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Differential Transmission of Two Isolates of *Wheat streak mosaic virus* by Five Wheat Curl Mite Populations

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Abstract

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Wheat streak mosaic virus (WSMV), type member of the genus *Tritimovirus* in the family *Potyviridae,* is an economically important virus causing annual average yield losses of approximately 2 to 3% in winter wheat across the Great Plains. The wheat curl mite (WCM), *Aceria tosichella*, transmits WSMV along with two other viruses found throughout the Great Plains of the United States. Two common genotypes of WSMV (Sidney 81 and Type) in the United States share 97.6% nucleotide sequence identity but their transmission relationships with the WCM are unknown. The objective of this study was to determine transmission of these two isolates of WSMV by five WCM populations ('Nebraska', 'Montana', 'South Dakota', 'Type 1', and 'Type 2'). Nonviruliferous mites from each population were reared on wheat source plants mechanically inoculated with either Sidney 81 or Type WSMV isolates. For each source plant, individual mites were transferred to 10 separate test plants and virus transmission was determined by a double-antibody sandwich enzyme-linked immunosorbent assay. Source plants were replicated nine times for each treatment (90 individual mite transfers). Results indicate that three mite populations transmitted Sidney 81 at higher rates compared with Type. Two mite populations (Nebraska and Type 2) transmitted Sidney 81 and Type at higher rates compared with the other three populations. Results from this study demonstrate that interactions between virus isolates and mite populations influence the epidemiology of WSMV.

Wheat streak mosaic virus (WSMV) is the type species of the genus *Tritimovirus* within the family *Potyviridae*, the largest group of plant viruses (Brunt et al. 1996; Stenger et al. 1998). This virus is a pathogen of wheat and other cereals in the Americas, Europe, Asia, and North Africa (Brunt et al. 1996; Dwyer et al. 2007; Sánchez-Sánchez et al. 2001; Stenger et al. 1998). It is an especially serious pathogen in the Great Plains of North America, where it causes 2 to 3% annual yield loss in wheat (Appel et al. 2012). In serious outbreaks, WSMV often causes up to 100% yield loss in individual fields (Wegulo et al. 2008).

The wheat curl mite (WCM), *Aceria tosichella* Keifer, is the only known vector of WSMV (Slykhuis 1955; Staples and Allington 1956). This mite also vectors two other viruses in wheat in the Great Plains: Wheat mosaic virus (WMoV), also known as High Plains virus, a tentative member of the genus *Emaravirus* (Seifers et al. 1997; Tatineni et al. 2014); and *Triticum mosaic virus* (TriMV; *Poacevirus, Potyviridae*) (Seifers et al. 2009; Tatineni et al. 2009). These three wheat viruses are widely spread across the Great Plains; however, WSMV has been shown to be the predominant virus in this complex (Burrows et al. 2008; Byamukama et al. 2013).

Field populations of WSMV are complex and consist of numerous genotypes (Fuentes-Bueno et al. 2011; McNeil et al. 1996; Montana et al. 1996; Robinson and Murray 2013) but different genotypes rarely occurred (approximately 2%) in the same plant (McNeil et al. 1996). Three WSMV isolates (Sidney 81, Type, and El Batán 3) have been completely sequenced (Choi et al. 2001; Stenger et al. 1998). Sidney 81, the most characterized isolate (Brakke et al. 1990; Choi et al. 1999; Hall et al. 2001b; Stenger et al. 1998), was isolated in 1981 from an infected wheat plant from western Nebraska (Brakke et al. 1990). The Type isolate was originally isolated in 1937 from infected wheat plants from Kansas (McKinney 1937). Sidney 81 and Type are the representative American isolates of WSMV, sharing 97.6% nucleotide sequence identity, and they produce similar symptoms in wheat (Choi et al. 2001; Hall et al. 2001a). Sidney 81 systemically infects and produces symptoms in *Zea mays* inbred line SDp2 but Type does not (Tatineni et al. 2011). El Batán, from Mexico (Sánchez-Sánchez et al. 2001), has diverged from the American strains and retains 79% nucleotide sequence identity to Sidney 81 and Type (Choi et al. 2001). According to McNeil et al. (1996), predominant WSMV isolates in Nebraska were indistinguishable from Sidney 81 but no isolates were identical to Type.

A. tosichella is a complex of multiple cryptic lineages with diverse but distinct host ranges (Skoracka et al. 2012, 2014). In North America, two distinct genotypes of WCM have been identified and designated as 'type 1' and 'type 2' (Hein et al. 2012). The original designation of these two types was made from Australian mite populations by Carew et al. (2009) and later shown to match populations found in North America (Hein et al. 2012). These two genotypes are known to coexist as mixed populations within wheat fields and even within wheat heads (Schiffer et al. 2009; Siriwetwiwat 2006). These WCM genotypes vary in their response to different resistant genes in wheat (Harvey et al. 1999). They also differ in their ability to transmit the three wheat viruses found in the Great Plains. The type 2 genotype transmits TriMV and WMoV more efficiently compared with the type 1 genotype (McMechan et al. 2014; Oliveira-Hofman et al. 2015; Seifers et al. 2002). Oliveira-Hofman et al. (2015) found that transmission of WSMV by type 2 mites is higher from singly infected source plants than those coinfected with TriMV. In addition, Schiffer et al. (2009) demonstrated that the type 1 genotype from Australia was unable to transmit an unknown strain of WSMV. Available literature documents WSMV transmission using multiple mites (Seifers et al. 2002) or from source plants coinfected with WSMV or TriMV (Oliveira-Hofman et al. 2015). Little has been done to document specific effects of various virus strains or mite genotypes on WSMV transmission. In addition, much of this transmission work in recent years has been done on the same mite colonies that were maintained since 1996, according to Harvey et al. (1999). This transmission work has been of considerable value because it provided excellent comparisons of transmission of an established virus and two relatively new viruses. However, these colonies were collected almost two decades ago, and it is important to compare the old colonies to recently collected ones to determine whether there are any shifts in WSMV transmission rates.

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Evaluating the impact of genetic variability for both WSMV isolates and various WCM populations is essential to understand the impact of mite and virus variability on the epidemiology and management of this virus–mite complex. The objective of this study was to determine transmission rates of two isolates of WSMV (Sidney 81 and Type) by five populations of WCM, including three long-established populations ('Montana', 'Nebraska', and 'South Dakota') and two newly established populations (Type 1 and Type 2).

Materials and Methods

Mite populations. The study was conducted as a randomized complete block design with a factorial arrangement of treatments consisting of five mite populations and two virus treatments (two WSMV virus strains). The five mite populations were designated Montana (MT), South Dakota (SD), Nebraska (NE), Type 1 (T1), and Type 2 (T2). MT, SD, and NE are populations that were established 19 years ago and are the same as those used to evaluate mite resistance in WCM-resistant wheat varieties (Harvey et al. 1999), transmission of WMoV (Seifers et al. 2002), mite genotype characterization (Hein et al. 2012), TriMV transmission (McMechan et al. 2014), and cotransmission of WSMV and TriMV (Oliveira-Hofman et al. 2015). The WSMV Sidney 81 strain was collected from western Nebraska in 1981 (Brakke et al. 1990). The Type isolate was originally isolated in 1937 from infected wheat plants in Kansas (McKinney 1937).

T1 and T2 mites were established in the summer of 2011 by collecting 10 to 25 wheat tillers from a wheat field in each of three Nebraska counties (Box Butte, Scottsbluff, and Chase Counties). Field-collected wheat tillers were used to infest 14-day-old 'Millennium' wheat plants reared in 4-cm-diameter cone-tainers (Stuewe & Sons Inc., Tangent, OR) filled with standard greenhouse soil. Cone-tainers were covered with plastic cylindrical cages (5 cm in diameter and 50 cm in height), with two to three vents covered with Nitex screen (80-μm-mesh opening; BioQuip Products Inc., Compton, CA) after planting. One to two wheat tillers were used to infest each cone-tainer. Plants were transferred to a growth chamber with a photoperiod of 14 h of light and 10 h of darkness maintained at 27°C for 3 weeks. From this process, 13 different WCM colonies were established (1 in Box Butte County, 10 in Scottsbluff County, and 2 in Chase County). Subsequently, single mite transfers were done from these colonies onto 50 cone-tainers (approximately 4/colony) containing 14-day-old test plants. Each cone-tainer (clonal colony) was tested for WCM type using polymerase chain reaction (PCR) amplification and restriction enzyme digestion of an approximately 1,600-bp fragment of the nuclear ribosomal internal transcribed spacer (ITS) and associated 28S ribosomal DNA region. The PCR product was digested using *Hha*I restriction enzyme (Promega Corp., Madison, WI), and restriction fragment length polymorphism (RFLP) scored by visual analysis was compared with the DNA ladder (Hein et al. 2012; Siriwetwiwat 2006). To establish nonviruliferous colonies, five eggs were transferred from each cone-tainer to a 14-day-old test plant. After 4 weeks, WCM of the same genotype were combined together onto 14-day-old plants in a single pot. Thereafter, mite colonies were maintained by transferring mites from the original pot to 14-day-old plants in new pots every 3 weeks. Plants from the original pots were tested for WSMV, TriMV, and WMoV using double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and reverse-transcription PCR, and all were confirmed negative for these viruses.

The colonies were maintained on 'Settler CL' wheat sown in 15-cm-diameter plastic pots and isolated with cages. Each cage was assembled from plastic sheeting molded into a cylinder 15 cm in diameter and 60 cm high. Two 8-cm-diameter holes were cut on opposite sides of the cage, approximately one-third of the way up the cage. The top of the cage and the side vents were covered with Nitex screen. The five mite populations were reared in separate growth chambers held at 24 to 27°C, with a photoperiod of 14 h of light and 10 h of darkness and 30 to 40% relative humidity. Mite populations were maintained by transferring 50 mites per pot to 14-day-old wheat plants approximately every 3 weeks.

Establishment of virus source plants. Settler CL wheat was sown in 4-cm-diameter cone-tainers filled with standard greenhouse soil prepared by mixing soil, sand, vermiculite, and peat moss in a ratio of 2:1:1:2. The cone-tainers were each seeded with three seeds, placed on greenhouse benches, and watered appropriately. The cone-tainers were covered with plastic cylindrical cages, as described above. The plants were fertilized three times a week with Scotts brand "Peter's Professional" water-soluble 20-10-20 general-purpose fertilizer (Everris NA, Inc., Dublin, OH). After 10 days, wheat plants were thinned to one seedling per cone-tainer, and this plant was mechanically inoculated with WSMV Sidney 81 or Type isolate. Three plants per mite population and virus combination were used for run 1 but some source plants died or did not have enough mites; thereafter, five plants were used to ensure sufficient source plants and mites. Sidney 81 and Type were obtained from an infectious cDNA clone whose in-vitro-generated RNA transcripts were inoculated to wheat seedlings at the single-leaf stage (Choi et al. 1999; Tatineni et al. 2011). The virus inoculum was prepared by grinding infected wheat tissue in sterile distilled water (1:10 [wt/vol]) using a mortar and pestle. The plant leaves were lightly dusted with carborundum and inoculated by gently rubbing the inoculum onto leaves using the pestle.

Four days after inoculation, 10 aviruliferous WCM were transferred from each of the five populations onto each of the source plants inoculated with WSMV Sidney 81 or Type strain. In all, 1 plant per treatment combination (10 plants per run) were also inoculated with sterile water and infested with mites to provide a mock as a check for contamination from virus or mites. To transfer mites to source plants, the mites were placed onto moist black insect-mounting triangles (1.3 by 0.4 mm) with a human eyelash attached to a wooden dowel. Triangles were placed into the axil of the newly emerging leaf of each source plant. After 24 h to allow mites to establish on the plants, plants were transferred to a growth chamber (14 h of light, 10 h of darkness, and 25 to 27°C). Mites were allowed to build up on source plants for a period of 4 weeks.

Single-mite transfers. Test plants used in this study were 14-day-old wheat plants (two- to three-leaf stage) grown in cone-tainers (three seedlings per cone-tainer that were thinned to one at the time of mite transfers). This experiment was conducted three times (runs) for a total of 9 source plants and 90 test plants per treatment combination, except for NE mites and Sidney 81, SD mites and Type, T2 mites and Type, and T2 mites and Sidney 81, which each had a total of 8 source plants and 80 test plants due to either dead source plants or insufficient mite numbers in run 1. Large, active adult mites from each source plant were individually transferred to 10 separate test plants. To enable this extensive number of transfers to be accomplished in a reasonable amount of time, single-mite transfers were done by three different persons for each run (one replicate per person). After transfers were completed, source plants were placed individually into plastic Ziploc bags and stored at −20°C for subsequent WSMV assay via DAS-ELISA. Test plants were arranged in a randomized complete block design and held in a growth chamber (14 h of light and 10 h of darkness and 23 to 25°C). Mock plants were incorporated (one per five test plants) among the treatment plants to monitor potential contamination. The test plants were held in the growth chamber for 4 weeks before harvesting. They were cut at soil level and examined under a stereomicroscope to determine mite presence. Each test plant was put into individual Ziploc bags and stored at –20°C until virus testing by DAS-ELISA. Some one or two test plants in MT and Type, T1 and Type, T1 and Sidney 81, and T2 and Sidney 81 died and, therefore, were excluded from mite scoring or virus testing. Although plants were established in an insect- and mite-free greenhouse and held in chambers with no other plants infested with either mites or viruses, source plants were also tested for TriMV and WMoV to ensure that they were not contaminated with these viruses.

DAS-ELISA assays on test plants. Duplicate samples were tested for WSMV using DAS-ELISA. Positive WSMV controls consisted of wheat tissue inoculated with WSMV, and healthy wheat tissue was used as a negative control. ELISA plates (96-well Flat-Bottom Immuno Plate; Maxisorp, Nunc, Thermo Fisher Scientific Inc., Waltham,

MA) were coated with WSMV capture (primary) antibody (Agdia Inc., Elkhart, IN) in 1× carbonate buffer at 1:400 dilution and stored overnight at 4°C. Each sample was prepared by adding wheat tissue (approximately 0.15 g) along with general extraction buffer (Agdia Inc.) at a 1:10 (wt/vol) ratio to a mesh bag (Agdia Inc.). The sample was ground using a tissue homogenizer (Agdia Inc.). Plant tissue solution (100 μl) was added to each of two sample wells of the ELISA plate. WSMV alkaline phosphate conjugate (secondary) antibody (Agdia, Inc.) in general extraction buffer (1:400 dilution) was added (100 μl per well). Plates were incubated at 37°C for 1 h and rinsed seven times with phosphate-buffered saline-Tween buffer. Purine nucleoside phosphorylase (100 μl) was added to each well and incubated in the dark at room temperature for 1 h. Quantitative measurements of the reaction were determined using absorbance at 405 nm with a Multiscan FC Spectrophotometer (Thermo Fisher Scientific Inc.). Plants were considered positive for WSMV when the absorbance value was three times (or greater) that of a healthy (negative) control.

Data analysis. Data analysis was performed using SAS software (version 9.4; SAS Institute Inc., Cary, NC). Proportions of WSMV-infected plants and presence of mites for the five mite populations and two WSMV virus isolates were tested for differences using PROC GLIMMIX with binomial distribution. The LSMEANS statement was used to obtain least squares means and the Tukey-Kramer test at $P = 0.05$ was used for pairwise comparison of treatment means. Posthoc contrast analyses were also done to compare transmission rates among groups of mite populations. The effect of transfer person was analyzed as a fixed factor in a preliminary analysis to determine the appropriateness of considering these effects as replications within runs. In the final analysis, fixed factors were mite population and virus isolate, and run and replicate were included as random factors. Percent transmission means and standard errors were obtained using the PROC MEANS statement.

Results

The percentage of test plants with established mites ranged from 92.5% (NE mites and Sidney 81) to 77.5% (T1 mites and Sidney 81). However, mite presence did not differ between virus types ($F = 0.08$, df = 1, 68, $P = 0.773$) or mite populations ($F = 1.00$, df = 4, 68, $P = 0.413$). In addition, there were no significant interactions between these factors ($F = 1.97$, df = 4, 68, $P = 0.109$).

Throughout the study, no mock samples were found positive for WSMV, indicating that there was no contamination during the experiments. All source plants tested positive for WSMV, and no contamination occurred from TriMV or WMoV because they tested negative for these two viruses. A preliminary analysis of the effect of transfer person showed no effect ($F = 0.1$, df = 2, 4, $P = 0.866$) and no treatment–person interaction was observed. As a result, transfer person was considered as a replicate within runs, providing nine total replicates for the entire study.

WSMV isolate had a significant effect on virus transmission rate ($F = 18.3$, df = 1, 68, $P < 0.0001$; Table 1). Single-mite transmission of Sidney 81 (43.3%) was greater than transmission of Type isolate (25.7%). Transmission differences were also observed between the five mite populations ($F = 7.1$, df = 4, 68, $P < 0.0001$). The transmission rates for the MT, SD, and T1 populations across both WSMV isolates were 25.6, 25.4, and 25.4%, respectively, and the transmission rates for the NE and T2 populations across the two isolates were 43.3 and 52.8%, respectively. There was no significant interaction between virus isolate and mite population ($F = 1.5$, df = 4, 68, $P = 0.212$).

Table 1. Transmission of two isolates of *Wheat streak mosaic virus* (WSMV) by five *Aceria tosichella* populations (percent means ± standard error [SE])

Wheat curl mite population	WSMV isolate (% mean ± SE)[z]		Mean (%)
	Sidney 81	Type	
Montana	36. 7 ± 5.1 bc (90)	14.6 ± 3.8 d (89)	25.6
Nebraska	58.8 ± 5.5 a (80)	27.8 ± 4.7 bcd (90)	43.3
South Dakota	34.4 ± 5.0 bc (90)	16.3 ± 4.2 d (80)	25.4
Type 1	25.8 ± 4.7 bcd (89)	25.0 ± 4.6 cd (88)	25.4
Type 2	60.6 ± 5.6 a (78)	45.0 ± 5.6 ab (80)	52.8
Mean (%)	43.3	25.7	

[z] Means with same letter within rows and columns are not significantly different ($P = 0.05$, Tukey-Kramer test). Numbers in parentheses = total number of test plants per treatment.

The NE (58.8%) and T2 (60.6%) mite populations transmitted the Sidney 81 isolate at a significantly higher rate compared with MT (36.7%), SD (34.4%), and T1 (25.8%). T2 mites transmitted the Type isolate at a significantly higher rate (45%) than MT (14.6%) and SD (16.3%) mites but they did not transmit at a higher rate than T1 (25%) or NE (27.8%) mites.

Posthoc contrast analysis revealed that both populations classified as type 2 genotype (NE and T2) transmitted Sidney 81 strain (mean = 59.7 versus 32.3%, Student's t-distribution value (t) = 4.15, df = 30, $P = 0.0003$) and Type strain (mean = 36.4 versus 18.6%, t = 3.10, df = 30, $P = 0.0042$) at a significantly higher rate than type 1 genotype (MT, SD, and T1). The newer mite populations (T1 and T2) transmitted Type strain (mean = 35 versus 18.6%, t = 2.72, df = 30, $P = 0.0109$) at a significantly higher rate than older mite populations (MT, SD, and NE) but these groups did not differ in transmission of Sidney 81.

Discussion

No differences were seen in mite survival and presence on test plants, indicating that the impact of WSMV on mite survival between Sidney 81 and Type isolates is comparable for all five mite populations. The Sidney 81 isolate was shown to have a positive effect on survival and reproduction of the NE mite population (Siriwetwiwat 2006). However, TriMV has a negative effect on survival compared with noninfected wheat plants (McMechan et al. 2014; Oliveira-Hofman et al. 2015). The comparable response of mites to WSMV presence across both strains may provide a competitive advantage to WSMV over TriMV.

The mite populations tested (MT, SD, NE, T1, and T2) differentially transmit WSMV isolates Sidney 81 and Type. This shows that the extent of WSMV spread and intensity could be determined by prevailing mite genotype and virus strain. The capability of NE mites (19-year-old colony) to transmit Sidney 81 at the same level as T2 mites (3-year-old colony) indicated that laboratory conditions have not affected the mite's ability to transmit the virus. The lower transmission rate of Sidney 81 by T1 (from Nebraska), MT (from Montana), and SD (from South Dakota) compared with NE and T2 mites indicates that mite genotype (but not source location or colony age) has greater influence on WSMV transmission.

The NE, MT, and SD mite populations transmitted Sidney 81 at a significantly higher rate than Type, while T2 and T1 did not differ in their ability to transmit the two virus isolates. This observation, in which mites from the same genotype differed in their ability to transmit the two isolates of WSMV, was unexpected. A plausible explanation for this behavior could be that different WSMV isolates prevailed in the field during collection of NE, MT, and SD mites in 1996 and T1 and T2 mites in 2011. McNeil et al. (1996) reported that the most predominant WSMV isolates (46%) in Nebraska were indistinguishable from Sidney 81, and none of the isolates were similar to the Type isolate. No recent study has been carried out to determine the composition of WSMV isolates in Nebraska. A change in virus isolate presence could partly be responsible for similar transmission rates for Sidney 81and Type isolates by the recently collected T1 and T2 mites.

Other investigators have reported the differential virus transmission ability of MT, SD, and NE mite populations in wheat. The NE mite population transmitted WSMV Sidney 81 isolate at a higher rate (45.5%) than MT (20.9%) and SD (36.5%) from wheat source plants coinfected with TriMV (Oliveira-Hofman et al. 2015). The transmission rates of Sidney 81 in this study by NE (58.8%) and MT (36.7%) were higher, while transmission rates by SD (34.4%) were similar to those from source plants coinfected with TriMV (Oliveira-Hofman et al. 2015). The similar transmission rates of Sidney 81 from singly infected plants (in this study) and plants coinfected with TriMV (Oliveira-Hofman et al. 2015) by SD mites indicate that these mites'

ability to transmit Sidney 81 may not be influenced by the presence or absence of TriMV. However, the ability of NE mite populations to transmit Sidney 81 is reduced when source plants are coinoculated with TriMV (Oliveira-Hofman et al. 2015). Our findings also indicate that MT mite populations transmitted Sidney 81 at a higher rate than what was reported when transmission was from source plants coinoculated with TriMV (Oliveira-Hofman et al. 2015). Seifers et al. (2002), using multiple mite transfers (10 per test plant), observed similar transmission rates (82 to 100%) of WSMV (unnamed isolate) by MT, SD, and NE mite populations. The NE mite population is an efficient vector of TriMV (41% using single-mite transfers and 100% with multiple-mite transfers), whereas SD and MT are inefficient vectors (2.5% using multiple-mite transfers) (McMechan et al. 2014). WMoV isolates were differentially transmitted (using 10-mite transfers) by NE (9 to 74%), and MT (1 to 94%) but not SD mite populations (Seifers et al. 2002). No studies have been carried out to determine the ability of the recently collected T1 and T2 mite populations to transmit TriMV or WMoV virus species or their isolates.

This study demonstrates that T2 and NE mites (type 2 genotype) and MT, SD, and T1 mites (type 1 genotype) differ in their ability to transmit WSMV Sidney 81 and Type isolates. Genotypic characterization of the ITS1 region revealed small but very consistent (1.2%) nucleotide sequence diversity between NE mites (type 2 genotype) and SD and MT mites (type 1 genotype) but no diversity between MT and SD. However, characterization of the mitochondrial DNA partially spanning the CO1 and COII genes revealed at least 13.1% sequence diversity between NE mites versus SD and MT mites, and 0.5% diversity between MT versus SD (Hein et al. 2012). According to Carew et al. (2009), there is no evidence of genetic exchange between type 1 and type 2 genotypes when comparing 16SrNA and ITS1 regions but a small number of individuals displayed RFLP profiles of both genotypes, suggesting that genetic exchange is possible, though uncommon, considering the close contact of these genotypes in the same wheat fields and even the same wheat heads. This genotypic variability could contribute toward differences in WSMV transmission.

Although several other mite species in the family *Eriophyidae* are known to transmit economically important plant pathogens (Oldfield and Proeseler 1996), it is only recently, with the advance in use of molecular techniques, that cryptic species or genotypes have been discovered to exist among these species (Navajas and Navia 2010). Currently, with the discovery of cryptic species or genotypes, the majority of studies have focused on genotypic differences in relation to host plant response and rarely on pathogen transmission (Navajas and Navia 2010). Thus far, studies reporting differential virus transmission by different mite genotypes involve WCM (McMechan et al. 2014; Oliveira-Hofman et al. 2015; Schiffer et al. 2009; Seifers et al. 2002). Differences in transmission of viruses by vector types or clones have also been reported in insect virus–vector interactions (Fereres 2015; Jones 2014). Verbeek et al. (2010) reported differences in the ability of *Aphis nasturti* (Kaltenbach) biotypes (relative efficiency factors = 0.08 to 1.59) to transmit *Potato virus Y*. Lupoli et al. (1992) observed a 3.5-fold difference between the most and least efficient *A. gossypii* (Glover) clones in transmission of *Papaya ringspot virus*. In whiteflies (*Bemisia tabaci* Gennadius), Gottlieb et al. (2010) reported a more efficient transmission of *Tomato yellow leaf curl virus* in Israel by the B biotype (80%) than the Q biotype (<10%). These vector transmission differences influence the epidemiology of plant virus diseases by determining the extent of disease spread and severity. Therefore, it is important that researchers working on virus transmission by vector species complexes characterize the vector they are using to facilitate a more clear association with virus transmission.

Hall et al. (2001a) reported higher transmission rates of Sidney 81 (57%) than Type (30%) from source plants coinfected with both isolates by a mite population, which, although not referenced, was the NE population (type 2 genotype). These transmission rates are comparable with what we found in this study with the NE mite population transmitting Sidney 81 (58.8%) at a higher rate than Type (27.7%) from singly infected source plants. McNeil et al. (1996) reported that WSMV isolates rarely (approximately 2%) exist as coinfections in wheat fields. Additionally, in mechanically coinoculated (Sidney 81 and Type) wheat plants, virus strains often segregated into portions from individual leaves, and individual tillers were found to have either of the strains or both (Hall et al. 2001a). This could partly explain the similar transmission pattern of these two isolates from mixed-infected source plants (Hall et al. 2001a) versus singly infected plants used in this study.

The differences in transmission of Sidney 81 and Type could be associated with genetic variability in the helper component proteinase (HC-Pro) region, which is the determinant of WSMV transmission by WCM (Stenger et al. 2005). Choi et al. (2001) reported 97.6% nucleotide and 98.7% amino acid similarity between Sidney 81 and Type, with amino acid substitutions distributed along the entire genome, though variability mostly occurred in the P1, HC-Pro, and coat protein cistrons. Virus transmission by WCM was abolished by replacement of WSMV Sidney 81 HC-Pro with homologs of *Turnip mosaic virus*, *Agropyron mosaic virus*, and *Oat necrotic mottle virus* (ONMV; 72.9% nucleotide and 80% amino acid identity to WSMV) (Stenger et al. 2005). WSMV Sidney 81 chimeras with ONMV partial genome replacements in the 5′-proximal half of HC-Pro failed to be transmitted by WCM. In contrast, a chimeric WSMV Sidney 81 genome flanking partial replacement with the ONMV genome in the 3′-proximal half of HC-Pro was transmitted by WCM (10 mites per test plant), though at a reduced rate (70%) compared with Sidney 81 (92%). This indicates that, although the determinant for WCM transmission lies in the 5′-proximal half of HC-Pro, the 3′-proximal half also has a role in transmission (Stenger et al. 2005). Further work by Stenger et al. (2006) indicated that deletion of 24 to 120 nucleotides in the 5′-proximal half of HC-Pro abolished WCM transmission. In contrast, mutants lacking three to six codons and containing some progeny with G to A transition at nucleotide 1,190 (amino acid aspartic acid to asparagine) were transmitted by WCM (10 mites per test plant), though at a reduced rate (73%) compared with Sidney 81 (Stenger et al. 2006). The differences in WSMV transmission efficiency by WCM due to genetic modifications in the HC-Pro region (Stenger et al. 2005, 2006) indicate that genetic variability in this region in WSMV strains could influence transmission efficiency.

Seifers et al. (2002) also demonstrated differential transmission of five isolates of WMoV by NE and MT mite populations. Differences in transmission of virus strains have been observed in other virus systems. For example, *Barley yellow dwarf virus* isolate MAV2 was transmitted at a higher rate (35%) compared with isolate MAV11 (19%) by *Rhopalosiphum padi* L. clone Rp5 (Sadeghi et al. 1997). *Cucumber mosaic virus* CMV-T strain was transmitted more efficiently (90%) compared with CMV-6 strain (10%) by its vector, *A. gossypii*; this difference was attributed to variability in the coat protein (Gera et al. 1979).

These findings demonstrate that mite genotype and virus isolate do influence transmission rates of WSMV. Studies on WCM populations collected from wheat fields in Nebraska show that type 1 and type 2 genotypes coexist as mixed populations in fields and even within single wheat heads (Siriwetwiwat 2006). In Australia, these two genotypes coexist in wheat fields, though some sites were predominant with either type 1 or type 2 (Schiffer et al. 2009). A recent study of mites collected from wheat and other grass hosts indicates that WCM is a complex comprising numerous cryptic species (Skoracka et al. 2012). Field populations of WSMV are also complex and consist of numerous genotypes (Fuentes-Bueno et al. 2011; McNeil et al. 1996; Montana et al. 1996; Robinson and Murray 2013). The differential transmission of WSMV based on mite genotype and virus isolate indicates that the existence of cryptic species of WCM and numerous genotypes of WSMV complicates the epidemiology and poses a challenge to management of this virus. Greater knowledge of the genotype composition of WCM populations and WSMV isolates and their virus transmission rates in the Great Plains are essential for understanding the epidemiology and improving the management of WSMV.

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