

Occurrence of Viruses in Wheat in the Great Plains Region, 2008

Mary Burrows, Assistant Professor, Plant Sciences and Plant Pathology Department, Montana State University, Bozeman, MT 59717; **Gary Franc**, Professor, Plant Sciences Department, University of Wyoming, Laramie, WY 82071; **Charlie Rush**, Professor, Texas A&M Research and Extension Center, Amarillo, TX 79106; **Tamla Blunt**, Plant Disease Diagnostician, Bioagricultural Sciences & Pest Management, Colorado State University, Fort Collins, CO 80523; **Dai Ito**, Graduate student, Plant Sciences and Plant Pathology Department, Montana State University, Bozeman, MT 59717; **Kasia Kinzer**, Plant Diagnostician, Department of Plant Pathology, North Dakota State University, Fargo, ND 58108; **Jen Olson**, Plant Disease Diagnostician, Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK 74078; **Judy O'Mara**, Diagnostician and Director, Plant Disease Diagnostic Lab, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506; **Jacob Price**, Research Associate, Plant Pathology Department, Texas A&M Research and Extension Center, Amarillo, TX 79106; **Connie Tande**, Diagnostician, Plant Science Department, South Dakota State University, Brookings, SD 57007; **Amy Ziems**, Extension Educator, Plant Pathology Department, University of Nebraska, Lincoln, NE 68583; and **James Stack**, Director, Great Plains Diagnostic Network, Kansas State University, Manhattan, KS 66506

Corresponding author: Mary Burrows. mburrows@montana.edu

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Abstract

Field surveys in 2008 determined the prevalence and diversity of viruses present in the Great Plains wheat crops. Symptomatic plants ($n = 754$) in nine states were tested for *Wheat streak mosaic virus* (WSMV), *Wheat mosaic virus* (WMoV, formerly known as *High Plains virus*), *Triticum mosaic virus* (TriMV), *Barley yellow dwarf virus-PAV* (BYDV-PAV), and *Cereal yellow dwarf virus-RPV* (CYDV-RPV), using indirect ELISA. Virus prevalence varied greatly, with average frequency of detection highest for WSMV (47%), followed by WMoV (19%), TriMV (17%), BYDV-PAV (7%), and lowest for CYDV-RPV (2%). Most positive plant samples (37%) had one virus present, with decreasing frequencies for co-infection by two (19%), three (5%), or four viruses (1%). TriMV was detected for the first time in Colorado, Nebraska, Oklahoma, South Dakota, Texas, and Wyoming. WMoV was identified for the first time in Montana and Wyoming. Chlorotic streaks were more frequently associated with WSMV, WMoV, and TriMV ($R = 0.166$ to 0.342 ; $P < 0.05$), and stunting was more frequently associated with WMoV ($R = 0.142$; $P = 0.004$) or TriMV ($R = 0.107$; $P = 0.033$) than WSMV. Symptom severity did not increase with co-infection as compared to single virus infections, with the exception of plants co-infected with mite transmitted viruses in Texas.

Introduction

In the Great Plains of the United States, the concentration of wheat (*Triticum aestivum*) production has resulted in specialized pest complexes threatening the economic and environmental sustainability of the small grain production system (7). Extension specialists, plant pathologists, entomologists,

and producers across the Great Plains region are concerned about the impact that wheat viruses have on wheat yield and grain quality. Since much of the wheat in this region is grazed, there also is concern about the impact of virus diseases on wheat forage. The need to learn more about viruses in the Great Plains region was identified at a meeting of the Great Plains Diagnostic Network (GPDN) during the National Plant Diagnostic Network (NPDN) meeting held in January of 2007. At this meeting, GPDN diagnosticians from multiple states reported greater frequency of virus-like symptoms observed in wheat during the 2006 growing season than in previous years. During a subsequent meeting of the GPDN (2-4 October 2007, Manhattan, KS), the GPDN network coordinator agreed to fund a survey of the 2008 wheat crop in nine Great Plains states (Colorado, Kansas, Montana, Nebraska, North Dakota, Oklahoma, South Dakota, Texas, and Wyoming). The goals of this survey were to determine the prevalence and diversity of viruses present in our cropping systems. Additional goals were to improve initial diagnostic efforts by determining the range of symptoms associated with single and multiple infections, and to provide more accurate information to producers dealing with virus diseases in small grains. Plant materials collected during the survey also would enable development of improved diagnostic tools such as PCR primers, as well as contribute to research projects on epidemiology and management of plant viruses.

Rough estimates of chronic yield loss from wheat viruses range between 5 and 10% across the region, with up to 100% yield loss in severely infected fields. Estimates from a Kansas wheat disease survey place the “wheat streak complex” second only to leaf rust for yield loss over a 20-year period (1). Particularly of concern are the recently identified viruses *Wheat mosaic virus* (WMoV), formerly known as *High Plains virus* (HPV), and *Triticum mosaic virus* (TriMV) that were detected in *Wheat streak mosaic virus* (WSMV)-resistant wheat lines in Kansas (12,14). Co-infection of plants by two viruses causes greater symptom severity and potential yield loss, compared to infection by a single virus (16). Prior to 2008, TriMV had only been identified in Kansas.

Survey Methodology

Diagnosticians at the GPDN meeting prioritized five major wheat viruses for survey efforts. These viruses included WSMV, WMoV, and TriMV, which are all transmitted by the wheat curl mite (*Aceria tosichella* Keifer). Additional viruses included in the tests were *Barley yellow dwarf virus* (BYDV)-PAV and *Cereal yellow dwarf virus* (CYDV)-RPV, both of which are transmitted by various aphid species. These viruses were selected based on prior diagnostic results for the Great Plains states, symptomatology observed in the field, and the need to understand the role of mixed infections in symptom development.

Most survey samples were collected from wheat plants with virus-like symptoms including chlorosis, mosaic, and/or stunting. Samples for testing included those submitted by producers for diagnosis, as well as those collected from the field during disease surveys, or solicited from county extension agents and crop advisors. The majority of samples were winter wheat, with a minor number of spring wheat and durum wheat samples from northern states (North Dakota, South Dakota, and Montana). Data for samples included collection date, plant growth stage, variety (if known), location of collection, symptom type(s) and incidence. After collection, samples were tested to determine virus status of the plant material. Viruses were detected with standard indirect enzyme linked immunosorbant assay (ELISA) methods. Kits were prepared by Agdia Diagnostics (Elkhart, IN) and samples were processed according to the manufacturer’s instructions. Each kit consisted of a single noncoated ELISA plate (96-well formatted in 8-well break-apart “strips”) for each of the five viruses, appropriate positive and negative controls, and reagents required for the assays, including antibodies and buffers. Infected plant tissue was preserved by lyophilization or freezing at -80°C for use in future research projects.

Survey Results

A total of 754 wheat samples were collected in the nine Great Plains states during the 2008 survey (Table 1). Due to various factors a small number of the samples were not tested for all five viruses. Therefore, results were standardized and presented as the percentage of the plant samples tested for each virus. The number of samples from each state varied from 21 to 307 (Table 1). The predominant virus detected in each state was WSMV, and this virus was detected in 47% of the plant samples (Table 1). WMoV (19%) and TriMV (17%) were the second and third most commonly detected viruses (Table 1). The aphid-transmitted viruses BYDV-PAV (7%) and CYDV-RPV (2%) were infrequently detected compared to the mite-transmitted viruses. Co-infection of a single plant by WSMV plus WMoV (13%) or WSMV plus TriMV (13%) was the most common co-infection detected. Five percent of samples were co-infected by all three mite-transmitted viruses and only 0.4% of samples were co-infected by both aphid-transmitted viruses.

Table 1. Percentage of wheat samples that tested positive for virus during the 2008 Great Plains survey. Samples were tested for *Wheat streak mosaic virus* (WSMV), *Wheat mosaic virus* (WMoV), *Triticum mosaic virus* (TriMV), *Barley yellow dwarf virus* (BYDV-PAV) and *Cereal yellow dwarf virus* (CYDV-RPV) using indirect ELISA. Common co-infections and the percent of plants infected with all mite-transmitted viruses and all aphid-transmitted viruses are also presented^{x,y}.

State	n	Percent of wheat samples testing positive for virus									
		WSMV	WMoV	TriMV	BYDV PAV	CYDV RPV	WSMV + WMoV	WSMV + TriMV	WMoV + TriMV	All mite ^x	All aphid ^y
CO	51	61	10	10	10	4	8	8	0	0	0
KS	53	62	38	30	6	2	15	21	13	8	0
MT	23	43	9	0	9	0	9	0	0	0	0
NE	66	39	8	27	5	3	8	18	5	5	2
ND	44	40	12	0	2	0	9	0	0	0	0
OK	93	27	30	6	16	3	16	4	3	3	2
SD	96	28	7	2	3	0	7	2	1	1	0
TX	307	83	41	57	14	2	37	53	28	26	0
WY	21	38	19	24	0	0	5	10	10	0	0
Total	754	47	19	17	7	2	13	13	13	5	0.4

^x "All mite" includes plant samples that were co-infected by WSMV, WMoV and TriMV.

^y "All aphid" includes samples that were co-infected by BYDV-PAV and CYDV-RPV.

Data in Table 2 show an average of 39% of the samples had no viruses detected by the ELISA tests used in the survey. Of the samples that tested positive, the majority (37%) had only one virus, with decreasing frequencies resulting for co-infection by two viruses (19%), three viruses (5%), or four viruses (1%). No individual sample had all five viruses simultaneously present.

Table 2. Percentage of wheat samples that tested positive for co-infection by one to five viruses in a survey of wheat in the Great Plains region, 2008.

State	n	Number of viruses in a sample					
		0	1	2	3	4	5
CO	51	22	63	16	0	0	0
KS	53	8	55	30	8	0	0
MT	23	48	43	9	0	0	0
NE	66	47	29	20	5	0	0
ND	44	57	34	9	0	0	0
OK	93	49	28	17	3	2	0
SD	96	68	23	7	1	0	0
TX	307	8	23	37	28	4	0
WY	21	43	33	24	0	0	0
Total	754	39	37	19	5	1	0

TriMV was identified in seven states, six of these being the first reported incidence of TriMV infection. WMoV also was identified for the first time in Montana and Wyoming, although its presence was suspected based on symptomatology.

Foliar symptom type and severity was consistently recorded by diagnosticians in seven of the nine states surveyed. An attempt was made to correlate symptom descriptions and severity with the virus(es) detected in each samples. If a symptom was absent for a particular sample, the datum point was recorded as a "0" and, if present, was recorded as a "1." Symptom categories included: general chlorosis; chlorotic streaks; plant stunting; purpling stem; purpling leaves; other symptoms. Spearman Rank Correlations were calculated (Statistical Analysis System version 9.2, SAS Institute Inc., Cary, NC) to determine if any symptom was predictive for the type of virus infecting the plant. Correlations were calculated for each data set within each state, and also for the "regional" data set, which included data from all seven states. Correlations tended to be weak ($R < 0.5$), and an alpha of 0.05 was considered significant. Results described below were calculated at the "regional" level, unless otherwise noted.

General chlorosis was not consistently associated with any individual virus infection in wheat. Chlorotic streaks were associated with individual mite-transmitted viruses and co-infections by mite-transmitted viruses ($R = 0.166$ to 0.342 ; $P < 0.05$). Stunting was associated more frequently with single infections by WMoV ($R = 0.142$; $P = 0.004$) or TriMV ($R = 0.107$; $P = 0.033$), and less frequently with single infections by WSMV ($R = 0.085$; $P = 0.090$). Double or triple co-infections by mite-transmitted viruses were correlated with stunting ($R = 0.109$ to 0.170 ; $P < 0.05$), but single or co-infection by aphid transmitted viruses were not associated with stunting ($P > 0.05$). Purpling of leaves was predictive of BYDV-PAV infection in Montana ($R = 0.423$; $P = 0.045$), and purpling of stems and leaves was predictive of BYDV-PAV ($R_{\text{stems}} = 0.564$, $P_{\text{stems}} < 0.001$; $R_{\text{leaves}} = 0.352$, $P_{\text{leaves}} = 0.016$) and CYDV-RPV ($R_{\text{stems}} = 0.375$, $P_{\text{stems}} = 0.010$; $R_{\text{leaves}} = 0.503$, $P_{\text{leaves}} < 0.001$) infection in Nebraska. However, very few luteovirus-positive samples were found in these states (Table 1), and no correlation between purpling symptoms and luteovirus presence was identified in Texas, Kansas, or Oklahoma. No purpling symptoms were noted in South Dakota or Wyoming. Purpling of stems ($R = 0.102$ to 0.328 ; $P < 0.05$) and leaves ($R = 0.107$ to 0.181 ; $P < 0.05$) was correlated with single and co-infections of aphid-transmitted viruses at the regional level.

The number of different symptoms noted for each sample was used to indicate symptom severity, and data were analyzed to determine if co-infection by more than one virus increased symptom severity. Analysis revealed that symptom severity did not increase as the number of viruses increased, with the

exception of samples collected in Texas. In Texas, TriMV ($R = 0.297$; $P = 0.003$) had greater symptom severity compared to single infections by WSMV ($R = 0.113$; $P > 0.05$) or WMoV ($R = 0.099$; $P > 0.05$). Co-infection of plants with more than one mite-transmitted virus exhibited greater symptom severity than either virus alone. TriMV caused greater symptom severity when co-infecting plants with WSMV ($R = 0.317$; $P = 0.001$) and WMoV ($R = 0.215$; $P = 0.031$) than with any of those viruses alone. Also, co-infections of WSMV and WMoV ($R = 0.148$; $P > 0.05$) had greater symptom severity than infection by either virus alone. Co-infection by all three mite transmitted viruses was also correlated with increased symptom severity ($R = 0.215$; $P = 0.031$).

Wheat Viruses in the Great Plains Region

We can only speculate why an increase in virus-like symptoms in wheat has been observed in the Great Plains. Production practices such as the greater adoption of late weed control, earlier winter wheat planting in the northern Great Plains, greater frequency of continuous wheat cropping in Texas, as well as increased adoption of no-tillage or conservation tillage could all contribute. Continuous wheat cropping and no till or conservation tillage practices all favor maintenance of host plants or the "green bridge" on which viruliferous wheat curl mites and other potential vectors survive. Vector survival increases virus frequency in the new wheat crop as viruliferous vectors move from senescing and dying plants to young winter wheat plants (9,17). The density of grassy weeds, which could serve as virus and mite hosts, has been increasing in recent decades because widespread use of semi-dwarf cultivars, broadcast nitrogen fertilizer, and control of broadleaf weeds with in-crop herbicides (3,8). Additionally, corn acreage has increased, and corn is known to be an alternate virus and wheat curl mite source (18). The increased prevalence of monocots such as grassy weeds and corn is likely to continue and will become increasingly important if our climate warms. In addition to the cultural practices and potential environmental effects on virus prevalence mentioned above, recently described viruses such as TriMV (12) and a new variant of WMoV (11) also could be contributing to the observed increase. It has been suggested that plants infected by more than one virus may exhibit more severe symptoms (11). This was confirmed in our study with samples from Texas, where we had the greatest number of samples and data on symptom expression (Texas). Other possible explanations for the increase in virus prevalence include climate change, changes in crop germplasm, or that pathologists, entomologists, and other related crop specialists are increasingly cognizant of virus diseases and the range of symptoms they cause.

Native and non-native grass species can serve as virus and vector reservoirs (5,13,15). As viruliferous mites or aphids move out of Conservation Reserve Program (CRP) grasslands and native pastures, viruses are introduced into the wheat crop. Vector movement and virus introduction are dependent on a number of factors including virus species, vectoring capacity of mites and insects, survival of vectors in CRP and native pasture, ability of vectors to feed and reproduce on over-summering hosts and crop plants, susceptibility of over-summering hosts and crops to the viruses, and environmental conditions which favor vector reproduction and disease development. Populations of viruliferous vectors may also be influenced by increased prevalence of C3 grassy weeds such as wild oat (*Avena fatua*) (2), which would promote vector survival and reproductive rates (19). Preliminary studies have indicated that wild oat is very susceptible to WSMV (M. Burrows, *unpublished data*), however wild oat is not a host of the wheat curl mite (15). Other grasses may serve as hosts for mite reproduction while wild oat serves as a virus reservoir.

The relative importance of CRP and native pastures as over-summer reservoirs, and subsequently the primary sources of viruses and vectors for the fall planted wheat crop, is not well documented. It is a complex question, due to the varying life cycles of grasses and vectors, and how they are influenced by the environment. Previous studies have shown that the number of virus-infected plants required to start an epidemic can be very low ($< 1\%$ of the field), when vector populations are very high (6,10). Therefore, even trace amounts of virus

in a field, or adjacent pasture, early in the season could have a significant impact on disease development in the crop later in the season. In addition, varieties with delayed maturity or the “stay-green” trait have shown increased yields as compared to varieties without the stay-green trait, and breeders are increasingly interested in developing and deploying these varieties in the United States (4). However, these could ultimately increase disease potential by creating reservoirs for viruses and their vectors and increasing the length of the “green bridge.”

Impact of the Great Plains Wheat Virus Survey

The goals of this survey were to identify wheat virus prevalence and diversity in our Great Plains wheat cropping systems, to determine the symptoms of single and multiple infections, to increase communication both within the research community and directly with producers regarding virus diseases in small grains, and to provide plant materials for research. We met each of these goals. We found that WSMV was commonly detected in symptomatic wheat samples from all states, with a frequency of detection as high as 83% in Texas. Additionally, we found WMoV and TriMV are widespread throughout the Great Plains. The symptom data we obtained generally supported that typical virus symptoms were seen for the types of viruses present in the plant (e.g., chlorosis and streaking for WSMV, WMoV, and TriMV; purpling for BYDV-PAV and CYDV-RPV). With samples from Texas, we also determined that mixed infections of mite-transmitted viruses produced more severe symptoms than single virus infections. However, we did not find any symptom unique to mixed infections.

With this study, we increased awareness and communication pertaining to virus diseases in the Great Plains, formed a listserv and initiated several collaborative projects as well as improved extension programming efforts related to virus diseases in wheat and small grains. We have provided lyophilized tissue and germplasm for several research projects. In addition to the original goals, several training opportunities for diagnosticians on plant virus diseases and their identification was offered via the GPDN online seminar series, as well as an online workshop on plant virus identification and RNA workshop at the 2009 GPDN annual meeting. This survey represents not only a successful collaborative effort of the GPDN, it illustrates the strength and potential of the NPDN network to facilitate research and extension efforts in the United States.

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