0.01	0.03 ± 0.02	0 a
<i>S. viridula</i> ^c	<i>A. trachycaulum</i>	0.00	0.01 ± 0.01	0 a
	<i>B. inermis</i>	0.01 ± 0.01	0.01 ± 0.01	0 a
	<i>P. pratensis</i>	0.02 ± 0.01	0.02 ± 0.02	0 a
	<i>P. smithii</i>	0.00	0.01 ± 0.01	0 a
	<i>S. viridula</i>	0.03 ± 0.01	0.02 ± 0.01	1.0 a
	<i>T. aestivum</i>	0.01 ± 0.01	0.03 ± 0.01	0 a

^a Mean PAS-ELISA absorbance value (± STD) of three wells of 4 pooled leaf sections from individual grass plants of the same species; Absorbance values 4 X negative wheat were considered positive (14 days post-infestation negative control = 0.02 ± 0.01; positive wheat control = 0.54 ± 0.01 and 28 days post-infestation negative control = 0.01 ± 0.01; positive wheat control = 0.43 ± 0.01)

^b Mean number of WCM per 4 plants; Means within same column followed by the same letter are not significantly different at the 0.05 level of probability (Tukey's Studentized Range test).

^c *S. viridula* rearing host at the end of 28 days harbored 8 ± 3 WCMs per plant.

Discussion

My primary objective was to evaluate thirteen common annual and perennial grasses for their capacity to serve as reservoirs for a Montana WCM and WSMV. Such alternative grass hosts would be particularly important for monitoring the incidence of WSM disease in habitats that are low or void of volunteer wheat.

The results of the pathogenicity tests indicate that *A. cylindrica* and *A. fatua* are susceptible to both the WCM and WSMV. More importantly, WCMs that acquire the WSMV from these two graminaceous species can transmit the virus to non-infected wheat. Connin (13) demonstrated in the greenhouse that WCMs removed from *A. cylindrica* were able to transmit WSMV to wheat. This is the first report of WCMs from *A. fatua* transmitting WSMV to wheat under laboratory conditions. There have been conflicting reports on the host suitability of this species to both the WCM and WSMV. Sill and Connin (14) reported this annual grass to be immune to the virus, whereas Slykhuis (5) found this species to be susceptible to the virus by both manual and mite inoculation, but resistant to mite reproduction. Recently, *A. fatua* was reported by mechanical inoculation to be susceptible to six different WSMV isolates (12). Previous differences in results reported in the literature may have been due to different genotypes.

Repeated attempts to infect eleven feral grasses with WSMV by both manual and mite inoculations were unsuccessful. Thus, these grasses appear to be immune to the virus. However, I did achieve a positive PAS-ELISA absorbance values for *E. canadensis* and *S. comata* in the artificial inoculation and WCM exposure tests

respectively. This is the first reported case of *S. comata* becoming infected by viruliferous WCMs in the laboratory. Other studies (2, 5) have shown *E. canadensis* to be susceptible to WSMV transmission by both mite infestations and virus inoculations. My results should be interpreted cautiously since they were both isolated cases.

Wheat curl mite colonization levels were higher in *P. pratensis*, *B. inermis* and *S. comata* plants grown in pots than those grown in cones. One reason may be that plant density in pots provide a more suitable microclimate for the mites to survive and reproduce than single plants growing in cones. Also, the plants in cones are more likely to be subject to desiccation than those clustered in pots. Wheat curl mites thrive best when placed in 100 percent relative humidity at 25°C (5).

There was a large variation among the grasses tested in the extent of colonization by WCMs. The survivability of mites appeared to be directly correlated to the micro-environment supplied by the leaves, and this was determined by the condition of the leaves (5). Of all the grasses that tested immune to the virus, mite numbers were the highest on young (2-3 leaf) *B. inermis* plants. But as the *B. inermis* plants matured mite numbers began to decline. This may have been a result of the unfolding of the leaves as the plants matured which is a characteristic of this genus. Also, young *B. inermis* plants have pubescent leaves (high number of trichomes) which provide a larger surface area for mites than grass species with glabrous leaves. Grasses with pubescent leaves improve the ability of airborne WCMs to successfully land (15).

Results of this investigation demonstrate that *A. cylindrica* and *A. fatua* are a more

suitable host for both the WCM and WSMV than the other grasses that were examined. Several authors have questioned the existence of *A. cylindrica* as an important component of WCM and WSMV epidemiology (2, 11, 16, 17) because of its short growth period (May to June). *Aegilops cylindrica* is usually ripe before winter wheat is harvested so its contribution to a green bridge is negligible. This study has demonstrated that *A. cylindrica* is a good host for the mite and virus under a controlled environment and should be re-examined as a potential source for the virus and mite in the field. Since *A. cylindrica* and *A. fatua* are major weed problems in Montana, they could play a role in increasing the incidence and severity of WSM in wheat fields. These results point to the importance of weed control, not only for reducing their competition with wheat, but also in possibly reducing a potential WSM outbreak. In addition, further studies are needed to determine whether the WCMs that colonize grasses that are immune to WSMV are viruliferous, and if so for how long can mites retain the virus.

Failure to develop WSMV infection from field collected eriophyid mites indicates that the mites collected were not WCMs or if they were they were at such low numbers. This may be explained by interspecific competition with other eriophyid species. In general, eriophyid mites, with the exception of WCMs, are highly host specific (9). Another explanation is that the eriophyid mites may have been injured during mechanical transfer from wild to experimental grass hosts.

References

1. Edwardson, J. R. and R. G. Christie. 1991. The potyvirus group. Volumes 1-IV, Florida Agric. Exp. Stn. Monograph 16.
2. Somsen, H. W. and W. H. Sill Jr. 1970. The wheat curl mite, *Aceria tulipae* Keifer, in relation to epidemiology and control of wheat streak mosaic. Tech. Bull. Kan. Agric. Exp. Stn. 162: 1-15.
3. del Rosario, M. S. and W. H. Sill Jr. 1965. Physiological strains of *Aceria tulipae* and their relationship to the transmission of wheat streak mosaic virus. Phytopathology. 55: 1168-1175.
4. Gibson, W. W. 1957. Biological and ecological studies on the wheat curl mite, *Aceria tulipae* (K.) on winter wheat in Kansas. Ph.D. Dissertation. Kansas State Univ. Manhattan, 148 pp.
5. Slykhuis J. T. 1955. *Aceria tulipae* Keifer (Acarina: Eriophyidae) in relation to the spread of wheat streak mosaic. Phytopathology. 45: 116-128.
6. Carroll, T. W., S. K. Zaske and R. H. Bransky. 1982. Separation of Montana isolates of wheat streak mosaic virus on Michigan amber wheat. Plant Disease. 66: 916-918.
7. Starks, K. J., R. L. Burton, and O. G. Merkle. 1983. Greenbugs (Homoptera: Aphididae) plant resistance in small grains and sorghum to biotype E. J. Econ. Entomol. 76: 877-880.
8. Painter R. H. 1951. Insect resistance in crop plants. The Macmillan Co., New York. 520 p.
9. Amrine, J. W. and T. A. Stasny. 1994. Catalog of the Eriophyoidea (Acarina: Prostigmata) of the world. Indira Publishing Co., W. Bloomfield, MI, 798 pp.
10. Jepson, L. R., H. H. Keifer and E. W. Baker. 1975. Eriophyoidea Nalepa, 445-447. In Mites injurious to economic plants. Univ. California Press, Berkeley, 614 pp.
11. Burns, E. E., T. W. Carroll and B. C. Moebling. 1975. Wheat streak mosaic virus disease. Plant Diseases. 1162: 1-6.

12. Seifers, D. L., T. L. Harvey, K. D. Kofoed and W. D. Stegmeier. 1996. Natural infection of pearl millet and sorghum by wheat streak mosaic virus in Kansas. *Plant Disease*. 80: 179-185.
13. SAS Institute. 1989. SAS user's guide: statistics, version 5th ed. SAS Institute, Cary, N.C.
14. Connin, R. V. 1956. The host range of the wheat curl mite, vector of the wheat streak-mosaic. *J. Econ. Entomol.* 49: 1-4.
15. Sill, W. H. Jr. and R.V. Connin. 1953. Summary of the known host range of the wheat streak-mosaic virus. *Trans. Kan. Acad. Sci.* 56: 411-417.
16. Staples, R., and W. B. Allington. 1956. Streak mosaic of wheat in Nebraska and its control. *Univ. Neb. Agric. Exp. Stn. Res. Bull.* 178: 41 pp.
17. Watkins, J. E., R. C. French, J. B. Campbell, E. D. Kerr, and R. N. Klein. 1989. Wheat streak mosaic disease. *Nebraska Coop. Ext. Serv. EC* 89-1871.

CHAPTER 5

DETECTION OF WHEAT STREAK MOSAIC VIRUS IN GRAMINACEOUS HOSTS USING SEROLOGICAL AND NUCLEIC ACID BASED ASSAYS

Introduction

Wheat streak mosaic virus is composed of a messenger sense, single stranded RNA genome of about 8,500 nucleotides. The complete nucleotide sequence of the viral genome is unknown, however the nucleotide sequence of the 45 kDa coat protein has been characterized (1). Wheat streak mosaic virus is transmitted in a persistent manner by the wheat curl mite, *Aceria tosichella* (2). Wheat streak mosaic virus has an extensive host range comprising many cultivated (3) and native (4) plants in the family Gramineae.

Ever since WSMV was recognized in 1932, it has been known that virus isolates vary widely in symptom expression in *T. aestivum* (5, 6, 7). Recently with the advent of reverse-transcription polymerase chain reaction (RT-PCR), restriction fragment polymorphisms and the known sequence of the capsid protein region of WSMV, primers

have been designed for the specific identification of WSMV in wheat (5, 8). McNeil et al. (5) employing nucleic acid based assays demonstrated that genetic diversity does occur within WSMV subpopulations in and between wheat fields in Nebraska and Wyoming. Wheat streak mosaic virus is known to occur commonly in grasses, but it has not been determined whether virus isolates differ between and within feral grass hosts. Currently, there has been no report of a WSMV isolate originating from a feral grass host. In Montana, the importance of feral grasses as reservoirs for WSMV have not been closely studied.

The objectives of this study were to: 1) compare the sensitivity of protein antibody sandwich enzyme linked immunosorbent assay, and immunocapture reverse transcriptase polymerase chain reaction for the detection of WSMV in wheat and 2) confirm laboratory and field collected grasses that tested PAS-ELISA positive for WSMV with more sensitive serological and nucleic acid based assays.

Material and Methods

Wheat Streak Mosaic Virus Isolates

Ten wheat streak mosaic virus isolates (Table 5.1) from wheat and one isolate from corn were tested by protein antibody sandwich enzyme linked immunosorbent assay (PAS-ELISA) and immunocapture reverse transcription polymerase chain reaction (IC-RTPCR). All virus isolates were stored at -20°C in plant tissue with the exception of WSMV-84 which was stored in purified form.

Table 5.1. Eleven different wheat streak mosaic virus isolates.

Host	Isolate	Origin	Source
corn	MKB530		R. French
wheat	WYM	Wyoming	R. French
wheat	SID-81	Montana	R. French
wheat	ST960V	Kansas	R. French
wheat	WSMV-84		R. French
wheat	H-88		D. Seifers
wheat	PU57		D. Seifers
wheat	OSU	Oklahoma	D. Seifers
wheat	SDK-96	South Dakota	D. Seifers
wheat	Tex-96	Texas	D. Seifers
wheat	Mont-95	Montana	S. Blodgett

Serological Assays

Protein antibody sandwich ELISA was performed according to the protocol outlined in Chapter 3. Wheat streak mosaic virus was detected by Western blot assays as previously described with minor modifications by Titus (8). Proteins extracted from plant leaves were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. The proteins were transferred by electrophoresis to a nitrocellulose membrane (Nitroband). After soaking with 3% solution of dry milk powder in Tris-buffered saline 0.05 % Tween-20 (10mM Tris-HCL, pH 8.0 and 150mM NaCl) (TBST). for 30 minutes, to saturate nonspecific binding sites, the membrane was incubated with a 1: 25,000 dilution of primary anti HVA1 antibody for 30 minutes (supplied by S. A. Lommel, Oklahoma State University, Stillwater) followed by a 1:10,000 dilution

secondary antibody of goat anti-rabbit IgG alkaline-phosphatase conjugate (Promega Corp., USA) for an additional 30 minutes. After each incubation, the membrane was washed 3 times for 5 minutes in TBST. Substrates used to detect color development were nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (Nalgene). All reactions were conducted at room temperature under gentle agitation.

Nucleic Acid Based Assays

For RT-PCR assay, two oligonucleotide primers were designed based on the 3' end region sequence of WSMV RNA coat protein gene (1). The upstream primer (5'-CGGGATCCAACAATGTCAGGATCAGGTTCTG-3') is located between nucleotides 742 to 758, while the downstream primer (5'-CGCGGATCCTTATCACGCAAGAGCGTTTAC-3') is complementary to nucleotides 1646 to 1665, giving an amplification product of 944 base pairs. Primers were purchased from Integrated DNA Technologies (Coralville, IA).

Immunocapture

Immunocapture was performed from a modified procedure of (9). Antibody (80µl; WSMV#260) diluted 1:500 in grinding buffer [500 mM Tris-HCL (pH 8.2), containing 2% PVP-40, 1% PEG 6000, 140mM NaCl, 0.05% Tween-20] was added to a 0.5 ml Perkin Elmer microfuge tube, incubated overnight at 4°C, and then rinsed two times with grinding buffer. Leaf material (approximately 0.1 gm) was homogenized in

grinding buffer (1.0 ml), the suspension was centrifuged at 14,000 x g for 5 minutes and 50 μ l added to an antibody-coated 0.5 ml microfuge tube. The tube was then incubated overnight at 4°C, the next day sap was discarded and the tube was rinsed two times with nuclease free water. The tubes were immediately used or stored at -20°C indefinitely.

RNA Extraction

Total plant RNA extraction was conducted using a method adapted from McNeil (5). Approximately 0.1 gm of tissue was ground to a powder in liquid nitrogen. Next 500 μ l of Tris (pH 9.0) buffer was added, followed by 500 μ l of buffered phenol (pH 9.0), and the sample was mixed for 5 minutes. Following centrifugation at 7,000 x g for 2 minutes, 400 μ l of the upper aqueous layer was added to equal volume of phenol and vortexed for 30 seconds. After centrifugation at 7,000 x g for 2 minutes, 300 μ l of the aqueous layer was transferred to a fresh tube containing 30 μ l of 20% potassium acetate and 350 μ l of isopropanol. The tube was inverted 10 times before placing in a refrigerator at -20°C for 15 minutes. After refrigeration, the tube was centrifuged at 12,000 x g at 4°C for 15 minutes. The supernatant was removed, and the pellet was washed with 70% ethanol by centrifugation at 12,000 x g for 2 minutes at 4°C, followed by a 100% ethanol wash under the same conditions. The pellet was air dried for 20 minutes and then resuspended in 20 μ l of sterile double distilled water.

Reverse Transcription Polymerase Chain Reaction (RT-PCR)

A single reaction mix was used for the RT-PCR step (Access RT-PCR kit, Promega, Madison, WI), performed in a 0.5 ml Perkin Elmer microfuge tubes, containing 5.0 μ l 5X avian myeloblastis virus (AMV)/Tfl reaction buffer (Tris Base, glacial acetic acid, 0.5M EDTA, pH 8.0), 0.75 nM upstream primer (F 742-758), 0.75 nM downstream primer (R1646-1665), 1-3 U Tfl DNA polymerase, 2.5-5.0 U AMV reverse transcriptase, 25 mM MgSO₄, 10 mM each of dATP, dCTP, dGTP, and dTTP. The reaction mixture was made up of 25 μ l with nuclease free water and overlaid with mineral oil (50 μ l). The RT-PCR was carried out in Perkin Elmer DNA Thermal Cycler using the following program; 2 minutes denaturation 94°C, 2-3 minutes ice bath (DNA and RNA polymerase cocktail added), 45 minutes reverse transcription 48°C then for PCR, 30 seconds denaturation at 94°C, 1 minute annealing at 58°C, 2 minutes extension at 68°C, for 35 cycles. Following PCR the amplified product was detected by gel electrophoresis. For the gel electrophoresis, PCR product (10 μ l) was mixed with loading buffer (10:2 vol/vol, PCR product: loading buffer) and electrophoresed in 1% agarose gel containing ethidium bromide (0.5 μ g/ml), and visualized with ultraviolet light.

Northern Blot Hybridization

Northern blot hybridization was performed as described by Titus (8). RNA extracts of samples were electrophoresed in a 1 % agarose gel containing 0.2 M 3-(N-morpholino) propanesulfonic acid. The RNA was transferred unidirectionally onto a nitrocellulose

membrane by passive diffusion. The PCR coat protein probe (^{32}P) constructed using the Prime-It® II Random primer labeling kit (Stratagene) was hybridized to Hybond N+ nitrocellulose nylon membrane (Amersham, UK). Membrane was exposed to X-ray film for 16 hours at -80°C , and film was developed by the standard photographic procedure.

Results

WSMV Detection by Serological Assays

Wheat streak mosaic virus polyclonal antiserum #260 was used for serological detection in protein antibody sandwich enzyme linked immunosorbent assay (PAS-ELISA). Wheat streak mosaic virus antiserum reacted with all eleven WSMV isolates (Table 5.2). The ten WSMV isolates from wheat were easily detected ($A_{405\text{nm}} = 0.193 - 0.715$). However, the WSMV isolate from corn was weakly identified ($A_{405\text{nm}} = 0.078$). In other PAS-ELISA tests, WSMV antiserum did not react with either CCMV or AgMV (data not shown).

The specific serological reactivity of WSMV-95 isolate with a homologous polyclonal antiserum was further tested by a Western blot assay. The isolate was easily detected, as the WSMV coat protein of 32 Kda was consistently observed. Predominance of the smaller protein indicates degradation of the 45 Kda protein. This is not unexpected because the proportion of smaller capsid proteins increase with the age of the tissue (13). Such serological activity was not observed when AgMV, CCMV and healthy tissues were used (data not shown).

Table 5.2. Comparison of wheat streak mosaic virus (WSMV) isolates by protein antibody sandwich enzyme linked immunosorbent assay (PAS-ELISA) with WSMV #260 antiserum.

Isolates	(A _{405nm}) ^a
MKB530	0.078 ± 0.005
WYM	0.226 ± 0.034
SID-81	0.305 ± 0.010
ST960V	0.193 ± 0.029
H-88	0.490 ± 0.014
WSMV-84	0.413 ± 0.033
PU57	0.715 ± 0.048
OSU	0.693 ± 0.025
SDK-96	0.358 ± 0.015
Tex-96	0.433 ± 0.017
Mont-95	0.413 ± 0.033
Healthy wheat	0.026 ± 0.009

^a Absorbances were taken 1 hour after addition of substrate and represent the average (± STD) of three wells.

WSMV Detection by IC-RTPCR

When WSMV was immunocaptured and used as a template for RT-PCR, a single DNA product of 944 base pairs was observed in an agarose gel. No amplification products were observed from reactions where the sample, reverse transcriptase or DNA polymerase were omitted (data not shown); furthermore, no amplification products were seen when AgMV, CCMV and healthy wheat (Rambo variety) tissue were used in the presence of WSMV primers F742 and R1646 (Figure 5.1).



Figure 5.1. Agarose gel amplified products using immunocapture reverse transcription polymerase chain reaction (IC-RT-PCR). Lane 1, AgMV-infected wheat (1:100 wt/vol); lane 2, CCMV (100ng); lane 3, healthy wheat; lane 4, WSMV-infected wheat (1:100 wt/vol); lane 5, purified WSMV-84 (100ng). Lane 6, contains DNA size standards (1 Kb Gibco-BRL DNA Ladder).

The sensitivity of IC-RT-PCR and PAS-ELISA was directly compared by using a dilution series of pure WSMV (isolate WSMV-84) and infected wheat plants (isolate MONT-95). The same WSMV and infected plant source was used in both assays. As shown in Figure 5.2, WSMV was detected at dilutions equivalent to 62 nanograms (ng)

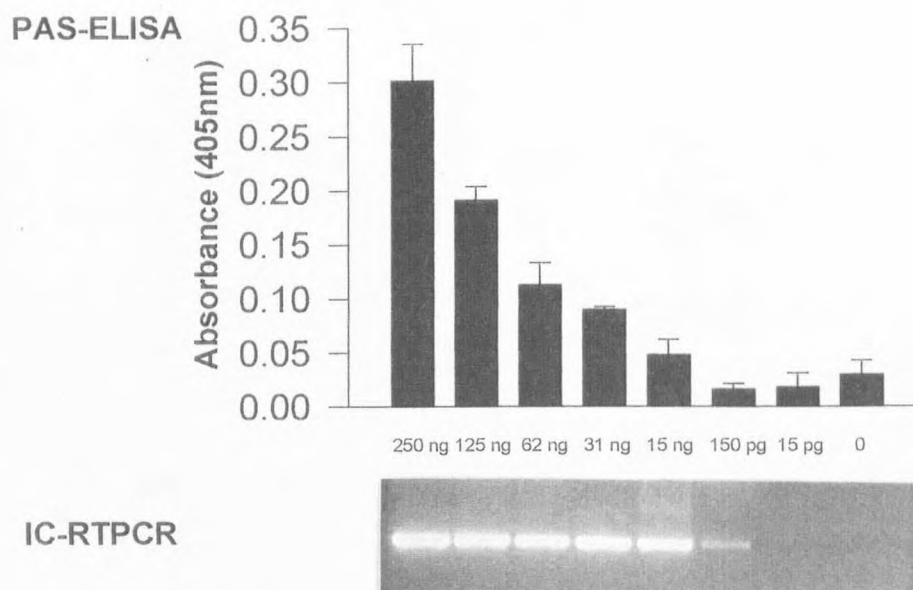


Figure 5.2. Sensitivity comparison of protein antibody sandwich enzyme linked immunosorbent assay (PAS-ELISA) and immunocapture reverse transcription polymerase chain reaction (IC-RTPCR) for detection of purified WSMV-84 isolate. The mean absorbance values in PAS-ELISA were the average of three wells. The IC-RTPCR assay amplified a DNA fragment approximately 944 base pairs.

and 150 picograms (pg) in PAS-ELISA and IC-RTPCR, respectively. The limit of the detection of the IC-RTPCR assay using purified WSMV was 150 picograms. In addition, leaf tissue from a WSMV-infected wheat plants was detected by IC-RTPCR at dilutions up to $1:10^5$, while PAS-ELISA was 1000X less sensitive at detecting WSMV in infected wheat plants (Figure 5.3).

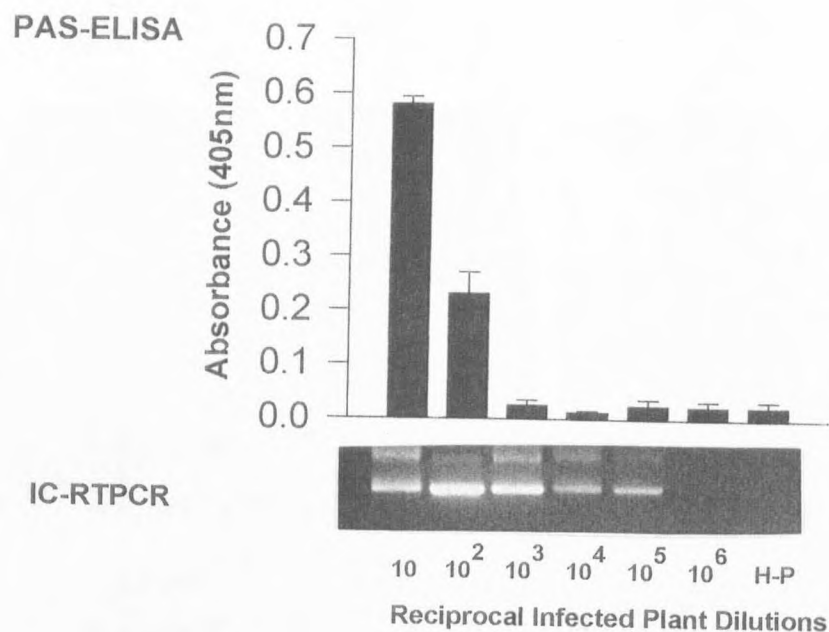


Figure 5.3. Sensitivity comparison of protein antibody sandwich enzyme linked immunosorbent assay (PAS-ELISA) and immunocapture reverse transcription polymerase chain reaction (IC-RTPCR) for detection of wheat streak mosaic virus (WSMV) infected wheat. The mean absorbance values in PAS-ELISA were the average of three wells. The IC-RTPCR assay amplified a DNA fragment approximately 944 base pairs from 10 fold dilutions of WSMV-infected wheat. H-P = healthy wheat plant (1:100 wt/vol).

All eleven of the WSMV isolates amplified a PCR product of the predicted length of 944 base pairs in the IC-RTPCR assay (data not shown). The intensity of the PCR band varied. The band product was directly correlated to its corresponding PAS-ELISA absorbance values.

Comparison of Serological and Nucleic Acid Based Assays for the Detection of WSMV from Field Collected Grasses

Field grass samples of *P. pratensis* and *B. inermis* that initially tested positive for PAS-ELISA in 1996 and 1997 when assayed a second time were negative for WSMV (Figure 5.4). All grass plants collected from the field were negative for WSMV by both IC-RTPCR and Northern blot analysis, with the exception of symptomatic wheat plants collected in 1997 (Figure 5.5).

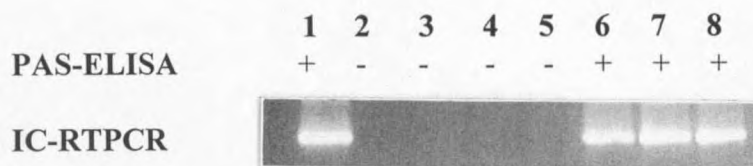


Figure 5.4. Sensitivity comparison of PAS-ELISA and IC-RTPCR for detection of WSMV in field collected grasses. Lane 1, WSMV-84 isolate (100ng); lane 2, *B. inermis* (1997); lane 3, *S. comata* (1997); lane 4, *P. pratensis* (1996); lane 5, *P. pratensis* (1997); lane 6, volunteer wheat (1997); lane 7, cultivated wheat (1997); lane 8 cultivated wheat (1997) (all grass samples were of a 1:20 wt/vol for PAS-ELISA and 1:50 wt/vol for IC-RTPCR and taken from 20 to 25 pooled grass plants of the same species). Samples were stored at -20°C for 6 to 24 months prior to nucleic acid and serological assays. Pooled grass samples for PAS-ELISA absorbance reading were considered positive for WSMV if they were 4X healthy wheat control. IC-RTPCR amplified a 944 base pair DNA fragment.

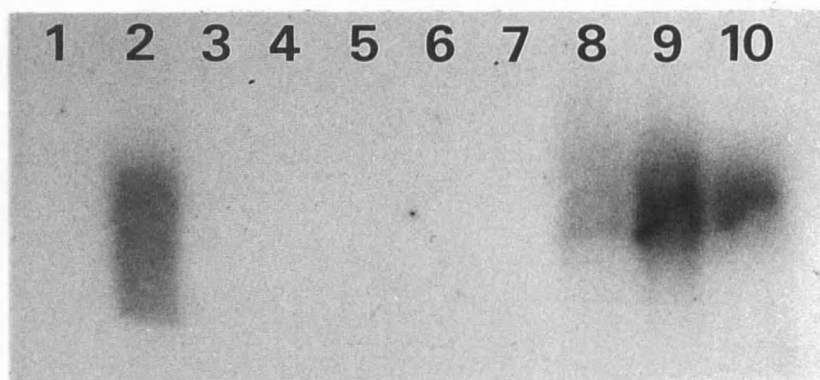


Figure 5.5. Northern analysis of nucleic acid extracts from 1996 and 1997 field collected grass samples. The gel was blotted to a nylon membrane and hybridized to a cloned ^{32}P -labeled DNA of the WSMV coat protein. The arrangement of samples is as follows: Lane 1, healthy Rambo wheat; lane 2, WSMV-infected Rambo wheat; lane 3, *S. comata* (1996); lane 4, *B. inermis* (1997); lane 5, *P. pratensis* (1996); lane 6 and 7, *P. pratensis* (1997); lane 8, WSMV symptomatic volunteer wheat (1997); lane 9 and 10 symptomatic cultivated wheat (1997).

Comparison of Serological and Nucleic Acid Based Assays for the Detection of WSMV from Viruliferous WCM Challenged Grasses: A Greenhouse Study.

The sensitivity of PAS-ELISA and IC-RTPCR were compared for the detection of WCM-transmitted WSMV in seven grass species. The results are shown in Figure 5.6. In the seven grass species tested, three were symptomatic (positive for PAS-ELISA) and four were asymptomatic (negative for PAS-ELISA) for WSM disease.

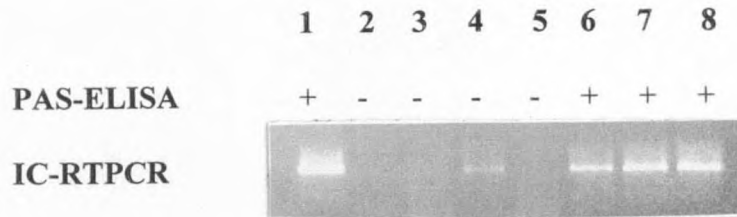


Figure 5.6. Sensitivity comparison of PAS-ELISA and IC-RTPCR for detection of WSMV-95 isolate in seven grass species exposed for 35 days to continuous viruliferous WCMs. There was no reaction to healthy control samples for each of the eight grass species in both assays. Lane 1, WSMV-84 isolate (100ng); lane 2, *B. inermis*; lane 3, *B. tectorum*; lane 4, *P. pratensis*; lane 5, *S. comata*; lane 6, *A. fatua*; lane 7, *A. cylindrica*; lane 8, *T. aestivum* (var. Rambo) (all grass samples were of a 1:20 wt/vol for PAS-ELISA and 1:50 for IC-RTPCR and taken from 20 to 25 pooled grass plants of the same species). IC-RTPCR amplified a 944 base pair DNA fragment.

Mosaic symptoms had a positive correlation with WSMV detection. This was demonstrated by WSMV detection in both serological and nucleic acid based tests for *A. cylindrica*, *A. fatua* and *T. aestivum* (Figures 5.6 and 5.7). In contrast, three of the four feral grasses, *B. tectorum*, *B. inermis* and *S. comata*, that showed no symptoms indicative of WSMV infection were correspondingly negative for the virus in both assays. These results strongly indicate that *B. tectorum*, *B. inermis*, and *S. comata* are non-hosts for WSMV isolate MONT-95.

Although, serological tests indicated *P. pratensis* as negative for WSMV infection, a single DNA product of the predict length of approximately 944 base pairs was

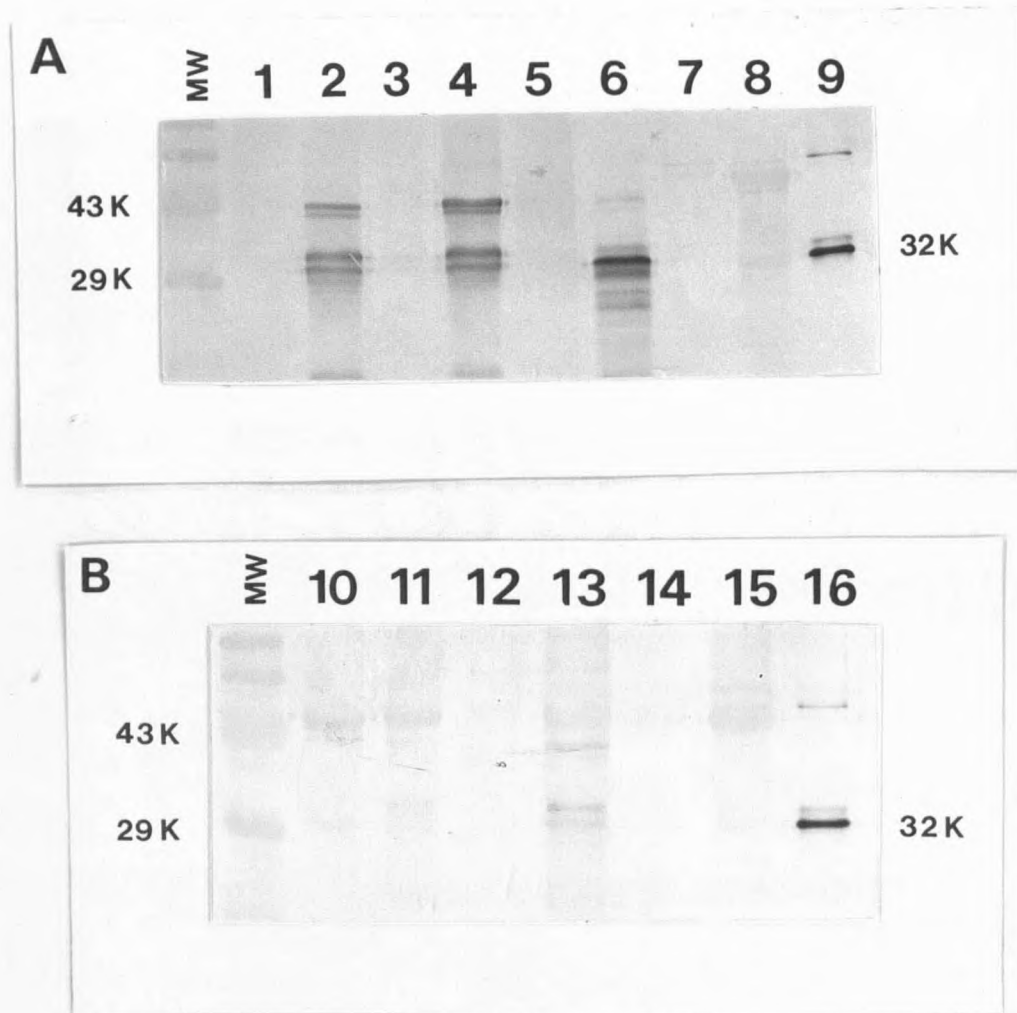


Figure 5.7. Analysis of wheat streak mosaic virus (WSMV) proteins extracted from eight grass species and separated by sodium dodecyl sulfate-polyacrylimide gel electrophoresis (SDS-PAGE) and probed with antibodies to WSMV in a Western blot. Lanes to the far left contain MW=molecular weight standards (K=kilodaltons). **(A)** Lane 1, healthy Rambo *T. aestivum*; lane 2, WSMV-infected Rambo *T. aestivum*; lane 3, healthy *A. cylindrica*; lane 4, WSMV-infected *A. cylindrica*, lane 5, healthy *A. fatua*, lane 6, WSMV-infected *A. fatua*, lane 7, healthy *B. tectorum*; lane 8, WSMV-infected *B. tectorum*; lane 9, purified WSMV. **(B)** Lane 10, healthy *P. pratensis*; lane 11, WSMV-infected *P. pratensis*; lane 12, healthy *B. inermis*; lane 13, WSMV-infected *B. inermis*; lane 14, healthy *S. comata*; lane 15, WSMV-infected *S. comata*; lane 16, purified WSMV.

detected in IC-RTPCR test for this grass species. However, the PCR signal was faint suggesting WSMV detection in WCMs and not the plant host. Attempts at obtaining similar results with *P. pratensis* leaves with WCMs and leaves with mites removed were negative in both PCR based tests (data not shown). Inconsistent results obtained in the nucleic acid and two serological tests for in *P. pratensis* prompted the use of a more reliable but less sensitive Northern blot assay. However, the low concentration of WSMV that was detected in *P. pratensis* samples by IC-RTPCR was undetectable with the Northern blot test (Figure. 5.8).

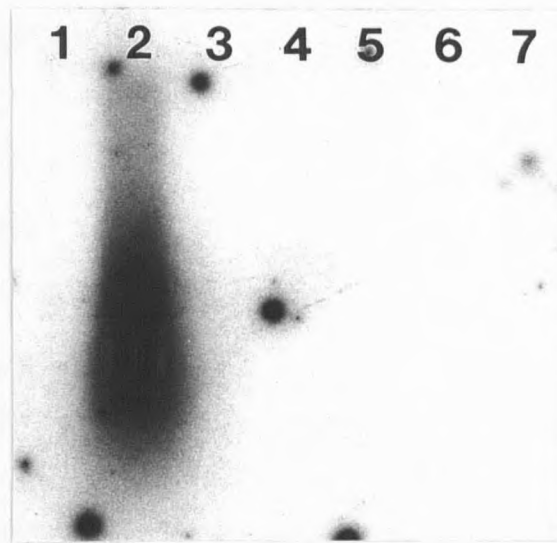


Figure 5.8. Northern analysis of nucleic acid extracts from *T. aestivum* and *P. pratensis* plants exposed for 35 days to continuous WSMV viruliferous WCMs. The gel was blotted to a nylon membrane and hybridized to a cloned ^{32}P -labeled DNA of the WSMV coat protein. The arrangement of samples is as follows: lane 1, healthy *T. aestivum*; lane 2, RNA of *T. aestivum* inoculated with WSMV by WCMs; lane 3, healthy *P. pratensis*; lanes 4 to 7, *P. pratensis* exposed to viruliferous WCMs.

Discussion

The results of PAS-ELISA indicate that WSMV #260 polyclonal antiserum against the eleven different WSMV isolates was suitable for WSMV detection in the one maize and ten infected wheat cultivars. In addition, WSMV #260 antiserum did not cross react with healthy wheat, AgMV and CCMV. For this reason, WSMV #260 antiserum was recognized to be a sufficient polyclonal antibody for WSMV screening in field collected and laboratory inoculated grass plants.

The sensitivity of PAS-ELISA and IC-RTPCR assays was compared for detection of WSMV from crude infected wheat extracts and purified WSMV-84 isolate dilutions. As expected, IC-RTPCR is the most sensitive assay of the two and might be used to confirm samples that are inconclusive in less sensitive serological and nucleic acid based assays. The increased sensitivity range between PAS-ELISA (300 fold) and IC-RTPCR (1000 fold) for WSMV infected wheat compared to purified WSMV dilutions may be attributed to plant inhibitors (10). This was further evident by the low band intensity of the lowest dilute wheat sample (1:10 wt/vol) when compared to the higher wheat plant dilutions. A previous study by Hu et al. (11) found dilutions of extracts from banana leaf samples improved the reliability of RTPCR assay for detection of cucumber mosaic virus in banana plants.

Protein antibody sandwich-ELISA absorbance values and its corresponding IC-RTPCR band intensity varied among isolates. Theoretically, IC-RTPCR should have amplified a band of approximately 944 base pairs with the same intensity for all eleven

isolates. Possible reasons for various band intensities may have been the response to different cereal cultivars, presence of plant inhibitors and/or initial virus titers in plant samples were low. A potential solution would have been to perform a series of plant dilutions in addition to varying the RT and PCR parameters in order to achieve optimal band amplification for each independent plant sample. However, for the purpose of this study nucleic acid verification of WSMV isolates was sufficient for confirmation of PAS-ELISA results.

Field collected *P. pratensis* and *B. inermis* plants that initially tested positive for PAS-ELISA, when re-tested after -20°C storage for 6 to 24 months with a second PAS-ELISA and a series of serological and nucleic acid based assays all tested negative for WSMV. Possible reasons for loss of WSMV signal may be an under estimation of PAS-ELISA positive threshold, and/or a degradation of virus coat protein and single strand viral RNA during prolonged cold storage. One major disadvantage in accurately reporting ELISA data for field plant surveys is the lack of a negative control match (i.e., same species, similar plant age, and plants subject to similar environmental conditions) for each test host plant (12). Although, initially a high frequency of positive samples were recorded over a three year period in *B. inermis* and *P. pratensis* species a lack of molecular evidence suggests a rapid degradation of WSMV capsid proteins and RNA viral nucleic acid by plant proteases and nucleases. Proteolysis *in vivo* of the capsid proteins in potyviruses is common and occurs to a greater degree in WSMV than other potyviruses (1,13). This viral degradation may occur to a greater extent in feral grasses

than domestic cereals which may contain lower inhibitory enzymes (14). Another possible explanation for the inability to detect WSMV in repeat PAS-ELISA tests might have been the absence of the virus or a low virus titer in the plant tissues that were tested. Previous reports have shown that the virus titer of cucumber mosaic virus to vary among plants and even between leaves on the same plant (11). Also, constant freeze/thaw cycles of the samples may have contributed to the negative serological and nucleic acid analysis. Recent tests by Mathews et al. (15) has shown that one freeze/thaw cycle is the limit for acceptable IC-PCR data from plant tissues. Another possible reason might be a cross reaction with a heterologous plant protein, and/or unrelated virus capsid protein. Serological relationships between WSMV and other potyviruses such as Johnsongrass mosaic virus core coat protein antibody have been shown to cross react with the capsid protein of WSMV (16).

The detection of WSMV in *P. pratensis* plants after 35 days of continuous viruliferous WCM exposure indicates virus presence in the mite and not the plant host. However the period of how long the WCMs remain viruliferous on *P. pratensis* is unknown. Reverse transcription-PCR has been used to detect citrus tristeza virus in three aphid species (17) and dot immunobinding assay has been developed to detect WSMV in a single WCM (18). Understanding the interaction of WSMV in WCMs on immune grass hosts may lead to novel ways to disrupt acquisition or transmission of the virus in susceptible hosts (i.e., wheat).

The results of laboratory tests show that WSMV-95 was easily detected by the

two serological and two nucleic acid tests for infected *T. aestivum*, *A. cylindrica* and *A. fatua* grown in the greenhouse and symptomatic *T. aestivum* plants collected in the field. Previous investigators have shown *A. cylindrica* and *A. fatua* to be reservoir hosts for WSMV through mechanical inoculation and WCM transmission (4, 19). This study indicates *A. cylindrica* and *A. fatua* are reservoir hosts for WSMV. This is the first report of serological and molecular evidence on the presence of WSMV in *A. cylindrica* and *A. fatua*.

In conclusion, ambiguous serological (i.e., PAS-ELISA) test results for field grass samples underscore the importance of multiple testing of individual grass samples and the use of independent confirmatory assays for the diagnosis of WSMV.

References

1. Niblett, C. L., K. R. Zagula, L. A. Calvert, T. L. Kendall, D. M. Stark, C. E. Smith, R. N. Beachy, and S. A. Lommel. 1991. cDNA cloning and nucleotide sequence of the wheat streak mosaic capsid protein gene. *J. Gen. Virol.* 72: 499-504.
2. Paliwal, Y. C. and J. T. Slykhuis 1967. Localization of wheat streak mosaic virus in the alimentary canal of its vector *Aceria tulipae* Keifer. *Arch. Virology.* 32: 344-353.
3. Sill, W. H. Jr, R. C. Bellingham, and H. Fellows. 1964. Reactions of wheat crosses, barley, rye, and oats to wheat streak mosaic virus. *Tech. Bull. Kan. Agric. Exp. Stn.* 132: 3-15.
4. Somsen, H. W. and W. H. Jr. Sill. 1970. The wheat curl mite, *Aceria tulipae* Keifer, in relation to epidemiology and control of wheat streak mosaic. *Tech. Bull. Kan. Agric. Exp. Stn.* 162: 1-15.
5. McNeil, J. E., R. French, G. L. Hein, P. S. Baenziger and K. M. Eskridge. 1996. Characterization of genetic variability among natural populations of wheat streak mosaic virus. *Phytopathology.* 86: 1222-1227.
6. Gao, J. G. and A. Nassuth. 1993. Alteration of major cellular organelles in wheat leaf tissue infected with wheat streak mosaic Rymovirus (Potyviridae). *Phytopathology.* 83: 206-213.
7. Carroll, T. W., S. K. Zaske and R. H. Brlansky. 1982. Separation of Montana isolates of wheat streak mosaic virus on Michigan amber wheat. *Plant Disease.* 66: 916-918.
8. Titus, D. 1991. Promega protocols and application guide. 2nd ed. Promega Corp. Madison, WI.
9. Nolasco, G., C. de Blas, V. Torres and F. Ponz. 1993. A method combining immunocapture and PCR amplification in a microtiter plate for the detection of plant viruses and subviral pathogens. *J. Virol. Methods.* 45: 201-218.
10. Slack, S. A., J. L. Drennan, A. A. G. Westra, N. C. Gudmestad and A. E. Oleson. 1996. Comparison of PCR, ELISA, and DNA hybridization for the detection of *Clavibacter michiganensis* subsp. *sepedonicus* in field-grown potatoes. *Plant Disease.* 80: 519-524.

12. Sutula, C. L., J. M. Gillett, S. M. Morrissey and D. C. Ramsdell. 1986. Interpreting ELISA data and establishing positive-negative threshold. *Plant Disease*. 70: 722-726.
13. Brake, M. K., R. N. Skoop and L. C. Lane. 1990. Degradation of wheat streak mosaic virus capsid protein during leaf senescence. *Phytopathology* 80: 1401-1405.
14. French, R. and N. L. Robertson. 1994. Simplified sample preparation for detection of wheat streak mosaic virus and barley yellow dwarf virus by PCR. *J. Virol. Methods*. 49: 93-100.
15. Mathews, D. M., K. Riley and J. A. Dodd. 1997. Comparison of detection methods for citrus tristeza virus in field trees during months of nonoptimal titer. *Plant Disease*. 81: 525-529.
16. Shukla, D. D., M. J. Frenkel, and C.W. Ward. 1991. Structure and function of the potyvirus genome with special reference to the coat protein coding region. *Can. J. Plant Pathology* 13: 178-179.
17. Mehta, P., R. H. Bransky, S. Gowda and R. K. Yokomi. 1997. Reverse-transcriptase polymerase chain reaction detection of citrus tristeza in aphids. *Plant Disease*. 81: 1066-1069.
18. Mahmood, T., G. L. Hein, and R. C. French. 1997. Development of serological procedures for rapid and reliable detection of wheat streak mosaic virus in a single wheat curl mite. *Plant Disease*. 81: 250-253.
19. Connin, R.V. 1956. The host range of the wheat curl mite, vector of the wheat streak-mosaic. *J. Econ. Entomol.* 49: 1-4.

CHAPTER 6

EFFECT OF GLYPHOSATE ON DISPERSAL OF WHEAT CURL MITE :

A WIND TUNNEL STUDY

Introduction

Wheat streak mosaic virus can spread through leaf abrasions, but it is primarily spread by the WCM, a minute (average adult length 250 μm), wingless, cigar shaped eriophyid mite. Since the WCM has no wings and two pairs of legs near its anterior cephalic region, its primary mode of dispersal is wind. The movement of viruliferous WCMs is influenced by plant nutrition. As the food supply decreases, e.g., host matures, the mites respond by moving up the plant where they undergo a dispersal behavior (1)

Volunteer wheat serves as the most important overwintering host for the WCM, and its etiological agent WSMV (1, 2, 3). The earlier that volunteer wheat plants are infested, relative to plant age, the greater opportunity for the development of large

viruliferous WCM populations (4). Wheat curl mites can survive for 4-6 days off a green host. Therefore, control of WSM is dependent on interrupting the life-cycle of the WCM (5). This is primarily accomplished by destroying volunteer wheat plants at least two weeks before planting winter wheat. Volunteer wheat can be managed by conventional tillage or by the use of herbicides (6). However, even when these control tactics were implemented in Kansas between 1987 to 1991 WSM damage still caused an estimated wheat loss of 15.5 million bushels (3). Incidence of WSM in locations where volunteer wheat was controlled by herbicide application suggests that WCMs either directly, by chemical irritation, or indirectly through a decrease in plant health, dispersed from volunteer wheat plants to adjacent grass hosts.

The most common agrochemical used to control volunteer wheat in north central Montana is the foliar-applied herbicide glyphosate. Glyphosate is a broad spectrum post-emergence herbicide that is effective on both annual and perennial weeds (7). Once applied, glyphosate blocks an enzymatic step in the plants shikimate pathway. Blockage of this pathway inhibits the synthesis of three aromatic amino acids, phenylalanine, tyrosine, and tryptophan that are essential for the plants survival and growth (8). Although translocation of glyphosate is rapid, within 4 hours of application, plants may not begin to show herbicide symptoms until 4 to 7 days post-treatment (9).

There are numerous studies on the effects of insecticides and herbicides on dispersal in the Tetranychidae mites (10,11,12), but there are no studies describing the effect of herbicides on the dispersal tendencies in eriophyid mites. Knowledge of the behavioral

response of WCMs to glyphosate may create a need to re-evaluate the use of this common herbicide as control for volunteer wheat particularly when the vector is present. The objective of this study was to assess the dispersal response of WCMs on wheat seedlings exposed to glyphosate at field rates of 420, and 840 ml/ha inside a laboratory wind tunnel.

Material and Methods

Viruliferous WCMs used in this study were collected from wheat fields in Teton County, Montana in 1995. Mite colonies were maintained in a growth chamber (Convion, Winnipeg, Manitoba, Canada) maintained at 22 to 25°C, photoperiod (400 $\mu\text{mol s}^{-1} \text{m}^{-2}$) 16 h L/8h D, relative humidity 50 to 80 percent. Infested plants at the 2- to 3- leaf stage grown in square plastic (10 X 10 cm) pots were used in the study.

Glyphosate N-(phosphonomethyl) glycine in the form of its isopropylamine salt (41 % wt:wt [AI]) (Monsanto, St. Louis, MO) was formulated with water. Formulations were mixed in 100-ml quantities before each trial. A spray tower cabinet (R & D Sprayers, Inc, Opelousas, LA) calibrated at 91 L/ha was used to deliver the treatments. Treatments consisted of application rates of water, 420, 840 ml/ha of technical grade glyphosate.

Experimental Design

Wheat curl mite dispersal trials were performed in two identical wind tunnels housed in an environmentally controlled walk-in growth chamber (Convion, Winnipeg, Manitoba, Canada) maintained at 22 to 25°C, photoperiod (600 $\mu\text{mol s}^{-1} \text{m}^{-2}$) 16 h L/8h D,

relative humidity 20 to 30 percent. The sides of the wind tunnels were assembled from clear acetate plastic sheets (height 0.5 m and length and width 1.0 m), with both ends of the tunnels covered with 0.25 mm² screen mesh. At one end of the tunnel, wind was generated at a constant air flow of 2.5 m s⁻¹ by an electric fan. Air flow was continuous throughout the duration of the study. Mite infested wheat plants were positioned 16 cm downwind from the electric fan (Figure 6.1).

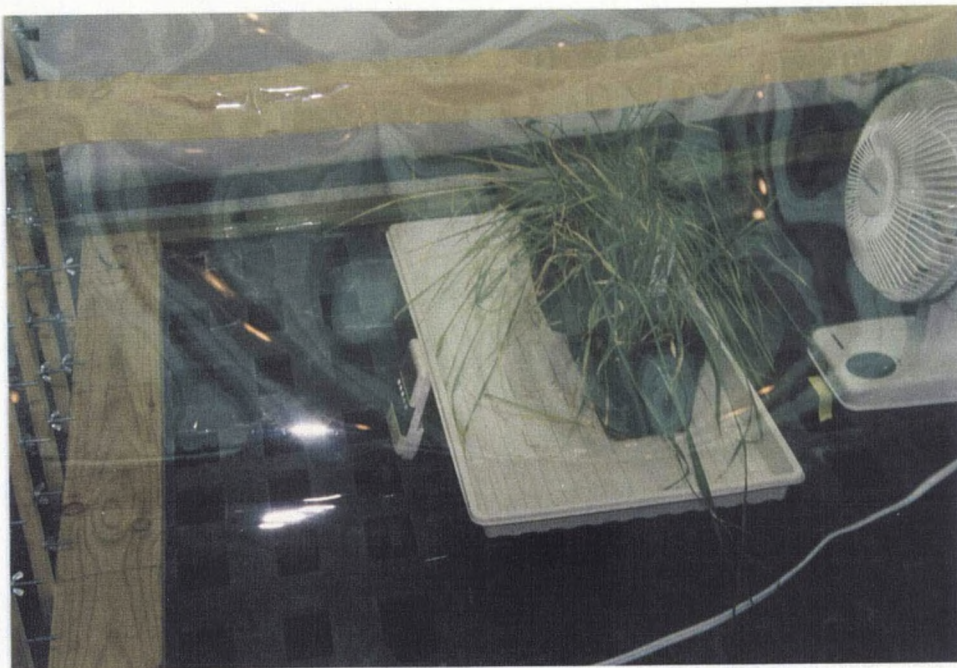


Figure 6.1. A wind tunnel showing an electric fan blowing onto a wheat infested source in the direction of a stand containing silicone grease coated slides (slides were coated on one side).

Approximately 0.75 m from the infested wheat source, twenty-nine silicone grease coated slides (2.5 X 7.5 cm) attached to a wooden stand by metal clips were used to capture

aerial-borne mites (Figure 6.2).

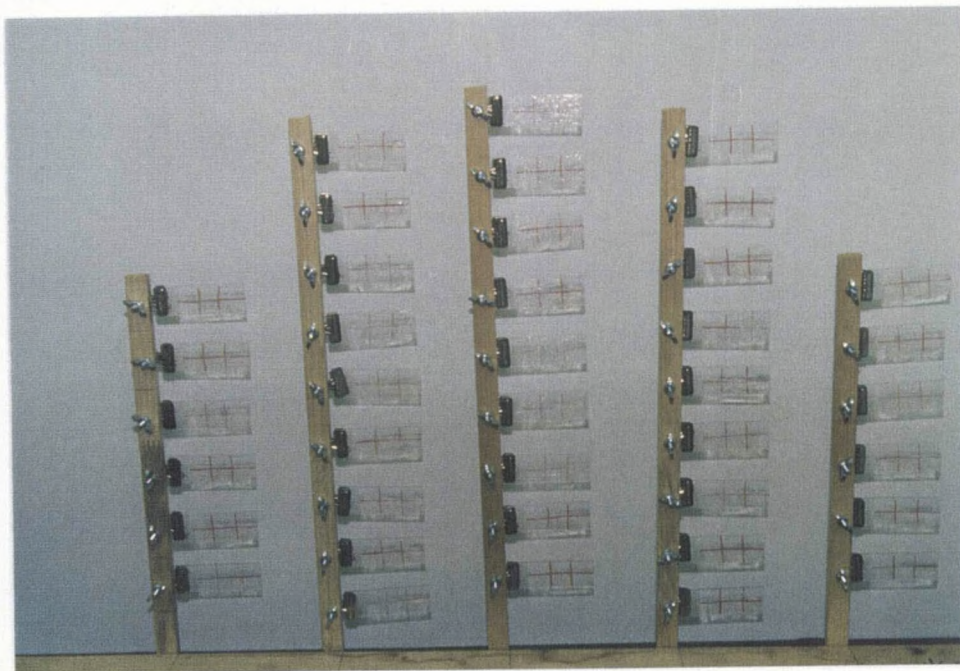


Figure 6.2. Wooden stand containing silicone grease coated slides used to capture aerial-borne WCMs.

A pre-treatment check 2 hours without air flow and 2 hours with air flow were conducted to estimate mite numbers dispersed by wind alone. Attempts were made to standardize the number of mites for each of the treatments. This was performed by removing or adding infested plants based on the number of mites capture by wind alone. For 6 hours, after glyphosate application, no air flow was generated, this was conducted to enhance glyphosate uptake and prevent herbicide evaporation. Post-treatment check times were at 12, 36, 60, 84, 108, 132, 156, 180, 204, 228, 252 and 276 hours from the start of each

replication (6 hours prior to treatment during no air flow). Slides removed at these time intervals were replaced by a new set of silicone greased slides. Removed slides were examined under a dissecting microscope (16X) and trapped mites were counted. A dilute fertilizer mixture (15:30:15, N: P: K) was applied to the infested plants daily. All treatments were replicated three times over a 35 day period.

Statistical Analysis

To stabilize variances, a square root transformation was applied to mites captured per treatment for each sampling time. The number of captured WCMs per treatment over time were analyzed using a repeated measures analysis of variance (13). Data were further analyzed with analysis of variance general linear model and Tukey studentized range test (significance levels were set at $\alpha < 0.10$ and $\alpha < 0.05$) was used to separate treatment means for each period (13). Nontransformed cumulative mite mean numbers over time were subject to a second order regression to determine overall mite dispersal trends for each treatment.

Results

Over all time periods there were no significant differences between total mean mite capture numbers for the two glyphosate rates and water control ($F = 0.12$; $df = 2,71$; $P > 0.47$). Also, there were no significant differences between the mean number of mites for each of the three pre-treatment checks (no glyphosate or water treatment only constant air

current for 2 hours) ($F = 2.92$; $df = 3,8$; $P > 0.16$). However, there were significant differences between treatment mean mite capture numbers at early and late sampling periods (Table 6.1). The number of mites captured after treatment with the high glyphosate rate were significantly higher than water control at 12, 36, and lower at 204, 228 and 252 hours post-treatment. Significant differences in mean mite capture numbers between the low and high glyphosate rate were observed at 12, 228, and 252 hours post-treatment. No differences were observed between the water and low glyphosate treatment, except at 228 and 252 hours post-treatment.

Mean number of captured mites peaked 12 hours post-treatment for each glyphosate treatment, with the higher rate having approximately 1000 more mites captured than the low treatment for this period (Figure 6.3). The large variability in the high rate is explained by one of the replications having a disproportionately large number of mites (3800 mites) captured compared to the other two replications. Captured mites for the high herbicide rate decreased from a high of 2000 mites at 12 hours to a low of 600 mites at 84 hours. A second spike occurred at 108 hours followed by continuous decline in mite capture numbers through out the remainder of the experiment. Observation at this second peak noted treated plants were beginning to exhibit early glyphosate symptoms of desiccation. A similar peak and glyphosate symptoms were observed in the low rate at 180 hours post-treatment.

Table 6.1. Mean number of WCMs captured from infested wheat plants after treatment with water, 840 and 420 ml/ha of glyphosate.

Treatment	Time (Hour)											
	12	36	60	84	108	132	156	180	204	228	252	276
840 ml/ha	42.4 a	38.5 a	31.4 a	25.4 a	28.9 a	23.5 a	23.3 a	22.1 a	19.1 b	17.3 c	15.5 c	15.5 a
420 ml /ha	33.2 b	29.3 ab	22.8 a	21.3 a	23.1 a	23.2 a	21.1 a	24.4 a	20.6 ab	21.5 b	24.3 b	23.6 a
Water	31.1 b	23.7 b	26.5 a	22.4 a	22.8 a	23.3 a	23.6 a	25.2 a	26.4 a	27.8 a	28.6 a	----

Statistical analysis was performed on transformed mite counts (square root). Means followed by the same letter within a column are not significantly different, Tukey's studentized range test ($\alpha = 0.1$).

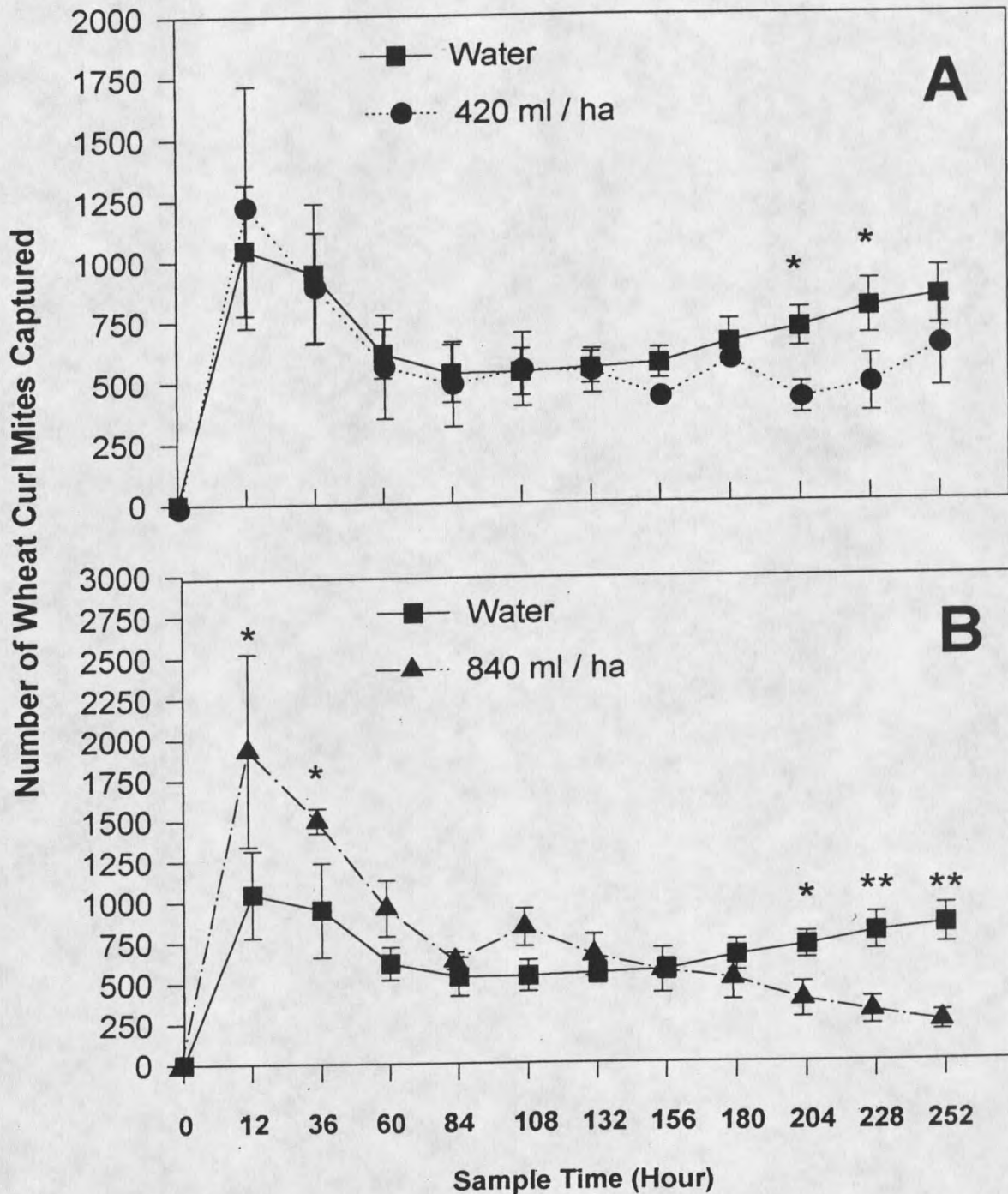


Figure 6.3. Mean number of wheat curl mites captured after infested plants were with water (A,B), 420 (A) and 840 ml/ha of glyphosate (B). Symbols indicate significant differences for (*) $\alpha = 0.10$ and (**) $\alpha = 0.05$ for Tukey's studentized range test for transformed (square root) mean mite numbers (actual mite numbers shown in graphs).

Cumulative mean number of WCMs captured at the end of 252 hours post-treatment was 1000 mites more than the water and low treatment (Figure 6.4). Interestingly, mites that were captured at 222 hours post treatment were alive in both herbicide treatments. It appears that glyphosate has no adverse effects directly on the mites, however indirectly through plant healthy it may be negatively correlated.

These results indicate peak mite dispersal for the high glyphosate rate occurs immediately at 12 hours post-treatment. After initial spike the mite numbers captured gradually decline, this trend may be a result of the majority of the mites disperse first, and the low number of mites that are left are comparable to the mite populations on the low glyphosate and water control treatments.

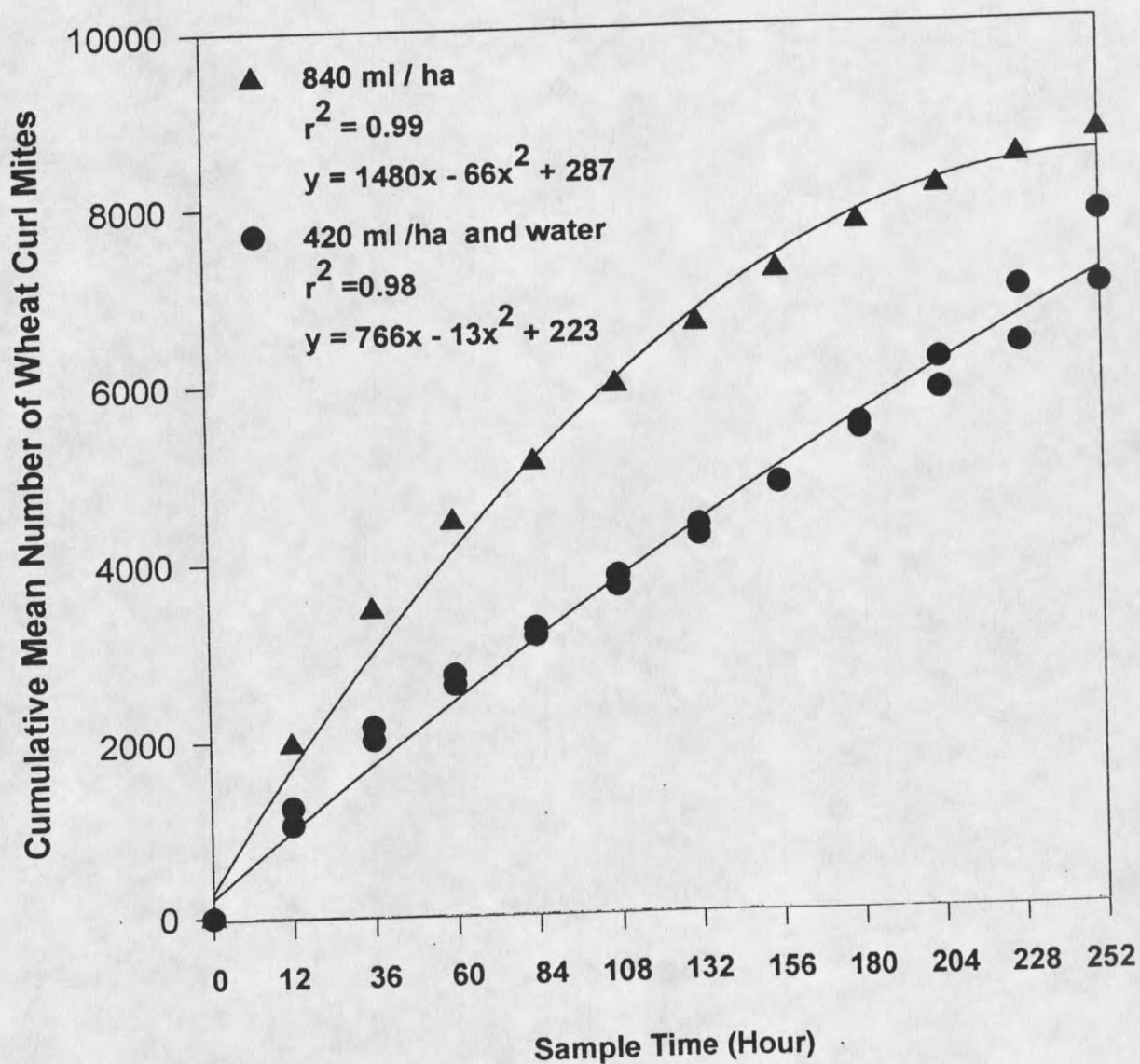


Figure 6.4. Comparison of the cumulative number of wheat curl mites captured from infested wheat plants after water, 420 and 840 ml/ha of glyphosate were applied (mean mite numbers captured were untransformed). A single regression was computed for both water and 420 ml/ha of glyphosate.

Discussion

This study demonstrated that the recommended field rate of 840 ml/ha of glyphosate caused 37% of the WCMs to disperse from infested wheat seedlings within 42 hours of application. This was significantly greater than the 21% of WCMs that dispersed from the low glyphosate rate and water control during this same period. Early mite dispersal behavior could be explained by mites that come in contact with the herbicide on the outside of the leaves are triggered to release first, while mites within the curled leaves are protected from herbicide contact. Similar WCM dispersal numbers would be magnified in the field, stimulating the potential spread of viruliferous WCMs to adjacent alternate grass and cultivated wheat hosts. A study by Flexner et al. (14) found the use of herbicides to control groundcover plants significantly increased the dispersal of *T. urticae* into fruit orchards.

Glyphosate had no apparent adverse effect on the survivability of WCMs treated with the low or high rate. This was indicated by the visible movement of WCMs captured after 200 h post-treatment in both herbicide treatments. Only one study involving woodlice has shown glyphosate treatment to have a negative effect on the survival of an arthropod (15). Most studies have shown glyphosate to be relatively harmless to arthropods (16).

The steady decline in WCM capture numbers after early peak dispersal in the high rate could be explained by a gradual reduction in host plant nutrition resulting from herbicide toxicity and/or the majority of mites dispersed within the first 42 h of

application. A combination of both is probable since symptoms of glyphosate desiccation were noted 84 hours post-application. However, in the low rate and water control WCMs after peak dispersal at 42 h post-treatment gradually declined until 84 h post-treatment before beginning to steadily increase for the remainder of the experiment.

In conclusion, the experiment described in this study was designed to determine under controlled conditions whether glyphosate application would increase dispersal of viruliferous WCMs and thus increase the chances of spreading a persistently borne plant pathogen. The major finding of this research is that the recommended rate (840 ml/ha) of glyphosate does increase WCM dispersal within 42 hours of initial application. Nonetheless, complimentary field studies are needed to address what effects glyphosate treatment has on WCM dispersal in relation to abiotic and biotic factors such as meteorological conditions, different host cultivars, host plant age, and mite densities. Field glyphosate trials incorporating these factors, coupled with our knowledge of WCM dispersal behavior, could help to identify an additional factor influencing WSMV spread and facilitate efforts to minimize such spread.

References

1. Harvey, T. L., T. J. Martin and D. L. Seifers. 1994. Importance of resistance to insect and mite vectors in controlling virus diseases of plants: Resistance to the wheat curl mite (Acari: Eriophyidae). *J. Agric. Entomol.* 11: 271-277.
2. Somsen, H. W. and W. H. Jr. Sill. 1970. The wheat curl mite, *Aceria tulipae* Keifer, in relation to epidemiology and control of wheat streak mosaic. *Technical Bull. Kan. Agric. Exp. Stn.* 162: 1-15.
3. Staples, R., and W. B. Allington. 1956. Streak mosaic of wheat in Nebraska and its control. *Univ. Nebraska Agric. Exp. Stn. Res. Bull.* 178: 41 pp.
4. Connin, R. V. 1956. The host range of the wheat curl mite, vector of the wheat streak-mosaic. *J. Econ. Entomol.* 49: 1-4.
5. Slykhuis, J. T. 1955. *Aceria tulipae* Keifer (Acarina: Eriophyidae) in relation to the spread of wheat streak mosaic. *Phytopathology.* 45: 116-128.
6. Watkins, J. E., French, R. C., Campbell, J. B., Kerr, E. D. and R. N. Klein. 1989. wheat streak mosaic disease, Nebraska Coop. Ext. Serv. E.C. 89- 1871: 1-5.
7. Baird D. D., Upchurch, R. P., Homesley, W. B. and J. E. Franz. 1971. Introduction of a broad spectrum postemergence herbicide class with utility for herbaceous perennial weed control. *Proc. North Cent. Weed Contr. Conf.* 26: 64-68.
8. Baillie, A. C., J. R. Corbett, J. R. Dowsett, and P. McCloskey. 1972. Inhibitors of shikimate dehydrogenase as potential herbicides. *Pesticide Sci.* 3: 113-120.
9. Sprankle, P., W. F. Meggitt and D. Penner. 1975. Absorption, action, and translocation of glyphosate. *Weed Sci.* 23: 235-240.
10. Bynum, E. D. Jr. and T. L. Archer. 1992. Banks grass mite (Acari: Tetranychidae) response to selected insecticides used for the control of Southwestern corn borer. *J. Agric. Entomol.* 9: 189-198.
11. Berry, J. S., T. O. Holtzer and H. J. Ball. 1990. Ambulatory dispersal of spider mites (Acari: Tetranychidae) on whole, untreated maize plants after exposure to fenvelerate and permethrin. *J. Econ. Entomol.* 83: 217-220.

12. Wilson, L. T., J. M. Smilanick, M. P. Hoffmann, D. L. Flaherty and S.M. Ruiz. 1988. Leaf nitrogen and position in relation to population parameters of pacific spider mite, *Tetranychus pacificus* (Acari: Tetranychidae) on grapes. Environ. Entomol. 17: 964-968.
13. SAS Institute. 1989. Users guide: statistics. SAS Institute. Cary, NC.
14. Flexner, J. L., P. H. Westigard, P. Gonzalves and R. Hilton. 1991. The effect of groundcover and herbicide treatment on twospotted spider mite density and dispersal in southern Oregon pear orchards. Entomol. Exp. Appl. 60: 111-123.
15. Mohamed, A. I, G. A Nair, H. L. Abbas, H. H. Kassam. 1992. Effects of pesticides on the survival, growth and oxygen consumption of *Hemilepistus reaumuri* (Audouin and Saviigny 1826) (Isopoda Oniscidea). Tropical Zoology. 5: 145-153.
16. Teran, A. L., R. A. Alvarez and C. A. Orlando. 1993. Effects of currently used pesticides in citrus orchards on two aphelinid parasitoids: laboratory tests. J. Appl. Entomol. 116: 20-24.

CHAPTER 7

CONCLUSIONS

- 1) In the field study (non-outbreak years 1995 to 1997), *Aceria tosichella* was recovered from *Bromus inermis*, *Poa interior* and *Poa pratensis*. This is the first report of *A. tosichella* collected from *P. interior*. Survey data indicated that *A. tosichella* does not develop large populations on feral grasses.
- 2) Field collected *Poa pratensis* and *B. inermis* tested PAS-ELISA positive for WSMV each of the three years. All grass samples that tested positive for WSMV were asymptomatic, except cultivated and volunteer wheat plants collected in 1997. This survey indicated that WSMV infecting perennial and annual grasses does not develop to epidemic levels.
- 3) Laboratory tests confirm previous reports that *Aegilops cylindrica* and *Avena fatua* are suitable grass hosts for the WCM and WSMV. In mechanical and viruliferous WCM

transmission tests, WSMV infection developed on both grass species. In addition, WCMs removed from these two species when transferred to WSMV non-infected wheat transmitted the virus to the wheat plants.

4) Repeated attempts to infect eleven feral grass species with WSMV by mechanical and viruliferous WCM inoculations were unsuccessful, except for *Elymus canadensis* and *Stipa comata*. However, these two cases were isolated incidences, and repeated tests on these species were unsuccessful.

5) Wheat curl mite populations developed on *B. inermis*, *P. pratensis*, and *S. comata*.

Only after continuous viruliferous WCM exposure for 35 days were significant WCM populations observed. The presence of eggs were discovered on these grass species indicating successful WCM colonization.

6) Field collected grass samples that initially tested PAS-ELISA positive for WSMV when retested by serological and nucleic acid based assays after 6-24 months of cold storage (-20°C) were all negative, except for symptomatic wheat plants collected in 1997. Possible reasons for loss of WSMV signal may have been an under estimation of PAS-ELISA positive threshold, and/or a degradation of virus coat protein and RNA by plant host protease and nucleases.

7) Molecular analysis showed that WSMV-95 was easily detected by the two serological and two nucleic acid based tests for infected *T. aestivum*, *A. cylindrica* and *A. fatua*

grown in the greenhouse. Although, serological tests indicated *P. pratensis* as negative for WSMV infection, a single DNA product of the predict length of approximately 944 base pairs was detected in IC-RT-PCR test. However, the PCR signal was faint suggesting WSMV detection in WCMs and not the plant host. Attempts at obtaining similar results with *P. pratensis* leaves harboring WCMs and leaves with mites removed were all negative in RT-PCR and Northern blot tests.

8) The wind tunnel study indicated that the recommended rate (840 ml/ha) of glyphosate did increase WCM dispersal within 42 hours of initial application. Nonetheless, complimentary field studies are needed to address what effects glyphosate treatment has on WCM dispersal in relation to abiotic and biotic factors such as meteorological conditions, different host cultivars, host plant age, and mite densities.

My investigation with serological and nucleic acid based assays does not support the hypothesis that feral grass species may function as ecologically important reservoirs of WSMV (A summary of field and laboratory grasses tested for WSMV and WCM susceptibility are shown in Table 7.1). Initial, serological evidence (PAS-ELISA) indicated that grasses collected in 1995, 1996 and 1997, contained WSMV, but I had no evidence to confirm these results. In order to prove that the grass species I tested were infected with WSMV, it would have been necessary to reisolate this pathogen from the grasses and then reproduce the transmission from grasses to wheat, but I was never able

Table 7.1 Summary of field and grass species tested for susceptibility to WSMV and WCM.

Grass Species	Field		Laboratory			
	WSMV	WCM	WCM ^a	WSMV		Transmission ^b
				Mechan.	WCM	
<i>A. cylindrica</i>	-	-	+	+	+	+
<i>A. dasystachum</i>	-	-	-	-	-	-
<i>A. fatua</i>	-	-	+	+	+	+
<i>A. trachycaulum</i>	+	-	-	-	-	-
<i>B. inermis</i>	+	+	+	-	-	-
<i>B. tectorum</i>	-	-	-	-	-	-
<i>E. canadensis</i>	-	-	-	+	-	-
<i>K. cristata</i>	-	-	-	-	-	-
<i>P. pratensis</i>	+	+	+	-	+ ^c	-
<i>P. smithii</i>	-	-	-	-	-	-
<i>S. comata</i>	+	-	+	-	+	-
<i>S. viridula</i>	+	-	-	-	-	-
<i>T. aestivum</i>	+	+	+	+	+	+

^a Wheat curl mite eggs present; 10 > mites observed per plant.

^b Wheat curl mites from grass species transmitted WSMV to wheat.

^c Wheat streak mosaic virus detected by IC-RTPCR (possible detection from WCMs).

to isolate the virus from field collected samples or develop consistent infection in laboratory grasses grown from seeds. My repeated failures to reisolate WSMV from field collected and laboratory inoculated grasses (mechanical and mite virus transmission) with multiple isolation techniques have lead me to conclude that the feral grass species I tested are not important reservoirs of inoculum. However, the development of WCM populations and the apparent detection of WSMV in WCMs on laboratory infested *P. pratensis* indicates that this species may play a role in perpetuating WSMV in the field. As a result of this finding, further studies should be focused on examining the vector competence of WCMs on *P. pratensis*.

Poa pratensis appears to have the greatest potential to develop into an important alternate host for the WCM and WSMV. This study has shown that *P. pratensis* is a suitable host for the WCM in the field and laboratory. This species is common and abundant in grassy areas near wheat fields increasing its selection pressure for the mite and virus over less common grass species. It is a perennial that is green from May to August and it grows in dense patches that provides a favorable microclimate for eriophyid mites. Also, the physical characteristics of the natural folding of its leaves are advantageous for eriophyid mite survival.

Agelopsis cylindrica and *A. fatua* are a more suitable host for both the WCM and WSMV than the other feral grasses that were tested. Since *A. cylindrica* and *A. fatua* are major weed problems in Montana, they could play an important role in heightening the incidence and severity of WSM in wheat fields. This re-emphasizes the importance of

weed control, not only for reducing their competition with crops, but also in possibly reducing a local WSM outbreak.

Volunteer wheat still appears to be the most important source for WCM and WSMV. This was supported by the increase in volunteer wheat in 1997 which resulted in low level of WSMV detected in wheat fields that year. Thus, growers should continue to practice field sanitation at the end of the growing season and voluntarily create a wheat free period during spring and winter wheat planting to help management of WSM disease.

Future Directions

Many interesting questions remain to be answered. An additional field grass survey should be conducted during an outbreak period to compliment this field survey (non-outbreak years). The possibility that WCM may survive on *P. pratensis* and how long they remain viruliferous on grasses deserves further study. More research is needed to determine whether *A. fatua* and *A. cylindrica* are important reservoirs for the WCM and WSMV in the field. More importantly can they serve as an intermediate bridge host to wheat for the virus and mite. Studies are needed to more effectively elucidate what caused the false positive PAS-ELISA readings in the field collected grasses and what were the factors that resulted in some grass species to be nonhosts for the virus and mite while others such as wheat are superior hosts.

AppendixAbbreviations

A ₄₀₅	absorbance (405 wave length)
AI	active ingredient
AgMV	agropyron mosaic virus
bp	base pair
CCMV	cowpea mosaic virus
dATP	deoxy adenosine triphosphate
dCTP	deoxy cytosine triphosphate
dGTP	deoxy guanine triphosphate
DNA	dioxyribonucleic acid
dTTP	deoxy thymidine triphosphate
ELISA	enzyme linked immunosorbent assay
EDTA	ethylenediaminetetraacetic acid
g	gravity
gm	gram
ha	hectare
IC-RTPCR	immunocapture reverse transcription polymerase chain reaction
Ig	immunoglobulin
ISEM	immunological specific electon microscopy
Kda	kilodalton

km	kilometer
μl	microliter
μm	micrometer
μmol	micromolar
mM	milimolar
M	molar
ng	nanogram
nm	nanometer
PAS-ELISA	protein antibody sandwich - enzyme linked immunosorbent assay
PBS	phosphate buffered saline
PEG	polyethelene glycol
pg	picogram
PVP	polyvinylpyrrolidone
³² P	phosphorus (radio isotope)
n. sp.	new species
RT-PCR	reverse transcriptase polymerase chain reaction
RNA	ribonucleic acid
RFLP	restriction fragment length polymorphism
STD	standard deviation
TBST	Tris buffered saline tween
SDS	sodium dodecyl sulfate

WCM	wheat curl mite
WSMV	wheat streak mosaic virus
WSM	wheat streak mosaic
WspMV	wheat spot mosaic virus

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