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Impact of *Wheat streak mosaic virus* and *Triticum mosaic virus* Coinfection of Wheat on Transmission Rates by Wheat Curl Mites

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Abstract

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Wheat streak mosaic virus (WSMV) and *Triticum mosaic virus* (TriMV) are transmitted by the wheat curl mite (WCM, *Aceria tosichella*), and coinfections of wheat by these viruses are common in the field. Previous work has shown that mite genotypes vary in their ability to transmit TriMV. However, the degree to which coinfection of wheat modifies WCM vector competence has not been studied. The objective was to determine whether mite genotypes differed in virus transmission ability when feeding on wheat coinfecting by WSMV and TriMV. First, WCM genotype type 2 was used to determine virus transmission rates from mock-, WSMV-, TriMV-, and coinfecting wheat plants. Transmission rates were determined by using single-mite transfers from replicated source plants. Coinfection reduced WSMV transmission by type 2

WCM from 50 to 35.6%; however, coinfection increased TriMV transmission from 43.3 to 56.8%. Mite survival on single-mite transfer test plants indicates that the reduction in WSMV transmission may result from poor mite survival when TriMV is present. In a second study, two separate colonies of WCM genotype type 1 were tested to assess the impact of coinfection on transmission. Type 1 mites did not transmit TriMV from coinfecting plants but the two colonies varied in transmission rates for WSMV (20.9 to 36.5%). Even though these changes in mite transmission rates are moderate, they help explain the high relative incidence of TriMV-positive plants that are coinfecting with WSMV in field observations. These findings begin to demonstrate the complicated interactions found in this mite–virus complex.

The wheat curl mite (WCM, *Aceria tosichella* Keifer) is the only known vector of *Wheat streak mosaic virus* (WSMV, genus *Tritimovirus*, family *Potyviridae*), *Wheat mosaic virus* (WMoV, also known as High Plains virus; tentative member of the genus *Emaravirus*) and *Triticum mosaic virus* (TriMV, genus *Poacevirus*, family *Potyviridae*) (15,16,21,25). These viruses are widespread across the Great Plains of the United States and cause significant yield losses to wheat in the region (2–4,26). Kansas's disease reports estimate that the average annual loss due to the WCM-vectored virus complex was 1% through the past 20 years (1); however, severely affected fields often have 100% yield loss. In field surveys, WSMV is the most prevalent of these viruses, followed by WMoV and TriMV (2,3). Triple infection by the viruses can occur at low rates, but co-infections are more common (2,3). Single infections in the field are most likely to be WSMV. TriMV infections appear to be dependent on WSMV because 91% of TriMV-positive samples were coinfecting with WSMV (3). WSMV and TriMV exhibit synergism when they coinfect wheat through increased titers of both viruses, greater symptom expression, and increased yield loss (4,23).

Field populations of WCM are made up of different biotypes, strains, and genotypes. Biotypic differences in WCM response to resistant wheat lines have been observed (7–9). Host-specific strains of the WCM were shown when mites reared on various grass hosts could not survive on wheat and vice versa (20). Mites reared on western wheatgrass (*Agropyron smithii* Rydb.) transmitted WSMV at significantly lower rates than mites reared on wheat. Once these mites adapted to wheat, they transmitted WSMV at rates comparable with those of colonies that were always reared on wheat (6). More recently, Skoracka et al. (19) found multiple cryptic lineages of *Aceria*

tosichella with diverse but distinct host ranges. This shows that *A. tosichella* is a genetically heterogeneous species complex.

Two distinct WCM genotypes were found in Australia and named as type 1 and type 2 based on nuclear and mitochondrial DNA (5). Also, using nuclear and mitochondrial DNA, Hein et al. (10) were able to separate five mite colonies, originally isolated from collections made in South Dakota (SD), Montana (MT), Texas (TX), Kansas (KS), and Nebraska (NE), into two distinct groups that corresponded genetically to the type 1 (SD, MT, TX, and KS) and type 2 (NE) mites found in Australia (5). These two mite types also correspond to two genotypes that were collected from wheat (20). The state locations where these mites were collected are not representative of the genetic diversity present in the field. Mixed populations of type 1 and type 2 were found within fields and even within wheat heads collected in NE, KS, and MT (18). These five mite colonies (10) originated from the exact colonies used in making biotype comparisons (9) and in establishing differential transmission of WMoV by WCM (14). Thus, the type 1 and 2 mite genotypes differ in relation to biotypic and virus transmission characteristics.

While mite types can vary in their ability to transmit viruses, there is also variation within types. In the United States, both type 1 and type 2 mite genotypes were shown to transmit WSMV at varying rates (14). However, in Australia, only type 2 mites were able to transmit WSMV (13). Type 2 mites (NE colony) transmitted WSMV at an average rate of 43 to 68%, depending on the vector's phenological stage (18). WMoV was transmitted by type 1 mites (KS, TX, SD, and MT colonies) at lower rates than type 2 (NE colony) mites (14). TriMV was transmitted by single type 2 mites at a rate of 41% but not by type 1 mites. However, type 1 mites transmitted TriMV at a lower rate (2%) when allowing continuous movement of large numbers of mites from infected to uninfected plants (12).

Coinfection of wheat with WCM-transmitted viruses may affect transmission rates of individual viruses. Low WMoV transmission rates on barley increased by type 1 mites (MT colony only) when coinfecting with WSMV (14). Using an unknown mite source, Seifers et al. (16) obtained their highest TriMV transmission rate (21%) when single mites were transferred from source plants coinfecting with WSMV.

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Given the increases in transmission rates for WMoV in the presence of WSMV and the uncertainty of transmission of TriMV from coinfecting plants, a better understanding of the nature of these viral coinfections and their impact on transmission and epidemiology is needed. Because coinfection of wheat with these viruses readily occurs in nature (2,3), there is a need to evaluate WSMV and TriMV transmission in the presence of other WCM-transmitted viruses. The objective of this research was to determine how coinfection of wheat by WSMV and TriMV affects individual virus transmission by the WCM. Two studies were undertaken to (i) establish whether differential transmission of TriMV and WSMV by the type 2 WCM occurred from coinfecting wheat and (ii) determine whether TriMV-WSMV coinfection enhanced the ability of type 1 WCM to transmit TriMV.

Materials and Methods

Mite colony maintenance. Established aviruliferous colonies of type 1 (MT and SD) and type 2 (NE) WCM were used. Mite colonies were maintained under artificial lights (cycle of 14 h of light and 10 h of darkness) in either a growth chamber or a colony room maintained at approximately 22°C. Colonies were maintained on 'Millennium' wheat grown in 15-cm-diameter pots by regularly (approximately every 3 weeks) transferring mites to new wheat plants. Cylindrical cages were placed over each pot to prevent contamination. These cages contained two vents on opposite sides and an open top, all covered with Nytex screen (80-micron mesh opening; BioQuip Products).

Transmission by type 2 WCM. This experiment compared the transmission of WSMV and TriMV by individual type 2 WCM from single- and coinfecting wheat. Millennium wheat was seeded in 4-cm-diameter cone-tainers (Stuewe & Sons Inc.) filled with autoclaved greenhouse soil. Plastic cylindrical cages (5 cm in diameter and 50 cm in height) with two to three Nytex vents were used to cover the cone-tainer plants. Three source plants (replicates) for each of four treatments were inoculated with sterilized water (mock), TriMV, WSMV, or WSMV + TriMV at 21 days after seeding. Crude sap of a 1:10 (wt/vol) ratio of infected tissue in sterilized water was extracted for each virus with a mortar and pestle. For single inoculations, 10 ml of the crude sap was combined with 10 ml of sterilized water. For coinoculations, 10 ml of each virus crude sap were combined. Thus, all inocula resulted in a 1:20 dilution. Plants to be inoculated were sprinkled with carborundum to allow scarring of the plant tissue and initiation of virus infection. Rub inoculation was performed by dipping the pestle in the inoculum and gently rubbing the entire length of the exposed leaves.

Within a week after inoculation, 10 WCM were placed on a point-mount triangle (card stock material, 11-mm height by 3-mm base) and carefully placed into the leaf axil of the newest leaf on each source plant. A mite transfer tool, made from a wood dowel with a single human eyelash attached, was used for mite transfers. Plants were then placed in a growth chamber (cycle of 14 h of light and 10 h of darkness) maintained at 27°C for 2 weeks.

After 2 weeks, single mites were transferred from each source plant to each of ten 14-day old test plants (two- to three-leaf stage). Source plants were cut and viewed under the microscope and mites were picked up with the transfer tool. A test plant was placed on an adjacent microscope and one mite was transferred directly to the whorl of the newest leaf. Only large (adult or late nymph) mites exhibiting normal movement were transferred to test plants. Test plants were immediately covered with cages and left overnight to allow mite establishment. Test plants were then transferred to a growth chamber held at 27°C (cycle of 14 h of light and 10 h of darkness). After the single-mite transfers, approximately 0.15 to 0.2 g of plant tissue from each source plant was stored at -20°C and later tested for WSMV and TriMV via double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). Only test plants from sources testing positive for the respective virus treatment were included in the statistical analysis.

Single-mite transfer test plants were harvested 21 to 24 days after infestation. Mite survival was determined by presence or absence for

each of the test plants. At harvest, leaf pieces from test plants were sampled and stored at -20°C until assayed for WSMV and TriMV via DAS-ELISA. Because of the extensive labor involved in mite transfers, the number of replicates for each run was limited to 3; however, this was conducted four times for a total of 12 source plants (replicates) for each treatment.

Type 1 versus type 2 transmission. The objective of this experiment was to determine whether WSMV + TriMV coinfection would influence virus transmission rates for type 1 WCM. Type 2 WCM was included as a comparison. Cone-tainer planting, inoculation procedures, and mite transfers were performed as described in the previous section. This experiment was conducted two times. The first run included three source plants each for a mock and WSMV + TriMV treatment for each of three WCM colonies tested: type 2 (NE) and type 1 (MT and SD). For the second run, the mock was eliminated to enable testing of six coinfecting source plants. For each virus treatment, mites were transferred individually from each source plant to 10 separate test plants. For the mock treatment used in the first run, only five single-mite transfers were made for each source plant. Nine WSMV + TriMV coinfecting source plants (90 test plants) were used for each colony, except the MT colony that only had 8 source plants (80 test plants) because one source plant tested negative for WSMV.

Test plants were harvested 21 days after single-mite transfers in the first run. Due to advanced symptom development in the second run, test plants were harvested only 14 days post WCM transfers. Plants were cut at the soil level and inspected for mite survival. Leaf tissue (approximately 0.15 to 0.2 g) for each test plant was placed into a mesh bag and stored at -20°C until DAS-ELISA testing for WSMV and TriMV.

Virus assay. DAS-ELISA for WSMV and TriMV was performed for all test plants. For each sample, approximately 0.15 to 0.2 g of plant tissue was added to a mesh bag (Agdia, Inc.). General extraction buffer (GEB) was added to the mesh bags at a 1:10 (wt/vol) ratio and then tissue was ground using a tissue homogenizer (Agdia Inc.). WSMV and TriMV tests were performed simultaneously, and leaf extract from the same mesh bag was used for both tests. ELISA plates (96-well Flat-Bottom Immuno Plate; Maxisorp, Nunc, Thermo Scientific Inc.) were coated with TriMV immunoglobulin G (IgG; 24) at 100 µl/well and 1:1000 (vol/vol) or WSMV capture antibody (Agdia Inc.) at 1:400 (vol/vol) in carbonate buffer, and stored overnight at 4°C. The following morning, plates were rinsed three times with phosphate-buffered saline with Tween (PBST). Extract (100 µl) of each sample was added to each of two wells of the WSMV- and TriMV-IgG-coated plates and incubated for 1 h at 37°C. Plates were washed with 1× PBST. Rabbit anti-WSMV or TriMV IgG-ALP conjugate antibody diluted in GEB (100 µl) was added to the plates at 1:400 (vol/vol) for WSMV and 1:500 (vol/vol) for TriMV, and incubated for 1 h at 37°C. Plates were washed with 1× PBST. p-Nitrophenyl phosphate (100 µl of 1 mg/ml) in 0.1 M diethanolamine buffer, pH 9.8, was added to each well, and plates were incubated at room temperature in the dark for at least 1 h. Absorbance estimates at 405 nm were obtained with a Multiskan FC Spectrophotometer (Thermo Scientific Inc.). A sample was considered positive if absorbance value was at least two times higher than that of negative controls (buffer and healthy extract) (16,17).

Data analysis. Data were analyzed using direct comparisons of transmission rates between WSMV and WSMV + TriMV and between TriMV and WSMV + TriMV treatments. PROC GLIMMIX (SAS, v. 9.3; SAS Institute Inc.) was used, specifying a binomial distribution for WSMV and TriMV presence because a plant was either positive or negative for each virus. Type III tests of analysis of variance and least significant differences for virus presence were used to generate differences between WSMV and TriMV transmission in single- and coinfecting treatments; source plants were treated as random effects. Interactions of mite survival and treatment response were also tested by including survival-treatment interaction as a response in the model statement. Separate analyses of virus presence were performed for plants with surviving mites and for plants with no surviving mites. Treatment effects and interactions at $P \leq 0.05$

were considered significant. Odds ratios were calculated by using PROC GLIMMIX (SAS, v. 9.3; SAS Institute Inc.) and compared the relative odds of WSMV transmission given its coinfection with TriMV, and TriMV transmission given its coinfection with WSMV.

Results

Transmission by type 2 WCM. All treatment source plants used in this study tested positive via DAS-ELISA for their respective viruses. All mock source plants and test plants tested negative for both WSMV and TriMV, indicating that no cross-contamination occurred. There was no significant treatment by run interaction, so data were combined across all four runs ($F_{3,320} = 0.49, Pr > F = 0.69$). The virus assay for WSMV indicated that type 2 WCM from single-infected source plants transmitted the virus at a 50.0% rate (standard error [SE] = 5.7, $n = 120$; Fig. 1). WCM feeding on plants coinfecting with WSMV and TriMV transmitted WSMV at a rate of 35.6% (SE = 5.3, $n = 120$; Fig. 1). WCM feeding on single-infected plants had a TriMV transmission rate of 43.3% (SE = 5.6, $n = 120$; Fig. 1). Mites feeding on plants coinfecting with WSMV and TriMV had a total TriMV transmission rate of 56.8% (SE = 5.6, $n = 120$; Fig. 1). Mites feeding on coinfecting source plants transmitted both viruses 23% of the time, transmitted TriMV alone at a rate of 33%, and transmitted WSMV alone at a reduced rate of 12.5% (Fig. 1). Transmission efficiency of WSMV was significantly reduced when TriMV was present in the source plant (50 versus 35.6%, $Pr > |t| = 0.0274$; Table 1). The opposite occurred for TriMV. The rate of TriMV transmission by the WCM increased significantly when WSMV was present in the source plant (43.3 versus 56.8%, $Pr > |t| = 0.0425$; Table 1). Overall, there was an odds ratio of 0.55 for WSMV transmission from

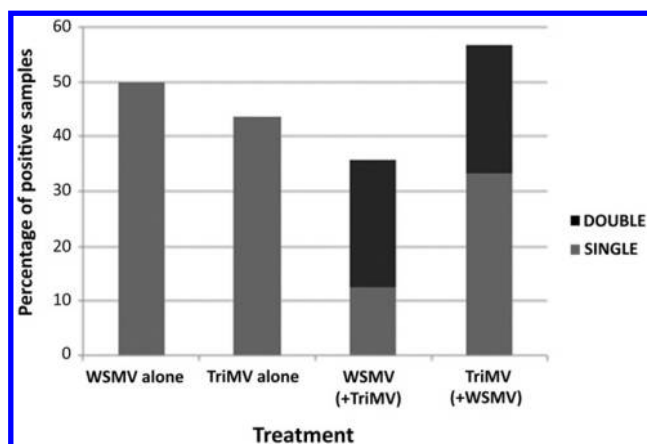


Fig. 1. Percentage of positive virus samples transmitted by type 2 wheat curl mites. WSMV = *Wheat streak mosaic virus* and TriMV = *Triticum mosaic virus*.

coinfecting plants over single-infected plants (Table 1). The TriMV transmission odds ratio was 1.72 for coinfecting compared with single-infected plants (Table 1). These odds ratios mean that mites exposed to coinfecting plants have lower odds of transmitting WSMV (0.55 times), but higher odds of transmitting TriMV (1.72 times) when compared with mites exposed to single-infected plants.

WCM survival rates on the test plants were 65, 55, 40, and 37% for WSMV, mock, TriMV, and coinfecting plants, respectively. WSMV and mock survival rates did not differ significantly from one another but they were significantly higher than the TriMV and coinfecting treatments. There was a significant interaction between mite survival and WSMV transmission rates ($F_{1,225} = 4.33, Pr > F = 0.0386$) but there was no interaction between mite survival and TriMV transmission rates ($F_{1,222} = 0.64, Pr > F = 0.4235$). Hence, if we restrict analysis to only those plants with surviving mites, WCM did not significantly differ in WSMV transmission rates between the WSMV single- and coinfecting treatments (Table 2). If mites survived, the rate of WSMV transmission was 59.7% when mites fed on single-infected plants and 68% when mites fed on coinfecting plants ($Pr > |t| > 0.05$, nonsignificant [NS]). If the mites did not survive to the end of the experiment, the transmission rate was 31.8% for the WSMV single-infected treatment and 16.1% for the coinfecting treatment ($Pr > |t| > 0.05$, NS).

Type 1 versus type 2 transmission. There was no colony-run interaction ($F_{2,254} = 0.45, Pr > F = 0.64$); therefore, virus transmission data were combined across runs. SD and MT colonies (type 1) were unable to transmit TriMV but NE (type 2) mites transmitted TriMV at a 47% rate (SE = 4.6, $n = 90$). NE mites transmitted WSMV at a 45.5% rate (SE = 11.8, $n = 90$), SD mites transmitted WSMV at a 36.5% rate (SE = 11.1, $n = 90$), and MT mites transmitted WSMV at a 20.9% rate (SE = 8.4, $n = 80$). NE and SD transmission rates were not significantly different from each other but they were both significantly higher than MT transmission rate (Table 3). There was no significant interaction between mite survival and transmission rates of WSMV ($F_{2,246} = 0.01, Pr > |t| = 0.98$). Mite survival in the test plants of the coinoculated treatments was 61% (SE = 5.8) for SD WCM, 53% (SE = 6.2) for MT WCM, and 42% (SE = 5.8) for NE mites. SD WCM survival was significantly higher than NE WCM survival ($Pr > |t| = 0.01$). MT WCM survival was not statistically different than SD WCM or NE WCM ($Pr > |t| > 0.05$, NS).

Discussion

In this study, coinfection of WSMV and TriMV in the source plant was found to alter the transmission efficiency of each virus by type 2 (NE colony) WCM when compared with single virus-infected source plants. The transmission rate of TriMV when WSMV was present in source plants was significantly increased. However, WSMV had a significant reduction in transmission when source plants were coinfecting with TriMV and WSMV. Type 1 mites (SD and MT colonies) did not transmit TriMV to test plants when they were reared on

Table 1. Differences of least square means for type 2 wheat curl mite (WCM) transmission from coinfecting *Wheat streak mosaic virus* + *Triticum mosaic virus* (WSMV + TriMV) plants and odds ratio for each virus (single-mite transfers)

Treatment	Virus present	Estimate	Standard error	df	t Value	Pr > t	Odds ratio
WSMV + TriMV	WSMV	-0.59	0.265	227	-2.22	0.0274	0.55
WSMV + TriMV	TriMV	0.54	0.265	224	2.04	0.0425	1.72

Table 2. Impact of wheat curl mite (WCM) survival post single-mite transfers on *Wheat streak mosaic virus* (WSMV) transmission rates

Treatment ^x	WCM survival (%)	Mite survival	WSMV transmission (%)	Mean SE ^y	t Grouping ^z
Double	37	Yes	68.3	6.8	a
		No	16.1	4.1	b
WSMV	65	Yes	59.7	5.4	a
		No	31.8	7.1	b

^x Double treatment: wheat plants were infected with a mixed solution of WSMV and *Triticum mosaic virus*.

^y SE = standard error.

^z For treatment least square (LS) means, LS means with the same letter are not significant at $P = 0.05$. Numerator df = 1 and denominator df = 225.

WSMV + TriMV coinfecting plants. McMechan et al. (12) showed extremely low TriMV transmission rates for type 1 mites. The present study also documents that, unlike type 2 WCM, coinfection of wheat plants does not improve TriMV transmission for type 1 mites.

TriMV has been strongly associated with WSMV in the Great Plains, being detected primarily in coinfections (2,3). Our study indicated that TriMV transmission efficiency increases when WSMV is present in the plant. However, only type 2 (NE) mites were able to transmit both viruses at the same time. Even though the increased TriMV transmission rate for mixed infections was modest, these findings help explain why Byamukama et al. (3) found that 91% of TriMV-positive samples were coinfecting with WSMV. TriMV may be strongly associated with WSMV partly because of a fitness advantage (i.e., increased transmission rate) in the presence of WSMV.

Field surveys have shown that WSMV is the predominant WCM-vectored virus, followed by WMoV and TriMV, the last two not always in this order (2,3). A high rate of TriMV infections occurred in coinfection with WSMV, with 76% (2) and 91% (3) of TriMV infections occurring with WSMV. TriMV can also co-occur with WMoV (2). Other studies have focused on effects of WSMV presence on transmission of WMoV or TriMV. Type 1 (MT) mites increased transmission of WMoV if they were already viruliferous for WSMV (14), and an unknown mite genotype transmitted TriMV primarily in association with WSMV (16). However, the present study is the first to show that TriMV hinders WSMV transmission. Specifically, this study demonstrated that WCM transmission of WSMV is negatively affected by TriMV presence. Lower WSMV transmission for coinfecting plants may be related to reduced mite survival. WCM survival on coinfecting plants was significantly reduced when compared with mite survival on WSMV-infected plants and the mock treatment in the first experiment. The second experiment was not set up to compare mite survival on coinfecting plants with mite survival on healthy plants but survival rates for the three mite sources varied, with significant differences between SD and NE mites. NE mite survival on coinfecting test plants in experiment 2 was similar to the previous experiment (42 versus 37%). Previous work has shown that type 1 and type 2 mites reared on TriMV-infected wheat had lower survival and reproductive rates (11) and that type 2 WCM feeding on WSMV-infected plants had enhanced reproductive rates (18). Given that type 1 and type 2 populations coexist in the field, it is likely that these transmission and mite survival interactions influence the transmission and epidemiology in the field. These effects need to be further investigated in the field.

The exact mechanisms of the interactions between WSMV and TriMV in terms of transmission rates are not known. One explanation for the increase in TriMV transmission might be that coinfection with TriMV and WSMV increases the concentration of both viruses in susceptible cultivars, as demonstrated by Tatineni et al. (23), thus making the virus more readily available for mite acquisition. Titer appears to be dependent on how long the plants have been infected. In coinfections, WSMV concentration decreased at 28 days postinoculation (dpi), when compared with single-infected plants, but TriMV concentration in coinfecting plants remained higher than in single-infected plants (23). Stenger et al. (22) found a similar pattern in the coinfection of corn with WSMV and *Maize chlorotic mottle virus* (MCMV). Titers of both viruses were higher in coinfecting plants at

15 to 17 dpi. However, WSMV titers in coinfecting plants decreased at 28 to 30 dpi and were comparable with single-infected WSMV plants. At 28 to 30 dpi, MCMV concentration in coinfecting plants remained higher than in single-infected plants (22). In this study, WCM fed on the source plants for about 2 weeks and, at the time of single-mite transfers, each source plant had been infected for at least 16 or 21 days. WSMV titer may have been decreasing in concentration in coinfecting plants by the time mites were transferred to test plants. But WSMV is retained through molting, so it is also plausible that any given mite used for transmission acquired the virus prior to titers decreasing.

An increase in TriMV transmission was found along with reduced WSMV transmission rates using type 2 WCM. This suggests not only that WSMV enhances TriMV transmission by type 2 WCM but also that TriMV interferes with WSMV transmission. The differences in transmission, 14.3% decrease in WSMV transmission in coinfecting plants and 13.5% increase in transmission of TriMV, were statistically significant but were not drastic increases and should be interpreted with caution. In these experiments, we are only making inferences about the interaction of WSMV and TriMV on single-mite transmission.

Future studies should investigate the mechanisms involved in the increase of TriMV transmission rate and the decrease in WSMV transmission rate when WCM are fed on coinfecting source plants. Even though type 1 WCM does not transmit TriMV, it is necessary to know whether coinfection of wheat with WSMV and TriMV can reduce WSMV transmission rates by the type 1 mite. More in-depth research needs to be done to determine the implications of these findings and determine whether coinfection by WSMV and TriMV affects vector capabilities and mite biology under field conditions.

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Table 3. Wheat streak mosaic virus transmission in coinfecting plants by type 1 (South Dakota [SD] and Montana [MT]) and type 2 (Nebraska [NE]) wheat curl mites (WCM)

Colony	Positive samples/total	Mean transmission (%)	Mean SE	t Grouping ^z
NE	44/90	45.5	11.8	a
SD	36/90	36.5	11.1	a
MT	20/80	20.9	8.4	b

^z For treatment least square (LS) means, LS means with the same letter are not significant at $P = 0.05$. Numerator df = 2 and denominator df = 256.

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