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Incidence of *Wheat streak mosaic virus*, *Triticum mosaic virus*, and *Wheat mosaic virus* in Wheat Curl Mites Recovered from Maturing Winter Wheat Spikes

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Abstract

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Wheat curl mites (WCM; *Aceria tosichella*) transmit *Wheat streak mosaic virus* (WSMV), *Triticum mosaic virus* (TriMV), and *Wheat mosaic virus* (WMoV) to wheat (*Triticum aestivum* L.) in the Great Plains region of the United States. These viruses can be detected in single, double, or triple combinations in leaf samples. Information on incidence of viruses in WCM at the end of the growing season is scant. The availability of this information can enhance our knowledge of the epidemiology of WCM-transmitted viruses. This research was conducted to determine the frequency of occurrence of WSMV, TriMV, and WMoV in WCM populations on field-collected maturing wheat spikes and to determine differences in WCM densities in three geographical regions (southeast, west-central, and panhandle) in Nebraska. Maturing wheat spikes were collected from 83 fields across Nebraska in 2011 and 2012. The spikes were placed in proximity to wheat seedlings (three- to four-leaf stage) in WCM-proof cages in a growth chamber and on sticky tape. WCM that moved off the drying wheat spikes in cages infested the wheat seedlings. WCM that moved off wheat spikes placed on sticky tape were trapped on the tape and were counted under a dissecting microscope. At 28 days after infestation, the wheat plants were tested for the presence of WSMV, TriMV, or WMoV using enzyme-linked immunosorbent assay and multiplex polymerase chain reaction. WSMV was the most predominant virus detected in wheat seedlings infested with WCM from field-collected spikes. Double (TriMV+WSMV or WMoV+WSMV) or triple (TriMV+ WMoV +WSMV) virus detections were more frequent (47%) than single detections (5%) of TriMV or WSMV. Overall, 81% of the wheat seedlings infested with WCM tested positive for at least one virus. No significant association ($P > 0.05$) was found between regions for WCM trapped on tape. These results suggest that WCM present on mature wheat spikes harbor multiple wheat viruses and may explain high virus incidence when direct movement of WCM into emerging winter wheat occurs in the fall.

Wheat (*Triticum aestivum* L.) contributes US$4.8 billion annually to the economy of the Great Plains region of the United States (NASS 2014). Viruses transmitted by wheat curl mites (WCM; *Aceria tosichella* Keifer) are a major wheat production constraint in this region (Appel et al. 2007). These viruses are *Wheat streak mosaic virus* (WSMV), *Triticum mosaic virus* (TriMV), and *Wheat mosaic virus* (WMoV, formerly High Plains virus) (Burrows et al. 2009; Byamukama et al. 2013; Seifers et al. 1997, 2008, 2009). WSMV was first reported in Nebraska in 1954 (Slykhuis 1955) whereas the latter two viruses have only recently been found in the Great Plains region (Jensen et al. 1996; Seifers et al. 1997, 2008). Most virus epidemics in the region have been attributed to WSMV; however, with the discovery of WMoV in 1993 (Jensen et al. 1996; Tatineni et al. 2014) and TriMV in 2006 (Seifers et al. 2008; Tatineni et al. 2009), coinfections of wheat with all or two of the three viruses have been found (Burrows et al. 2009; Byamukama et al. 2013).

The three viruses cause significant yield loss when they infect wheat singly or in combination, with yield loss exacerbated when coinfection occurs (Byamukama et al. 2014; Seifers et al. 2011). In field experiments, yield loss of up to 96% occurred when susceptible winter wheat 'Millennium' was coinoculated with TriMV and WSMV compared with single inoculations with WSMV or TriMV (up to 53 and 70% yield loss, respectively) (Byamukama et al. 2014). The level of yield loss caused by these viruses is influenced by time of infection (Hunger et al. 1992), cultivar (Byamukama et al. 2014), and weather conditions in the fall and spring (Atkinson and Grant 1966).

WCM are microscopic (250 μm) and are limited in their ability to move from one host plant to another. When the mites are ready to disperse due to poor food quality, host maturity, or overcrowding, they use their caudal pad to assist in dispersal from the plant (Navia et al. 2013; Thomas and Hein 2003). They will crawl to the surface of the leaf and are carried by wind currents. They are deposited in nearby areas where they land on bare ground or vegetation.

WCM transmission efficiency varies with virus and WCM genotype. The WCM has been grouped into two distinct genotypes (type 1 and type 2) based on the polymerase chain reaction (PCR) restriction fragment length polymorphism of the mitochondrial cytochrome oxidase subunit I and cytochrome oxidase subunit II and the ribosomal internal transcribed spacer region (Hein et al. 2012). Both WCM types can transmit WSMV (Oliveira-Hofman et al. 2015) but differ in their efficiency of transmission of WMoV and TriMV. Seifers et al. (2002) found that WCM from South Dakota and Texas within the type 1 genotype did not transmit WMoV whereas WCM from Nebraska (type 2 genotype) efficiently transmitted the virus. Only Montana WCM within the type 1 genotype transmitted WMoV. McMechan et al. (2014) found only type 2 WCM to be efficient in transmitting TriMV. Both mite types were found to be widely present in all fields sampled in Nebraska, Kansas, and Montana (Siriwetwiwat 2006). WCM disperse from maturing wheat in the summer to a suitable host ("green bridge" hosts such as volunteer wheat, corn, and grassy weeds). In the fall, they disperse from the green bridge hosts to the newly planted wheat crop to which they transmit WSMV, TriMV, or WMoV (Navia et al. 2013).

The three viruses can be transmitted by WCM singly as well as in various combinations. Recent surveys of wheat fields in the Great Plains indicated that single, double, or triple infections of wheat plants by these viruses are not uncommon (Burrows et al. 2009;

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Byamukama et al. 2013). These surveys also found that WSMV and WMoV were detected mostly as single infections whereas TriMV was detected mostly as a double infection with WSMV (Byamukama et al. 2013). However, information on incidence of viruses in WCM at the end of the wheat growing season is scant. Mahmood et al. (1998) reported incidence of WSMV and WMoV in WCM at the end of the growing season but, at that time, TriMV was not reported in the Great Plains. Knowledge of the occurrence of WCM-transmitted viruses and their combinations in WCM on maturing wheat spikes can enhance our understanding of the epidemiology of diseases caused by these viruses. Such knowledge is essential in developing effective management strategies for the mite–virus complex. In this study, the incidence of viruses present in WCM on maturing wheat spikes at the end of the growing season was determined.

Materials and Methods

Wheat spike sampling. Six to nine counties from the southeast, west-central, and panhandle regions in Nebraska were arbitrarily selected in the 2011 and 2012 growing seasons. Within each county, two to four winter wheat fields were arbitrarily selected. In each field, six locations were selected based on a modified "W" pattern. At each location, five wheat spikes (30 spikes/field) at the soft or hard dough growth stage were picked and placed in a prelabeled Ziploc bag. Two spikes from three locations per field were randomly selected and each spike fastened on HD sticky tape (Mahmood et al. 1998) mounted on 7-by-18-cm cardboards for later counting of mites. As the spikes dried, WCM moved off and were trapped on the sticky tape. To ensure that all WCM moved onto the sticky tape, spikes on the tape were kept for 6 weeks in clear plastic containers, after which WCM were counted. To count mites, the spike was removed and the tape was placed in the viewing area of a stereomicroscope. The number of WCM trapped on the entire tape was recorded.

Growth chamber transfer of WCM. In a greenhouse, six seeds of Millennium wheat were planted in 4-cm-diameter Cone-tainers (Stuewe & Sons Inc., Tangent, OR) and later thinned to three to four seedlings per Cone-tainer. The Cone-tainers were covered with clear plastic cylindrical cages (5 cm in diameter and 50 cm in height) with two mite-proof vents (Oliveira-Hofman et al. 2015). At the three- to four-leaf growth stage, three wheat spikes from each sampled location within a wheat field (a total of six Cone-tainers and 18 spikes per field) were transferred to each Cone-tainer. To check for cross contamination, eight Cone-tainers of Millennium seedlings were not infested with WCM (no spikes transferred to them). After transfer of spikes to the Cone-tainers, the plants were moved to a growth chamber and maintained at 27°C with a 14-h photoperiod. The seedlings were watered as needed and kept with the wheat spikes in the growth chamber for 28 days. As the spikes dried, WCM moved onto and infested the seedlings. After 28 days, wheat seedlings from each Cone-tainer were cut at the soil level and examined for WCM infestation using a stereomicroscope. Heavy WCM infestation was also indicated by extensive leaf curling. Control (without wheat spikes) wheat seedlings remained erect. The level of WCM infestation was recorded on a 0-to-3 scale, where 0 = no WCM found and 1 = 1 to

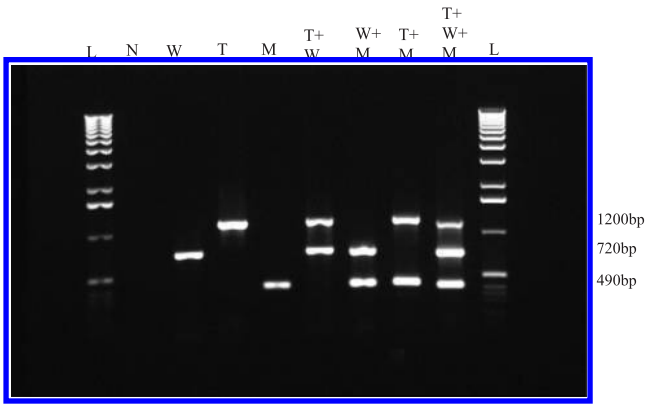

Fig. 1. Multiplex polymerase chain reaction detection of single, double, or triple infection of Millennium seedlings by *Triticum mosaic virus* (TriMV), *Wheat streak mosaic virus* (WSMV), and *Wheat mosaic virus* (WMoV). Lanes: L = loading ladder, N = negative control (healthy), W = WSMV, T = TriMV, M = WMoV, T+W = TriMV+WSMV, W+M = WSMV+WMoV, T+M = TriMV+WMoV, and T+W+M = TriMV+WSMV+WMoV.

Table 1. Primers used to test for the presence of *Wheat streak mosaic virus* (WSMV), *Triticum mosaic virus* (TriMV), and *Wheat mosaic virus* (WMoV)

Virus	Forward primer	Reverse primer	Product size (bp)
TriMV	CTT AAG CAC ATG TTA CAA TC	GTC CCT GAT AAC TAA TTC TA	1,200
WSMV	GTT GGG AGG CTT AAT TGA AGT G	CAG CCA TTA CTC GTG TTA TCC A	720
WMoV	GTTCCAATTCCTGTGCTTGATCTGTC	AACAATGACATAGCAATTACCTCAGCA	490

Table 2. Prevalence (%) of *Wheat streak mosaic virus* (WSMV), *Triticum mosaic virus* (TriMV), or *Wheat mosaic virus* (WMoV) and their combinations in Millennium wheat seedlings infested with wheat curl mites that moved off maturing wheat spikes onto the seedlings[a]

			Virus or virus combination[b]					
Year, region[c]	Fields[d]	WSMV	TriMV	WMoV	WSMV+TriMV	WSMV+WMoV	WSMV+TriMV+WMoV	Overall
2011								
PH	13	7.7	7.7	0.0	7.7	15.4	61.5	100
WC	13	0.0	0.0	0.0	0.0	7.7	92.3	100
SE	14	35.8	7.1	0.0	35.7	14.3	7.1	100
Overall	40	15.0	5.0	0.0	15.0	12.5	52.5	100
2012								
PH	16	12.5	0.0	0.0	6.3	50.0	31.2	100
WC	16	0.0	0.0	0.0	0.0	75.0	25.0	100
SE	11	27.2	0.0	0.0	9.1	63.6	9.1	100
Overall	43	11.6	0.0	0.0	4.7	62.8	23.3	100

[a] Prevalence is the percentage of fields that had at least one sample test positive for WSMV, TriMV, or WMoV alone or in combination with one or two of the other viruses.
[b] The TriMV+WMoV combination was not detected. Single occurrence indicates that no other virus was detected in that field.
[c] Wheat spikes were sampled from the panhandle (PH), west-central (WC), and southeast (SE) regions in Nebraska in 2011 and 2012 and placed on the seedlings in a cage in a greenhouse.
[d] Number of fields.

10, 2 = 11 to 100, and 3 = >100 WCM. After WCM assessment, the leaf samples were put in prelabeled Ziploc bags and kept at −80°C until they were tested for the presence of WSMV, TriMV, or WMoV using enzyme-linked immunosorbent assay (ELISA), as described by Byamukama et al. (2013), and multiplex PCR (Fig. 1, Table 1).

Isolation of total RNA for multiplex PCR. In order to confirm ELISA results, all samples were also tested for the presence of WSMV, TriMV, or WMoV using multiplex PCR. Total RNA from wheat leaf samples was isolated as follows. Leaf samples (0.2 to 0.4 g) from each Cone-tainer were manually ground in a mesh grinding bag (Agdia Inc.) with glycine extraction buffer (0.1 M glycine, 0.1 M NaCl, and 10 mM EDTA, pH 9.5). An aliquot of 500 µl of the resultant sap was transferred to a 1.5-ml Eppendorf tube containing 50 µl of 10% sodium dodecyl sulfate. After a vortex for 15 s, 500 µl of phenol-chloroform (Tris-buffered 50% phenol, 48% chloroform, and 2% isoamyl alcohol solution) was added followed by a vortex for 30 s. The mixture of sap and phenol-chloroform was then clarified at 12,000 × *g* for 5 min at 4°C. Then, 350 µl of the upper aqueous phase was transferred to Eppendorf tubes and 17.5 µl of 3 M NaOAc and 920 µl of 100% ethanol was added. The RNA was pelleted at 12,000 × *g* for 10 min at 4°C and the pellet was washed with 500 µl of 70% ethanol. The RNA pellet was then vacuum dried and suspended in 125 µl of sterile distilled water and stored at −70°C until reverse-transcription PCR was performed.

First-strand cDNA synthesis. Total RNA (2 µl) extracted from samples was used to synthesize the first strand of cDNA in a 20-µl reaction volume in the presence of 1× first-strand reaction buffer, random primers (Promega Corp.) at 2.5 ng/µl, 400 µM dNTP, and 8.8 U of AMV reverse transcription (Roche) at 42°C for 60 min followed by 5 min of incubation at 95°C. The first-strand cDNA (2 µl) was used in the multiplex PCR in a 25 µl volume.

Primers and multiplex PCR optimization. The primers were designed for TriMV and WSMV from the PIPO region and for WMoV from RNA3 (Table 1). The primer concentration, TaqMan enzyme volume, annealing temperature, and dNTP were optimized until clear bands of PCR products were obtained (Fig. 1). The best PCR conditions were used to test the rest of the samples. PCR was carried out in a total reaction volume of 25 µl containing 2 µl of cDNA template, 2 µl of TaqMan enzyme, 0.5 µl of 10 mM dNTP, and 2 µl of 5 µM each forward and reverse primer for TriMV, WSMV, and WMoV. The thermal settings were 95°C for 2 min for denaturation followed by 35 cycles of 95°C for 30 s, 45°C for 30 s, and 72°C for 2 min. Final extension was at 72°C for 10 min. PCR products were run on 1% agarose gels in Tris-acetate-EDTA buffer.

Data analysis. The number of samples from WCM-infested seedlings that tested positive for WSMV, TriMV, or WMoV was used to obtain regional prevalence (the percentage of fields with at least one sample testing positive for WSMV, TriMV, or WMoV) and incidence (the percentage of samples testing positive for WSMV, TriMV, or WMoV in each region). ELISA results of single, double, and triple infections were confirmed by multiplex PCR (Fig. 1). Leaf samples in which the viruses were detected singly or in double or triple combinations indicated single, double, or triple occurrence of viruses in WCM. Single, double, or triple occurrence was recorded at the sample and field levels. Association between occurrences of viruses (single, double, or triple) at the field and sample levels or between viruses and WCM numbers was tested using the χ^2 test at $P = 0.05$. Differences among regions in WCM numbers counted on sticky tape were detected using analysis of variance and considering fields in each region as replications. Mean separations showing these differences were done using Fisher's least significant difference test at $P = 0.05$ in SAS (version 9.2; SAS Institute Inc., Cary, NC).

Table 3. Among three regions in Nebraska in 2011 and 2012, χ^2 test for prevalence of single, double, or triple occurrences of *Wheat streak mosaic virus* (WSMV), *Triticum mosaic virus* (TriMV), or *Wheat mosaic virus* (WMoV)[a]

Year, region	Number of fields	Prevalence (%)			χ^2	*P* value[b]
		Single	Double	Triple		
2011						
PH	13	15.4	23.1	61.5	20.53	0.0004
WC	13	0.0	7.7	92.3	…	…
SE	14	42.9	50.0	7.1	…	…
2012						
PH	16	12.5	56.3	31.3	5.87	0.2091
WC	16	0	75.0	25.0	…	…
SE	12	25.0	66.7	8.3	…	…

[a] Prevalence (percentage of fields with single, double, or triple virus occurrences) was determined by enzyme-linked immunosorbent assay detection of virus in Millennium wheat seedlings on which maturing wheat spikes sampled from the panhandle (PH), west-central (WC), and southeast (SE) regions in Nebraska were placed in a cage in a greenhouse. Wheat curl mites moved off the wheat spikes, infested the seedlings, and transmitted virus to the seedlings.

[b] The χ^2 *P* value indicates the probability that prevalence of single, double, or triple occurrences of WSMV, TriMV, or WMoV was the same across regions.

Results

Field prevalence. Prevalence of WSMV, TriMV, or WMoV in wheat seedlings infested with WCM from maturing wheat spikes varied between regions and years. WSMV was the most detected virus in single or multiple infections with TriMV or WMoV in the panhandle, west-central, and southeast regions in Nebraska (Table 2).

Table 4. Incidence (%) of *Wheat streak mosaic virus* (WSMV), *Triticum mosaic virus* (TriMV), or *Wheat mosaic virus* (WMoV) in Millennium wheat seedlings infested with wheat curl mites that moved off maturing wheat spikes[a]

Year, region[c]	Samples[d]	Virus or virus combination[b]						Overall
		WSMV	TriMV	WMoV	WSMV+TriMV	WSMV+WMoV	WSMV+TriMV+WMoV	
2011								
PH	60	16.7	5.0	1.7	20.0	13.3	28.3	85.0
WC	73	20.5	1.4	1.4	23.3	6.8	41.1	94.5
SE	77	54.5	5.2	0.0	3.9	3.9	0.0	67.5
Overall	210	31.9	3.8	1.0	15.2	7.6	22.4	81.9
2012								
PH	84	22.6	0.0	8.3	1.2	28.6	10.7	71.4
WC	64	21.9	0.0	4.7	1.6	48.4	10.9	87.5
SE	43	39.5	0.0	0.0	2.4	48.8	2.3	93.0
Overall	191	26.2	0.0	5.2	1.6	39.8	8.9	81.7

[a] Incidence is the percentage of samples that tested positive for WSMV, TriMV, or WMoV alone or in combination with one or two of the other viruses.

[b] The TriMV+WMoV combination was not detected.

[c] Wheat spikes were sampled from the panhandle (PH), west-central (WC), and southeast (SE) regions in Nebraska in 2011 and 2012 and placed on the seedlings in a cage in a greenhouse.

[d] Number of samples.

All three viruses were detected in the majority of fields (53%) in 2011 across the three regions. In 2012, WSMV and WMoV co-occurred most frequently. Single occurrence of WMoV in a wheat field was not detected in either year. Single occurrence of TriMV was found in only 5% of the fields in 2011 and in none of the fields in 2012. At least one of the three viruses was detected in all fields sampled in both years. Prevalence of single, double, or triple virus occurrences differed across regions and years (Table 3). In 2011, the panhandle and west-central regions tended to have more double and triple than single virus detections ($P = 0.0004$). In the southeast region, the majority of detections were double. In 2012, there was no association between regions and single, double, or triple virus occurrences.

Virus incidence. In both years, at least one virus was detected in 82% of samples from wheat seedlings infested with WCM from maturing wheat spikes (Table 4). The highest virus incidence occurred in the west-central region in 2011 (95%) and in the southeast region in 2012 (93%). WSMV was the most frequently detected virus across regions in both years. TriMV was the second most frequently detected virus in 2011 whereas WMoV was the second most frequently detected virus in 2012. Two or three viruses were most frequently detected in a sample, except in the southeast region in 2011. In 2012, WSMV+WMoV was the most frequent double detection. In both years and across regions, χ^2 analysis indicated that detection of two or more viruses in a sample was more common than detection of a single virus (Table 5). In both years, there was a strong association between region and single, double, or triple virus occurrence. The three regions had low to moderate frequencies of single, double, and triple virus occurrences in both years, except the southeast region, which had a high frequency of single and double occurrences in 2011 and 2012, respectively (Table 5). The sampled location in a field was not associated with virus detection (2011: $\chi^2 = 2.78$, $P = 0.7337$; 2012: $\chi^2 = 5.9066$, $P = 0.3154$). Within positive samples, single, double, or triple virus detections differed among the three viruses. WSMV was mainly detected alone whereas TriMV and WMoV were detected mostly as double or triple infections with WSMV in both years (Table 6).

WCM on wheat spikes. The number of WCM that moved off wheat spikes and got trapped on sticky tape varied from year to year (Fig. 2). In 2012, no significant differences in numbers of WCM were detected among the three regions. However, the average number of WCM per spike was much greater in 2012 (n = 1,000) than in 2011 (n = 200). In 2011, the panhandle region had significantly more WCM per spike than the southeast region. The χ^2 analysis showed that the number of WCM found on a leaf sample (following infestation by placing field-collected spikes on wheat seedlings in the greenhouse) was not associated with the number of viruses detected in that sample in 2011. In 2012, however, the number of WCM was significantly associated with the number of viruses detected in a sample (Table 7). More WCM per sample tended to be associated with a single virus detected in a sample, whereas samples that had fewer WCM tended to have more than one virus detected. For individual viruses, WSMV and TriMV were more associated with medium to low WCM populations in both years whereas WMoV was not associated with WCM populations (Table 8).

Confirmation of ELISA results using multiplex PCR. Detection of all three viruses and their double and triple combinations by ELISA was confirmed by multiplex PCR (Fig. 1; Table 1).

Table 5. Occurrence of *Wheat streak mosaic virus* (WSMV), *Triticum mosaic virus* (TriMV), or *Wheat mosaic virus* (WMoV) single, double, or triple infections among positive plant samples resulting from wheat curl mites that moved off wheat spikes onto seedlings of Millennium wheat[a]

Year, region	Number of samples	Incidence (%)			χ^2	*P* value[b]
		Single	Double	Triple		
2011						
PH	60	27.5	29.2	33.3	61.03	<0.0001
WC	73	24.6	31.9	43.5		
SE	77	88.5	11.5	0		
2012						
PH	84	43.3	41.7	15.0	22.69	0.0001
WC	64	30.4	57.1	12.5		
SE	43	11.9	72.5	15.6		

[a] Wheat spikes were sampled from the panhandle (PH), west-central (WC), and southeast (SE) regions in Nebraska in 2011 and 2012 and placed on the seedlings in a cage in a greenhouse.
[b] The χ^2 *P* value indicates the probability that incidence of single, double, or triple infections of seedlings by WSMV, TriMV, or WMoV was the same across regions.

Table 6. Occurrence of *Wheat streak mosaic virus* (WSMV), *Triticum mosaic virus* (TriMV), or *Wheat mosaic virus* (WMoV) in single, double, or triple infections of Millennium seedlings resulting from wheat curl mites that moved off maturing wheat spikes[a]

Year	Virus	Positive[b]	Incidence (%)			χ^2	*P* value[c]
			Single	Double	Triple		
2011	WSMV	162	41.4	29.6	29.0	62.74	<0.0001
	TriMV	87	9.2	36.8	54.0		
	WMoV	65	3.1	24.6	72.3		
2012	WSMV	146	34.3	54.1	11.6	82.70	<0.0001
	TriMV	20	0	15.0	85.0		
	WMoV	103	9.7	73.8	16.5		

[a] Wheat spikes were sampled from the panhandle (PH), west-central (WC), and southeast (SE) regions in Nebraska in 2011 and 2012 and placed on the seedlings in a cage in a greenhouse.
[b] Number of positive samples.
[c] The χ^2 *P* value indicates that the probability of detecting single, double, or triple infections of seedlings by WSMV, TriMV, or WMoV was the same for each of the three WCM levels.

Discussion

This study demonstrated differences in the incidence of WSMV, TriMV, and WMoV in WCM at the end of the wheat growing season from the panhandle, west-central, and southeast regions in Nebraska. WSMV was the most detected virus in single, double, or triple infections of wheat seedlings infested by WCM moving off wheat spikes. TriMV and WMoV were mainly detected in double and triple infections, and low numbers of WCM on a wheat spike were associated with double or triple infections following infestation of wheat seedlings.

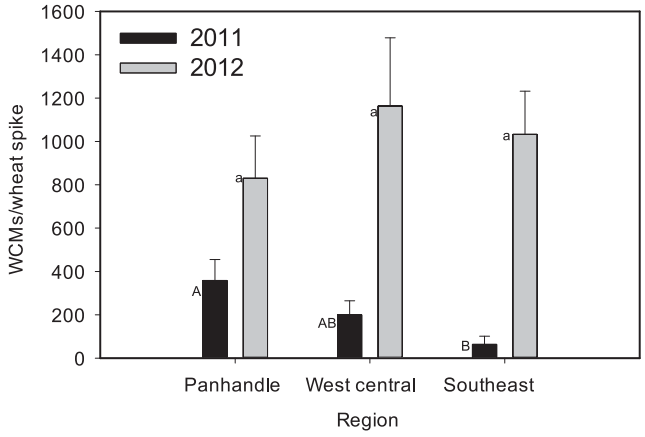

Fig. 2. Average number of wheat curl mites (WCM) that moved off maturing wheat spikes and got trapped on a high-definition tape in 2011 and 2012 in three regions in Nebraska. The tape was placed on a piece of cardboard measuring 7 by 18 cm. Bars with the same letter within a year are not significantly different according to Fisher's least significant difference test at $P = 0.05$. Between 11 and 26 fields were sampled in each region and six spikes from each field were placed on the tape. Uppercase letters denote mean separations in 2011 and lowercase letters denote mean separations in 2012.

The higher incidence of WSMV detection may be attributed, in part, to the fact that WSMV is transmitted by both type 1 and type 2 WCM whereas TriMV is transmitted only by type 2 WCM or at a very low rate by type 1 WCM (McMechan et al. 2014; Oliveira-Hofman et al. 2015). In addition, only Montana WCM within the type 1 genotype can transmit WMoV whereas South Dakota and Texas WCM within this genotype cannot (Seifers et al. 2002). Because both WCM genotypes are present in wheat fields (Siriwetwiwat 2006), it is expected that the virus that is efficiently transmitted by both genotypes will be predominant and, therefore, more frequently detected. Previous surveys in which symptomatic wheat leaves were collected from fields in the central Great Plains and tested for WCM-transmitted viruses also showed WSMV to be the predominant virus (Burrows et al. 2009; Byamukama et al. 2013).

The prevalence of viruses carried by WCM at the end of the wheat growing season was 100% and this result was expected because mite-transmitted viruses of wheat are widespread in the central Great Plains region of the United States (Burrows et al. 2009; Byamukama et al. 2013). Although previously reported prevalence of WCM-transmitted viruses was based on symptomatic plants arbitrarily sampled from wheat fields, not every field yielded samples that had WCM-transmitted viruses (Byamukama et al. 2013). Wheat plants infected later in the growing season may not show virus symptoms. Therefore, sampling wheat spikes may be a preferred method in detecting and establishing the presence of WCM-transmitted viruses in wheat.

Table 7. Frequency (%) of Millennium seedling samples with single, double, or triple virus infection detected for different levels of wheat curl mite (WCM) counts on the seedlings grown in a growth chamber at 21°C[a]

			Virus combinations				
Year	WCM	n[b]	0	1	2 or more	χ^2	P value[c]
2011	<10	24	8.3	25.0	66.7	9.81	0.1329
	11–100	41	2.4	34.2	63.4	…	…
	>100	78	16.7	34.6	48.7	…	…
2012	<10	21	4.8	23.8	71.4	25.55	0.0004
	11–100	39	5.1	53.9	41.0	…	…
	>100	50	32.0	36.0	32.0	…	…

[a] WCM moved off maturing wheat spikes that were sampled from the panhandle, west-central, and southeast regions in Nebraska in 2011 and 2012 and placed on the seedlings.
[b] Number of seedling samples.
[c] The χ^2 P value indicates that the probability of detecting single, double, or triple occurrences of WSMV, TriMV, or WMoV was the same for each of the three viruses.

The high incidence and prevalence of WCM-transmitted viruses at the end of a wheat growing season indicates the high risk of transmission of these viruses to volunteer wheat or grasses where they survive until winter wheat emergence in the fall. A high virus incidence in WCM at the end of the wheat growing season underscores the extreme risk from volunteer wheat that emerges before harvest, most often as a result of hail. This is the reason preharvest volunteer wheat, if not controlled, poses the highest risk for WSMV epidemics in the next wheat growing season.

WCM acquire WSMV at the larval stages and remain infective for at least 6 days but can remain infective for up to 2 months at 3°C (Navia et al. 2013; Orlob 1966). This indicates that viruliferous WCM that overwinter maintain sources of WSMV inoculum. However, in winter wheat, WSMV inoculum in overwintered WCM contributes minimally to disease initiation and development in the spring. The most severe epidemics seen in the spring result from infections that occurred in the fall.

The lack of correlation between WCM location in the wheat field and the incidence of WCM-transmitted viruses was likely due to a high incidence of mites and viruses developed over time, resulting in the loss of the edge effect (more intense virus symptoms at the edges of fields) commonly seen in high-risk areas during the growing season. WCM are generally thought to be deposited more at the edges of wheat fields and, normally, there is a gradient of symptomatic plants (Stilwell 2009; Workneh et al. 2009). The results from this study indicate that viruliferous WCM can be found in any part of the field by the end of growing season. The discrepancy between symptom expression and the presence of viruliferous WCM in the field may be attributed to time of infection. Other studies (Hunger et al. 1992; Somsen and Sill 1970) have shown that, as plants mature, they become more resistant to virus infection and, even when infected, they develop fewer and milder symptoms, if symptoms are seen at all.

The presence of more than one virus in a field was expected. The occurrence of WSMV and WSMV+TriMV was similar to that found in leaf samples collected in the spring (Byamukama et al. 2013). We expected to find some fields with the WMoV+TriMV combination but none of the samples was found with this combination. Fields that had WMoV and TriMV detected together also had WSMV. The lack of a significant association between region and the percentage of single, double, or triple virus occurrence in 2012 may have been due to severe drought (NOAA, 2012), causing all three regions to have comparable single and double or triple occurrences. The western region of Nebraska is usually drier than the eastern region and these conditions are more conducive for WCM population build up than in the eastern region. This is reflected in the results shown in Figure 2 for 2011, which was a "normal" (nondrought) year.

Table 8. Frequency (%) of detection of *Wheat streak mosaic virus* (WSMV), *Triticum mosaic virus* (TriMV), or *Wheat mosaic virus* (WMoV) in wheat seedling samples that had three categories (with increasing levels) of wheat curl mite (WCM) population densities and association between virus incidence and WCM population density

			WSMV		TriMV		WMoV	
Year	WCM[a]	n[b]	Positive	Negative	Positive	Negative	Positive	Negative
2011								
	<10	24	79.2	20.8	65.4	34.6	50	50
	11–100	41	97.6	2.4	61.0	39.0	39.0	61.0
	>100	78	79.5	20.5	36.4	63.6	37.2	62.8
	χ^2	…	7.4	…	10.06	…	1.28	…
	P value	…	0.0247	…	0.0065	…	0.5286	…
2012								
	<10	21	95.2	4.8	4.6	95.4	71.4	28.6
	11–100	39	97.4	2.6	10.3	89.7	43.6	56.4
	>100	48	62.1	37.9	0.0	100	50.0	50.0
	χ^2	…	21.83	…	5.08	…	4.36	…
	P value	…	<0.0001	…	0.0789	…	0.1133	…

[a] The χ^2 test P value indicates that the probability of detecting WSMV, TriMV, or WMoV is the same irrespective of the WCM population density on the sample. A χ^2 P value greater than 0.05 indicates a nonsignificant association between virus incidence and WCM population density.
[b] Number of seedling samples.

Lack of WMoV+TriMV detection was consistent with previous surveys (Byamukama et al. 2013), in which the TriMV+WMoV combination was not detected in nearly 13,000 symptomatic leaf samples collected in four central Great Plains states (Colorado, Kansas, Nebraska, and South Dakota). The significant association of single, double, or triple detections within regions in both years indicates that this is likely a common phenomenon. Of the three viruses, TriMV and WMoV were the most frequently detected in double or triple occurrence with WSMV. Lack of detection of TriMV and WMoV together may indicate that WSMV may be playing a helper role in the transmission efficiency of WMoV and TriMV.

The differences in the number of WCM trapped on sticky tape in the 2 years may be attributed to weather conditions. In 2012, when it was extremely dry and hot, considerably more WCM were trapped than in 2011. Dry and hot conditions favor the build-up of WCM populations (Orlob 1966). Higher WCM populations were found on WSMV-infected plants than on plants that had more than one virus. Siriwetwiwat (2006) found that type 2 WCM reproduced faster in the presence of WSMV. This may partly explain the high WCM populations on WSMV-infected plants. The low numbers of WCM found on plants with TriMV may indicate that TriMV-infected plants are associated with or support fewer WCM compared with WSMV-infected plants. This is in agreement with McMechan et al. (2014) and Oliveira-Hofman et al. (2015), who reported reduced survival of WCM when TriMV was present. Lack of correlation between the number of viruses detected in plants and the number of WCM on the plants was expected because one WCM can potentially transmit more than one virus.

This study demonstrated a high level of viruliferous WCM carrying WSMV, TriMV, or WMoV on maturing wheat spikes. This has implications on the epidemiology and management of diseases caused by WCM-transmitted viruses. The presence of large populations of WCM at the end of the wheat growing season ensures the infestation and subsequent virus infection of various green bridge hosts. If conditions allow survival of these hosts until fall-planted winter wheat emerges, the probability of virus transmission to fall-planted wheat increases, resulting in some level of disease and yield loss every year. Therefore, controlling volunteer wheat and other green bridge hosts for WCM before fall planting of winter wheat is critical in reducing damage from diseases caused by WCM-transmitted viruses.

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