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## Occurrence and Distribution of *Triticum mosaic virus* in the Central Great Plains

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### Abstract

Byamukama, E., Seifers, D. L., Hein, G. L., De Wolf, E., Tisserat, N. A., Langham, M. A. C., Osborne, L. E., Timmerman, A., and Wegulo, S. N. 2013. Occurrence and distribution of *Triticum mosaic virus* in the central Great Plains. *Plant Dis.* 97:21-29.

Wheat curl mite (WCM)-transmitted viruses—namely, *Wheat streak mosaic virus* (WSMV), *Triticum mosaic virus* (TriMV), and the High Plains virus (HPV)—are three of the wheat-infecting viruses in the central Great Plains of the United States. TriMV is newly discovered and its prevalence and incidence are largely unknown. Field surveys were carried out in Colorado, Kansas, Nebraska, and South Dakota in spring and fall 2010 and 2011 to determine TriMV prevalence and incidence and the frequency of TriMV co-infection with WSMV or HPV in winter wheat. WSMV was the most prevalent and was detected in 83% of 185 season-counties (= s-counties), 73% of 420 season-

fields (= s-fields), and 35% of 12,973 samples. TriMV was detected in 32, 6, and 6% of s-counties, s-fields, and samples, respectively. HPV was detected in 34, 15, and 4% of s-counties, s-fields, and samples, respectively. TriMV was detected in all four states. In all, 91% of TriMV-positive samples were co-infected with WSMV, whereas WSMV and HPV were mainly detected as single infections. The results from this study indicate that TriMV occurs in winter wheat predominantly as a double infection with WSMV, which will complicate breeding for resistance to WCM-transmitted viruses.

Winter wheat (*Triticum aestivum* L.) is an economically important crop in the Great Plains of the United States. In 2011, the Great Plains states of Colorado, Kansas, Nebraska, and South Dakota accounted for one-third of the total winter wheat production in the United States (13). Several viruses have been reported to infect winter wheat in this region, resulting in significant economic losses (12,27). They include *Wheat streak mosaic virus* (WSMV; 17), High Plains virus (HPV; 6), *Barley yellow dwarf virus* (14), *Wheat spindle streak mosaic virus* (24), *Wheat soilborne mosaic virus* (9), and *Triticum mosaic virus* (TriMV; 20). TriMV was discovered in 2006 in Kansas and subsequently shown to be transmitted by the wheat curl mite (WCM; *Aceria tosichella* Keifer) (19), which also transmits WSMV and HPV (16,21,23).

WSMV and HPV, the two viruses known to be transmitted by WCMs before TriMV's discovery, are widespread in the Great Plains (8,16). Recent studies have shown that co-infection of wheat with WSMV and TriMV results in synergism that increases symptom severity, elevates titers of both viruses (26), and exacerbates biomass and yield loss (2,28). Therefore, knowing the extent of the distribution of TriMV and the frequency of its co-infection of wheat with WSMV or HPV will assist in estimating the potential risk TriMV poses to wheat production. Recently, field surveys in nine Great Plains states detected TriMV for the first time in Colorado, Montana, Nebraska, North Dakota, Oklahoma, South Dakota,

Texas, and Wyoming (1; M. Burrows, *personal communication*), indicating that the virus is widespread in the Great Plains of the United States. However, information is lacking on the prevalence and incidence of TriMV and the frequency of its co-infection of wheat with WSMV or HPV in individual states in the Great Plains region. The objectives of this study were to (i) conduct detailed wheat surveys in four central Great Plains states (Colorado, Kansas, Nebraska, and South Dakota) to determine the distribution and prevalence of TriMV and (ii) assess the incidence of occurrence of TriMV singly or in double or triple infections with WSMV and/or HPV.

### Materials and Methods

**Field surveys.** Arbitrarily selected winter wheat fields were sampled in Colorado, Kansas, Nebraska, and South Dakota in spring 2010 and 2011; in Kansas, Nebraska, and South Dakota in fall 2010; and in Kansas and Nebraska in fall 2011 (Tables 1–3). In spring 2011, jointed goatgrass (*Aegilops cylindrica* Host.) was also collected from an area adjacent to a wheat field that had severe virus symptoms at a location in southwest Nebraska. In the spring, samples were collected from the current year's winter wheat crop. In the fall, samples were collected from volunteer wheat in fields previously cropped with winter wheat earlier during that year's growing season. Due to the low probability of finding volunteer wheat, fall sampling was not done in all states over the 2-year period. Fields in up to 31 counties were sampled in each state, and up to three fields were sampled in each county. In each field, up to 50 tillers were arbitrarily selected and collected. Although symptomatic tillers (with leaves showing greenish yellow, parallel, and discontinuous streaks) were targeted for collection, some asymptomatic tillers were collected as well. Symptoms at the field scale ranged from minimal (<1% incidence) to very severe (100% inci-

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dence, with the entire field having a yellow cast). The tillers were placed in self-sealing bags which were labeled with date, field number, county, and state; kept on ice in a cooler; and transported to the laboratory, where they were kept at 4°C. They were then mailed overnight or as soon as possible to the Agricultural Research Center, Kansas State University, Hays, where they were assayed for TriMV, WSMV, and HPV using enzyme-linked immunosorbent assay (ELISA).

**Antiserum sources and control viruses.** The TriMV antiserum was used as a whole serum at a 1:2,000 (vol/vol) dilution prepared

from a stock solution of protein at 1 mg/ml and was raised as described previously (20). WSMV antiserum (1:1,000, vol/vol) (15) and HPV antiserum (1:1,000, vol/vol) (HPV-WO) (7,19) dilutions were made from stocks adjusted to protein at 1 mg/ml. Positive controls consisted of the 06-123 TriMV isolate (20), the Sidney 81 WSMV isolate (20), and the 06-Maize A HPV isolate (7,19).

**Indirect ELISA.** Analyses used have been described previously (20). Plant tissue was ground in 800 µl of coating buffer (3) in a 1.5-ml microcentrifuge tube using a wooden applicator stick

**Table 1.** Prevalence of *Triticum mosaic virus* (TriMV), *Wheat streak mosaic virus* (WSMV), or High Plains virus (HPV) at the county scale in Colorado, Kansas, Nebraska, and South Dakota in fall and spring 2010 and 2011

State, season	Total counties (n)	County prevalence (%) <sup>a</sup>			
		TriMV	WSMV	HPV	Combined <sup>b</sup>
Colorado					
Spring 2010	13	53.8	69.2	61.5	92.3
Spring 2011	12	50	100	58.3	100
All seasons	25	52	84	60	96
Kansas					
Spring 2010	2	0	50	0	50
Fall 2010	17	5.9	64.7	58.8	82.4
Spring 2011	22	63.6	95.5	31.8	95.5
Fall 2011	7	57.1	85.7	71.4	85.7
All seasons	48	39.6	81.3	45.8	87.5
Nebraska					
Spring 2010	28	25	85.7	25	85.7
Fall 2010	10	10	20	20	20
Spring 2011	32	50	96.9	21.9	96.9
Fall 2011	9	11.1	100	66.7	100
All seasons	79	31.6	83.5	27.8	83.5
South Dakota					
Spring 2010	16	12.5	93.8	12.5	93.8
Fall 2010	3	0	66.7	0	66.7
Spring 2011	14	0	78.6	7.1	78.6
All seasons	33	6.1	84.8	9.1	84.8
Overall	185	31.9	83.2	33.5	86.5

<sup>a</sup> County prevalence was obtained by dividing the number of counties that had at least one sample positive for the respective virus by the total number of counties sampled in a state and multiplying by 100.

<sup>b</sup> Combined county prevalence was obtained by dividing the number of counties that had at least one virus detected in a sample by the total number of counties sampled in a state and multiplying by 100.

**Table 2.** Field prevalence of *Triticum mosaic virus* (TriMV), *Wheat streak mosaic virus* (WSMV), or High Plains virus (HPV) occurring alone or in combination in a field in Colorado, Kansas, Nebraska, and South Dakota in fall and spring 2010 and 2011

State, season	Total fields (n)	Field prevalence (%) <sup>a</sup>						Combined <sup>b</sup>
		TriMV	WSMV	HPV	TriMV+WSMV	WSMV+HPV	TriMV+WSMV+HPV	
Colorado								
Spring 2010	34	14.3	65.7	20.0	14.3	11.4	0	73.5
Spring 2011	53	5.7	77.4	11.3	28.3	5.7	11.3	79.2
Combined	87	9.2	73.6	14.9	23.0	8.0	6.9	77.0
Kansas								
Spring 2010	2	0	50.0	0	0	0	0	50.0
Fall 2010	27	0	53.8	30.8	3.8	23.1	0	69.2
Spring 2011	44	2.3	77.3	4.5	43.2	22.7	9.1	95.5
Fall 2011	19	5.3	68.4	63.2	26.3	15.8	0	89.5
Combined	91	2.2	68.1	24.2	27.5	20.9	4.4	84.6
Nebraska								
Spring 2010	57	8.8	75.4	17.5	7	3.5	0	75.4
Fall 2010	26	0	53.8	7.7	7.7	19.2	7.7	61.7
Spring 2011	57	14.0	94.7	8.8	52.6	7.0	0	94.7
Fall 2011	16	0	100	62.5	6.3	43.8	0	100
Combined	156	8.3	81.4	17.3	23.7	11.5	1.3	82.7
South Dakota								
Spring 2010	3	0	75.0	0	0	0	0	75.0
Fall 2010	33	2.9	60.0	0	2.9	5.7	0	60.0
Spring 2011	38	0	52.6	0	0	2.6	0	52.6
Combined	74	1.4	71.6	0	1.4	4.1	0	71.6
Overall	408	5.7	72.9	14.8	19.8	11.2	2.9	77.9

<sup>a</sup> Field prevalence was obtained by dividing the number of fields that had at least one sample positive for the respective virus by the total number of fields surveyed in a state and multiplying by 100. Single or mixed infections were detected in samples from fields in which more than one virus was found.

<sup>b</sup> Combined field prevalence was obtained by dividing the number of fields that had at least one virus-positive sample by the total number of fields sampled in a state and multiplying by 100.

**Table 3.** Incidence of *Triticum mosaic virus* (TriMV), *Wheat streak mosaic virus* (WSMV), and High Plains virus (HPV) in winter wheat samples collected from Colorado, Kansas, Nebraska, and South Dakota in spring and fall 2010 and 2011

Season, state	Total samples ( <i>n</i> )	Incidence (%)			
		TriMV	WSMV	HPV	All positive samples
Spring 2010					
Colorado	1,885	1.0	16.8	3.3	20.1
Kansas	69	0.0	8.7	0.0	8.7
Nebraska	2,411	1.1	31.8	1.6	34.5
South Dakota	1,098	0.2	32.9	0.4	33.5
All states	5,463	0.8	26.6	1.8	28.6
Fall 2010					
Kansas	289	1.4	54.0	40.8	74.0
Nebraska	645	1.1	24.5	3.9	25.0
South Dakota	18	0.0	16.7	0.0	16.7
All states	952	1.2	33.3	15.0	39.7
Spring 2011					
Colorado	1,199	30.0	52.0	6.1	54.8
Kansas	335	27.5	91.0	11.9	92.8
Nebraska	2,314	12.6	66.2	1.5	68.2
South Dakota	1,857	0.0	3.4	0.1	3.4
All states	5,705	13.0	44.2	2.6	45.7
Fall 2011					
Kansas	198	3.0	37.9	19.7	51.5
Nebraska	655	0.2	32.5	18.5	46.3
All states	853	0.8	33.8	18.8	47.5
All seasons					
Colorado	3,084	12.3	30.5	4.4	33.6
Kansas	891	11.4	60.8	22.1	71.0
Nebraska	6,025	5.4	44.3	3.6	47.2
South Dakota	2,973	0.1	14.4	0.2	14.5
Overall	12,973	6.2	35.3	4.3	38.1

**Table 4.** Percentage of virus-positive samples for *Triticum mosaic virus* (TriMV), *Wheat streak mosaic virus* (WSMV), or High Plains virus (HPV) that were detected as single, double, or triple infections among states in spring and fall 2010 and 2011

State, season	Infection status	Incidence (%) <sup>a</sup>				$\chi^2$ <sup>c</sup>	<i>P</i> value
		TriMV	WSMV	HPV	Combined <sup>b</sup>		
Colorado							
Spring 2010	Single	50.0	85.7	94.3	95.3	43.01	<0.0001
	Double	50.0	14.3	5.7	4.7	...	...
Spring 2011	Single	1.1	39.7	39.7	42.8	276.19	<0.0001
	Double	92.2	27.4	56.4	53.6	...	...
	Triple	6.7	32.9	3.9	3.7	...	...
Kansas							
Fall 2010	Single	0	41.0	49.2	70.1	7.36	0.0252
	Double	100	59.0	50.8	29.9	...	...
Spring 2011	Single	2.2	10.0	61.2	61.7	130.02	<0.0001
	Double	90.2	72.5	36.5	36.0	...	...
	Triple	7.61	17.5	2.3	2.3	...	...
Fall 2011	Single	16.7	76.0	66.7	82.4	9.62	0.0082
	Double	83.3	24.0	33.3	17.6	...	...
Nebraska							
Spring 2010	Single	29.2	81.8	97.0	97.1	213.8	<0.0001
	Double	70.8	18.2	3.0	2.9	...	...
Fall 2010	Single	0	80.8	10.3	82.9	101.39	<0.0001
	Double	69.2	17.3	75.9	15.1	...	...
	Triple	30.8	2.2	13.8	2.0	...	...
Spring 2011	Single	6.2	82.9	97.8	82.3	5,713.87	<0.0001
	Double	93.8	17.1	2.2	17.7	...	...
Fall 2011	Single	0	74.4	85.0	89.4	9.85	0.0073
	Double	100	25.6	15.0	10.6	...	...
South Dakota							
Spring 2010	Single	50.0	0	98.6	98.6	162.56	<0.0001
	Double	50.0	100	1.4	1.4	...	...
Fall 2010	Single	0	100	0	100	...	...
	Double	0	0	0	0	...	...
Spring 2011	Single	0	0	98.4	98.4	32	<0.0001
	Double	0	100	1.6	1.6	...	...
Overall	Single	5.2	79.3	61.3	81.1	1,823.82	<0.0001
	Double	90.5	20.0	34.2	18.2	...	...
	Triple	4.3	0.7	4.9	0.7	...	...

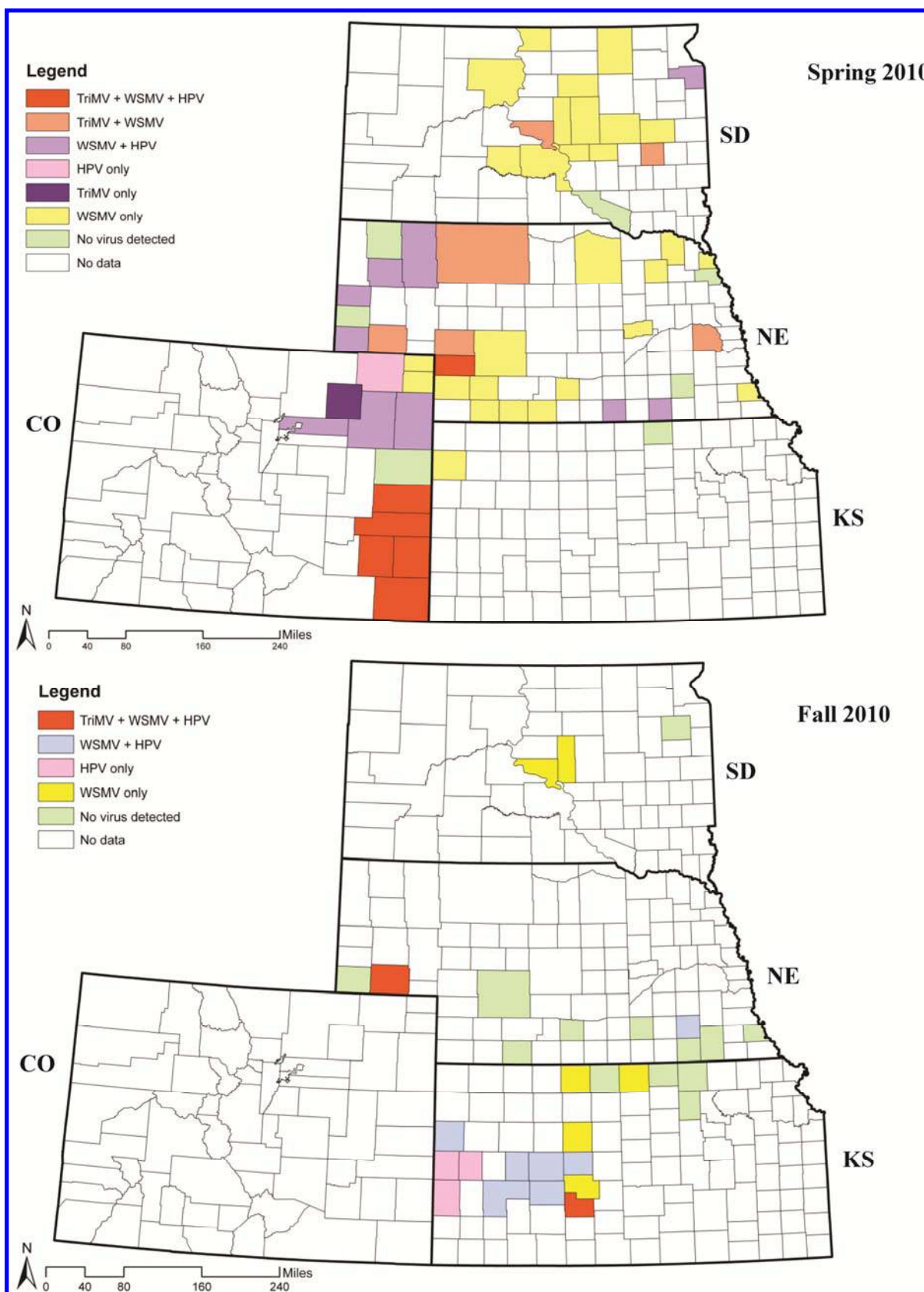
<sup>a</sup> Incidence was calculated as the percentage of virus-positive samples in single, double, or triple infections in a state and season.

<sup>b</sup> Combined incidence was obtained by dividing the number of single, double, or triple infections by the total number of virus-positive samples in a state and season.

<sup>c</sup> The  $\chi^2$  test examined the hypothesis that TriMV, WSMV, and HPV occurred in equal proportions as single, double, or triple infections.

(Fisher Scientific). Rather than weighing each sample, a piece of tissue the length of the microfuge tube was used for each sample. This was done because of the large sample number and the varying tissue amounts in each sample, so that some tissue could be retained for back inoculations to wheat if a sample reacted with

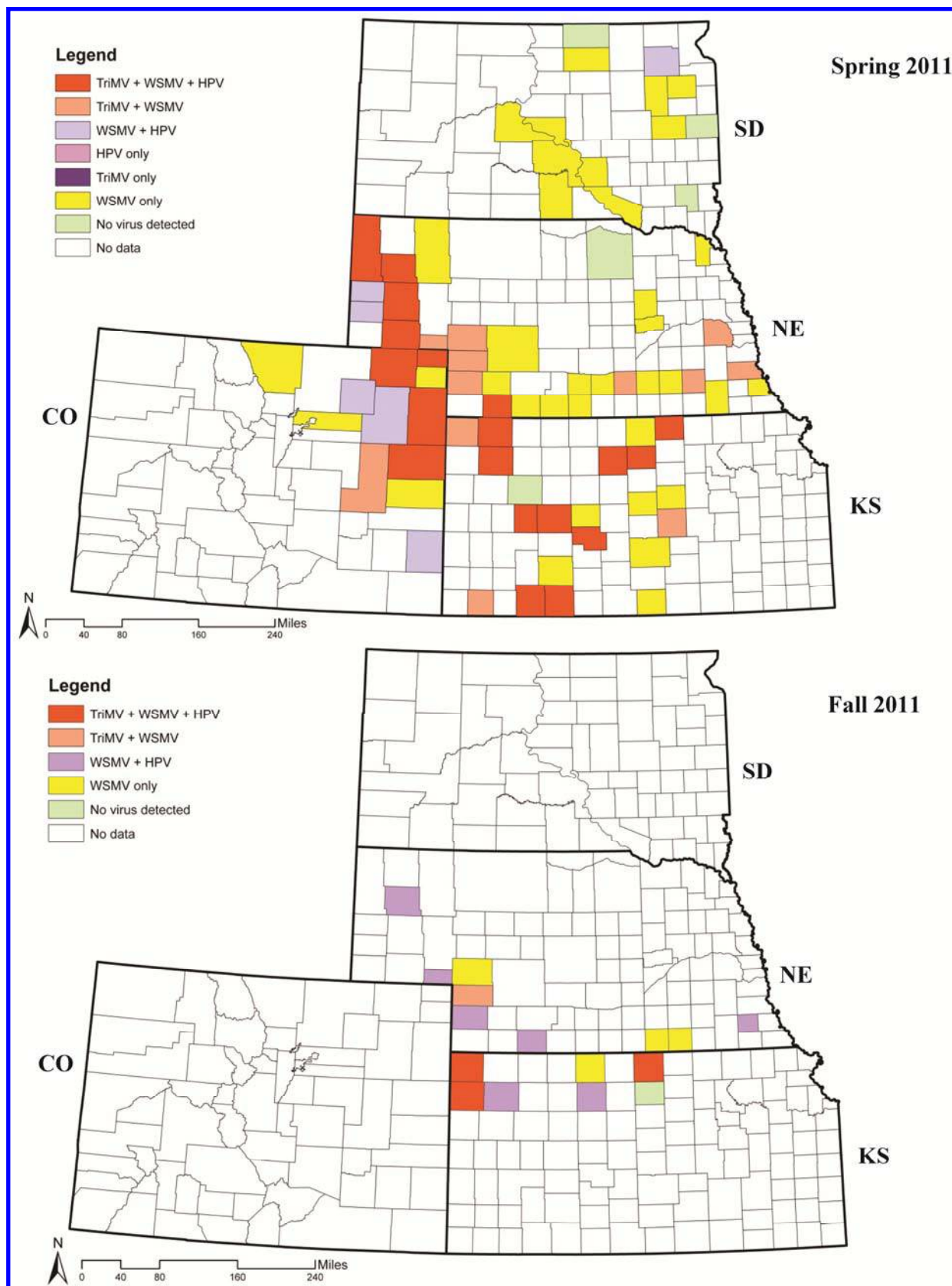
TriMV antibodies. To provide an estimate of the range of sample dilutions, 50 such samples were weighed initially, resulting in sample dilutions of 1:9 (wt/vol) to 1:188 (wt/vol). Extracts (200 µl) were placed in separate wells of ELISA plates (Immulon 1; Fisher Scientific) for 1 h at 37°C. The wells were then rinsed with a buffer



**Fig. 1.** Occurrence of *Triticum mosaic virus* (TriMV), *Wheat streak mosaic virus* (WSMV), or *High Plains virus* (HPV) among four Great Plains states in spring and fall 2010. Single or mixed infections were detected in samples from counties in which more than one virus was found.

consisting of 0.0137 M sodium chloride, 0.147 mM potassium phosphate, 0.81 mM sodium phosphate, 0.269 mM potassium chloride, 0.04 mM Tween 20, and 0.02 % sodium azide, with pH adjusted to 7.4 using 1 N NaOH. Following the rinse, the wells were incubated for 1 h at 37°C with the appropriate antiviral antibody

(as described above) in dilution buffer (3). The plates were then rinsed and blocked for 1 h in 1 ml of blocking buffer (5% nonfat dry milk, 0.01% antifoam A, and 0.02% sodium azide in phosphate-buffered saline, pH 7.4) at 37°C. Then, 200 µl of anti-rabbit antibody/alkaline phosphatase conjugate (Southern Bio-



**Fig. 2.** Occurrence of *Triticum mosaic virus* (TriMV), *Wheat streak mosaic virus* (WSMV), or *High Plains virus* (HPV) among four Great Plains states in spring and fall 2011. Single or mixed infections were detected in samples from counties in which more than one virus was found.



technology Associates) in dilution buffer (1:3,000 vol/vol) was added to each well. The plates were held at 37°C for 1 h. The plates were rinsed, and 200 µl of p-nitrophenyl phosphate substrate in substrate buffer (3) at 0.714 mg/ml was added to each well. The plates were then held on a bench top at 20 to 22°C for 30 min. Absorbance was measured at 405 nm using an iMark plate reader (Bio-Rad Laboratories). Absorbance values were arbitrarily considered positive if they were twice those of the healthy wheat leaf tissue.

**Data analysis.** Prevalence was calculated as the percentage of counties or fields with at least one sample testing positive for the respective virus. Incidence was calculated as the percentage of samples testing positive for single, double, or triple virus infections. Prevalence at the county scale was mapped with the ESRI ArcMap software (v.10; ESRI). The FREQUENCY procedure of SAS (SAS Institute) was used to test for equal occurrences of TriMV, WSMV, or HPV as single, double, or triple infections. The association of TriMV with WSMV or HPV was determined with the  $\chi^2$  test and Cramer's  $V^2$ . A Cramer's  $V^2$  value close to 1 indicates a strong association and a value close to 0 indicates a weak association (5).

## Results

**Virus distribution.** In spring 2010, TriMV and HPV were detected in Colorado, Nebraska, and South Dakota but not in Kansas whereas WSMV was detected in all four states. In fall 2010 (Kansas, Nebraska, and South Dakota surveyed), TriMV and HPV were detected in Kansas and Nebraska, whereas WSMV was detected in

all three states (Fig. 1). In spring 2011, TriMV was detected in Colorado, Kansas, and Nebraska but not in South Dakota, whereas WSMV and HPV were detected in all four states. In fall 2011 (Kansas and Nebraska surveyed), all three viruses were detected in both states (Fig. 2).

**Virus prevalence.** Over the four seasons during which virus surveys were conducted, TriMV, WSMV, and HPV occurred in 32, 83, and 34%, respectively, of 185 season-counties (= s-counties) (Table 1). Overall virus prevalence at the county scale (percentage of counties in which at least one virus was detected) over the four seasons was 87%. At the field scale, prevalence of TriMV, WSMV, and HPV was 6, 73, and 15%, respectively, over the four seasons (Table 2). Prevalence of TriMV+WSMV, HPV+WSMV, and TriMV+WSMV+HPV at the field scale was 20, 11, and 3%, respectively. Overall virus prevalence at the field scale (percentage of fields in which at least one virus was detected) over the four seasons was 78% (Table 2).

**Virus incidence.** Of 12,973 samples collected over the four seasons, the incidence of TriMV, WSMV, and HPV was 6, 35, and 4%, respectively (Table 3). In all, 38% of the samples tested positive for at least one virus. Analysis of virus incidence by season showed that WSMV was the predominant virus in each season and state and that the incidence of TriMV was much higher in spring 2011 than in the other three seasons (Fig. 3; Table 3).

**Single, double, and triple infections.** Over the four seasons for all states, TriMV was detected predominantly (91% of TriMV-positive samples) as a double infection with WSMV (Fig. 3; Table 4). These samples (doubly infected with TriMV and WSMV) in-

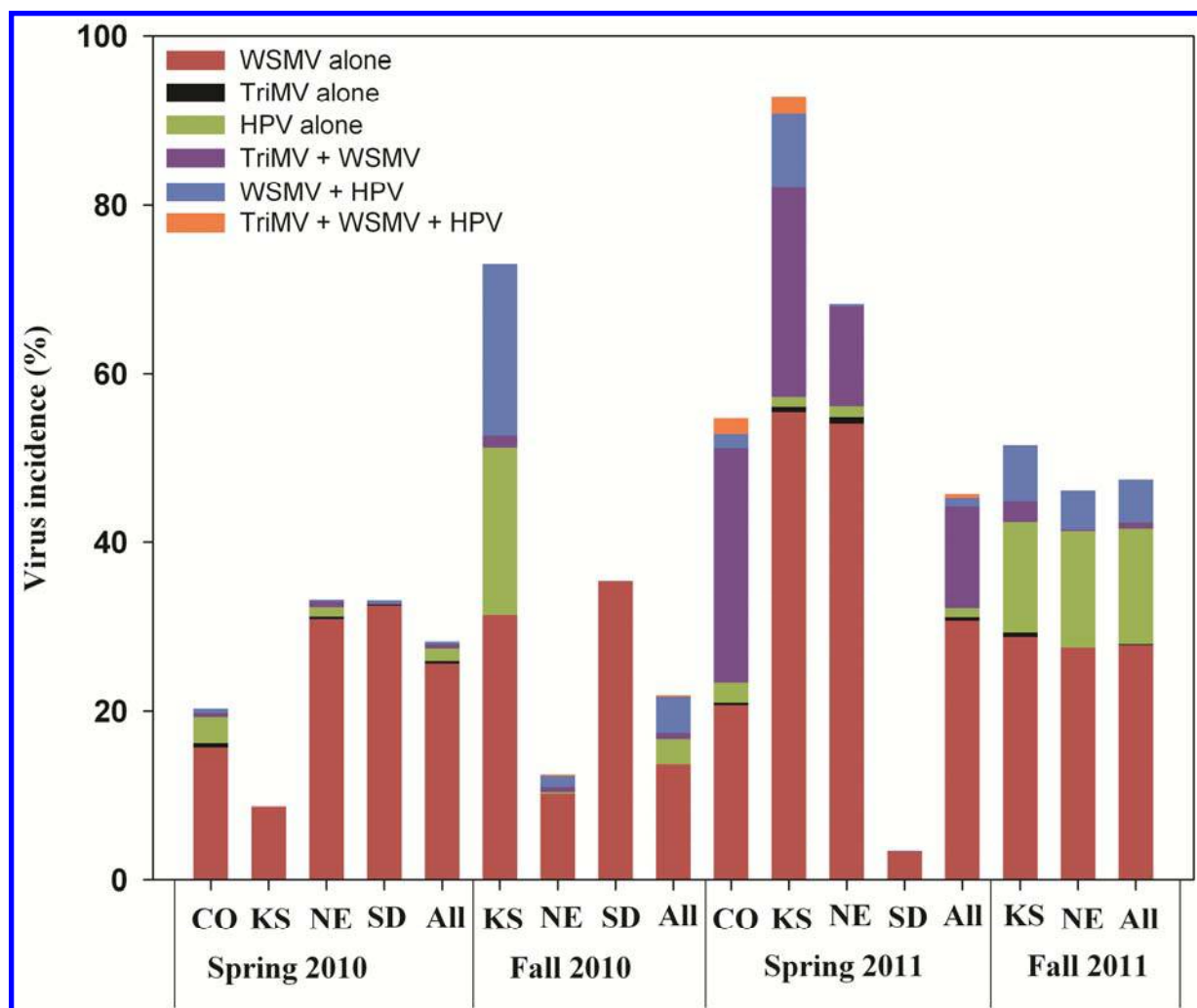


Fig. 3. Incidence of *Triticum mosaic virus* (TriMV), *Wheat streak mosaic virus* (WSMV), or *High Plains virus* (HPV) as single or mixed infections in winter wheat samples collected from fields in four Great Plains states in spring and fall 2010 and 2011.



cluded the jointed goatgrass samples collected in southwest Nebraska in spring 2011. In contrast, WSMV and HPV were detected predominantly (79 and 61%, respectively) as single infections. The incidence of WSMV and HPV occurring as double infections (WSMV+TriMV or WSMV+HPV) was 20 and 34%, respectively, whereas the incidence of the three viruses occurring as a triple infection was less than 5%. The  $\chi^2$  test showed that, over the four seasons, the three viruses differed significantly ( $P < 0.0001$ ) in their occurrence as single, double, or triple infections (Table 4).

**Association between viruses in double infections.** Double virus infections detected over the four seasons involved TriMV and WSMV or HPV and WSMV but not TriMV and HPV (Table 5). The  $\chi^2$  test showed that associations between TriMV and WSMV and between HPV and WSMV were both significant ( $P < 0.0001$ ). However, Cramer's  $V$  statistic indicated that the association between TriMV and WSMV was much stronger than the association between HPV and WSMV.

## Discussion

This study demonstrated that TriMV, a newly discovered WCM-transmitted virus of wheat originally reported in Kansas (20), is widely distributed in the central Great Plains states of Colorado, Kansas, Nebraska, and South Dakota. The current study involved detailed surveys to determine the distribution of TriMV and the

other two WCM-transmitted viruses (WSMV and HPV) in each of the four states. Results indicated that, at the county and field scales, WSMV was the most prevalent, followed by HPV and TriMV, with the prevalence of the latter two viruses being much lower than that of WSMV. Overall incidence (percentage of the total number of samples testing positive for virus over the 2 years) was highest for WSMV (35%) and lowest for TriMV (6%) and HPV (4%). In a 2008 Great Plains survey, Burrows et al. (1) similarly found that WSMV had the highest frequency of detection, followed by HPV and TriMV.

A higher percentage of samples were positive for at least one virus in spring 2011 compared with other seasons. This variability in virus incidence among seasons may be attributed to seasonal differences in weather conditions, including hail events. In Australia, Coutts et al. (4) reported a higher incidence of WSMV in a year when wind strength was high coupled with abundant grasses and volunteer cereals. The same study reported high WSMV incidence and WCM numbers when the preceding summer and autumn were wet, resulting in growth of grassy weeds and volunteer wheat, which are alternative hosts for WCMs and WSMV. Miller (11) reported that, in Wyoming, WSMV infection was most severe and losses were greatest in locations with volunteer wheat where hail had threshed maturing grain onto the ground the previous year. In Nebraska, Staples and Allington (25) reported a close association

**Table 5.** Association between *Triticum mosaic virus* (TriMV) and *Wheat streak mosaic virus* (WSMV), or High Plains virus (HPV) and WSMV, in winter wheat samples collected in spring and fall 2010 and 2011

State, season	Virus combination	Incidence <sup>a</sup>	$\chi^2$ <sup>b</sup>	$P$	$V^2$ <sup>c</sup>
Colorado					
Spring 2010	TriMV-WSMV	50	15.02	0.0001	0.09
	HPV-WSMV	14.3	0.21	0.65	-0.01
Spring 2011	TriMV-WSMV	98.9	471.14	<0.0001	0.63
	HPV-WSMV	60.27	25.24	<0.001	0.17
All seasons	TriMV-WSMV	96.56	929.7	<0.0001	0.55
	HPV-WSMV	56.61	154.72	<0.0001	0.22
Kansas					
Fall 2010	TriMV-WSMV	100	5.8	0.016	0.1558
	HPV-WSMV	50.9	0.244	0.6211	-0.029
Spring 2011	TriMV-WSMV	97.8	8.66	0.0033	0.168
	HPV-WSMV	90	0.16	0.6851	0.0255
Fall 2011	TriMV-WSMV	83.33	7.79	0.0053	0.198
	HPV-WSMV	33.3	0.0867	0.7684	-0.0209
All seasons	TriMV-WSMV	97.1	105.67	<0.0001	0.344
	HPV-WSMV	55.33	2.577	0.1084	0.1084
Nebraska					
Spring 2010	TriMV-WSMV	70.83	17.17	<0.0001	0.0842
	HPV-WSMV	18.18	0.168	0.6819	0.009
Fall 2010	TriMV-WSMV	100	74.05	<0.0001	0.214
	HPV-WSMV	88	144.49	<0.0001	0.298
Spring 2011	TriMV-WSMV	93.8	114	<0.0001	0.222
	HPV-WSMV	17.14	29.81	<0.0001	-0.12
Fall 2011	TriMV-WSMV	...d	...	...	...
	HPV-WSMV	25.62	3.086	0.079	-0.068
All seasons	TriMV-WSMV	92.4	439.24	<0.0001	0.25
	HPV-WSMV	31.65	0.741	0.3893	-0.01
South Dakota					
Spring 2010	HPV-WSMV	100	8.19	0.0042	0.086
All states					
Spring 2010	TriMV-WSMV	61.36	28.08	<0.0001	0.0718
	HPV-WSMV	19	2.74	0.0978	-0.02
Fall 2010	TriMV-WSMV	100	73.87	<0.0001	0.198
	HPV-WSMV	57.3	160.9	<0.0001	0.29
Spring 2011	TriMV-WSMV	96.8	979.8	<0.0001	0.42
	HPV-WSMV	58.39	31.43	<0.0001	0.079
Fall 2011	TriMV-WSMV	85.71	11.27	0.0008	0.12
	HPV-WSMV	27.5	2.75	0.0972	-0.057
Overall	TriMV-WSMV	94.74	1467.56	<0.0001	0.324
	HPV-WSMV	39.1	30.57	<0.0001	0.0468

<sup>a</sup> Number of samples in which TriMV or HPV was detected together with WSMV divided by the total number of samples positive for the first virus multiplied by 100.

<sup>b</sup> The  $\chi^2$  test examined the probability that the respective virus was or was not associated with WSMV.

<sup>c</sup> Cramer's  $V$  ( $V^2$ ) indicates the strength of association; a value close to 1 indicates a strong association and a value close to 0 indicates a weak association.

<sup>d</sup> Insufficient number of samples; statistical analysis not performed.

between hail injury in winter wheat and epidemics of WSMV in the following growing season in the areas where the hail occurred.

The finding in this study that the majority of TriMV-positive samples (91%) were doubly infected with TriMV and WSMV was surprising and warrants further investigation. It is possible that, evolutionarily, one virus benefits from the other or the two viruses benefit from each other by co-occurring in the WCM vector or the wheat plant host. Potential benefits of such co-occurrence include increased transmission efficiency from the vector to the plant host (19), increased replication, or increased infectivity. Tatineni et al. (26) showed that co-infection of winter wheat with TriMV and WSMV resulted in disease synergism and an increase in the titer of both viruses, indicating a fitness advantage when both viruses co-occur in a host plant. However, this synergism, which increases the severity of symptom expression (yellowing), may work against the viruses or mite vector by killing the host (volunteer wheat) in the summer, before the wheat crop is planted in the fall. The high frequency of TriMV and WSMV double infections may also be due to presence of a mite biotype that efficiently transmits both viruses. G. L. Hein (*unpublished*) and McMechan (10) demonstrated differential transmission of TriMV and WSMV among three WCM biotypes collected from the Great Plains, with only one biotype efficiently transmitting both viruses.

The high incidence of TriMV-positive samples co-infected with TriMV and WSMV prompted us to analyze the association between TriMV and WSMV and between HPV and WSMV in the virus-positive samples over the 2-year period during which the study was conducted. This analysis showed that, although both associations were significant as determined from the  $\chi^2$  test and Cramer's *V* statistic, the association between TriMV and WSMV was much stronger than the association between HPV and WSMV. The reason for the strong association between TriMV and WSMV is not known and, as mentioned in the preceding paragraph, warrants further investigation.

In contrast to the high incidence of TriMV double infections, the majority of WSMV and HPV infections occurred as single infections, followed by double infections (WSMV+TriMV or WSMV+HPV). Triple infections (WSMV+TriMV+HPV) were the least frequent. Burrows et al. (1) similarly found that, overall, most positive wheat plant samples had one virus present, with decreasing frequencies for co-infection with two, three, or four viruses. In the current study, co-infection of wheat samples with TriMV and HPV was not detected. However, Burrows et al. (1) detected wheat co-infection with TriMV and HPV in 13% of samples tested. Co-infection of wheat with two or more viruses can result in increased disease severity and, consequently, a significant negative impact on yield. Tatineni et al. (26) demonstrated increased symptom severity and titer of each virus when winter wheat was co-infected with TriMV and WSMV. Similarly, Byamukama et al. (2) and Wegulo et al. (28) showed that, under greenhouse and field conditions, co-infection of a WSMV-susceptible winter wheat cultivar with TriMV and WSMV significantly reduced yield and yield determinants and components compared with single infections.

Samples of jointed goatgrass taken from an area adjacent to a wheat field with severe virus symptoms tested positive for both TriMV and WSMV. Jointed goatgrass is a known host for WSMV (22) and was shown to be susceptible to TriMV in an experimental host range study (18). In this study, TriMV occurrence in jointed goatgrass was a result of natural infection, indicating that this grass species can be a reservoir of TriMV in addition to WSMV.

This study demonstrated that TriMV, a recently discovered virus of wheat, is widespread in the central Great Plains states of Colorado, Kansas, Nebraska, and South Dakota. The virus occurred in winter wheat mainly as a double infection with WSMV. This finding has significant implications in that previous studies have shown increased symptom severity and exacerbated yield loss in winter wheat co-infected with TriMV and WSMV. Further research is needed to determine the reasons for the high incidence of TriMV occurrence as a double infection with WSMV in winter wheat.

Occurrence of the two viruses together may negate recent progress in breeding for resistance to WSMV and poses greater challenges in breeding for resistance to WCM-transmitted viruses.

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