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Impact of Timing and Method of Virus Inoculation on the Severity of Wheat Streak Mosaic Disease

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Abstract

Wheat streak mosaic virus (WSMV), transmitted by the wheat curl mite *Aceria tosichella*, frequently causes significant yield loss in winter wheat throughout the Great Plains of the United States. A field study was conducted in the 2013–14 and 2014–15 growing seasons to compare the impact of timing of WSMV inoculation (early fall, late fall, or early spring) and method of inoculation (mite or mechanical) on susceptibility of winter wheat cultivars Mace (resistant) and Overland (susceptible). Relative chlorophyll content, WSMV incidence, and yield components were determined. The greatest WSMV infection occurred for Overland, with the early fall inoculations resulting in the highest WSMV infection rate (up to 97%) and the greatest yield reductions relative to the control (up to 94%). In contrast, inoculation of Mace resulted in low WSMV incidence (1 to 28.3%). The findings from this study indicate that both method of inoculation and wheat cultivar influenced severity of wheat streak mosaic; however, timing of inoculation also had a dramatic influence on disease. In addition, mite inoculation provided much more consistent infection rates and is considered a more realistic method of inoculation to measure disease impact on wheat cultivars.

Wheat streak mosaic (WSM) is a major disease of wheat (*Triticum aestivum* L.) in the Great Plains region of the United States. The causal pathogen of WSM is *Wheat streak mosaic virus* (WSMV), a member of the genus *Tritimovirus*, family *Potyviridae* (Stenger et al. 1998). WSMV infects wheat and other cereals in the Americas, Asia, Australia, Europe, and North Africa (Bockus et al. 2010; Brunt et al. 1996). WSMV is transmitted by the wheat curl mite (WCM), *Aceria tosichella* Keifer (Slykhuis 1955; Staples and Allington 1956). Two other wheat viruses in the Great Plains—*High Plains wheat mosaic virus* (HPWMoV), in the genus *Emaravirus* (Seifers et al. 1997; Tatineni et al. 2014), and *Triticum mosaic virus* (TriMV; genus *Poacevirus*, family *Potyviridae*) (Seifers et al. 2009; Tatineni et al. 2009) —are also transmitted by the WCM. These three viruses infect wheat across the Great Plains of the United States, although WSMV is the most common (Burrows et al. 2008; Byamukama et al. 2013).

Cultural practices to reduce WCM populations, and use of resistant or tolerant wheat cultivars, are major strategies employed to manage WSM disease (Graybosch et al. 2009; Sharp et al. 2002; Wegulo et al. 2008). Both virus and vector are dependent on the "green bridge" (hosts growing between the harvesting of one crop and the emergence of the next crop) (Somsen and Sill 1970). Therefore, the most effective management tactic for WSM is elimination of the green bridge. The few wheat cultivars with known resistance to WSMV are temperature sensitive, and usually become susceptible under high temperatures (Fahim et al. 2012; Price et al. 2014; Seifers et al. 1995, 2006, 2007; Tatineni et al. 2010). Temperature sensitive resistant genes *Wsm1* in Mace and *Wsm2* in cultivars RonL and Snowmass confer resistance to WSMV at temperatures below 18°C but infection and symptom expression increases when plants are exposed to temperatures ranging between 20 and 28°C (Fahim et al. 2012; Seifers et al. 2006, 2007; Tatineni et al. 2010, 2016; Wosula et al. 2017). Delayed planting also is a common management strategy to reduce infection by WSMV because lower temperatures later in the fall slow WCM population buildup, limit WCM movement, and limit WSMV titer buildup (Hunger et al. 1992; Price et al. 2014; Wosula et al. 2017).

Wheat susceptibility to WSMV and impact of WSM disease on biomass, grain yield, and grain quality can be affected by timing of infection, intensity of inoculation, host genotype, and environmental conditions such as temperature and soil water availability. WSMV symptoms were severe and yield loss higher in susceptible cultivars compared with the resistant Mace (Byamukama et al. 2014; Tatineni et al. 2010). Hunger et al. (1992) reported yield losses of up to 87% in susceptible cultivars when infected with WSMV during the fall season, whereas spring inoculations did not consistently show symptoms. In the northern Great Plains of the United States, spring inoculation of WSMV in winter wheat resulted in 45 to 57% yield reduction, compared with only 5 to 7% in fall inoculations (Miller et al. 2014). Knowledge of the effects of time of infection (early fall, late fall, or spring) of winter wheat by WSMV on disease intensity and yield will enable development and deployment of improved management tactics. In addition, information on the effects of inoculation method (mites versus mechanical) on disease intensity and yield will be useful to researchers comparing response of wheat cultivars to WSMV infection.

Laboratory experiments revealed a higher incidence of WSMV in barley plants inoculated using WCM compared with mechanical inoculation in the field (Ito et al. 2012; Lehnhoff et al. 2015), indicating that the method of inoculation can influence disease severity. A similar study by Miller et al. (2014) revealed severe symptoms and yield loss in wheat plants mechanically inoculated with WSMV in the spring but not those inoculated during the fall. They attributed this phenomenon to postinoculation temperatures being warmer in the spring and cooler in the fall in Montana. In the central Great Plains of the United States, temperatures are generally warmer than in Montana, and the dynamics of fall and spring temperature are quite different. Therefore, there was a need to determine the response of resistant and susceptible wheat cultivars to seasonal inoculation with WSMV. Additionally, information is lacking on the effectiveness of inoculation method on infection levels and severity of WSMV under field conditions. Under natural conditions, WSMV inoculation is by mites; therefore, comparing this to mechanical inoculation is essential to determine the effectiveness of the latter. The objective of this study was to determine the impact of timing of inoculation and method of inoculation on severity of WSM disease and yield in winter wheat cultivars Mace (resistant) and Overland (susceptible) grown in Nebraska.

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Materials and Methods

The study was conducted at the University of Nebraska's Agricultural Research and Development Center near Mead, NE. The experimental design was a split plot in randomized complete blocks with six replications. The main plot treatments were a two-by-two factorial, with two inoculation methods (mite and mechanical) and two cultivars. Two hard red winter wheat cultivars used in this study were Mace (WSMV resistant) and Overland (WSMV susceptible). Four split-plot treatments included (i) no mites or WSMV, (ii) early fall WSMV inoculation (two-leaf stage; Zadoks stage 12 to 13), (iii) late fall WSMV inoculation (one- to two-tiller stage; Zadoks stage 21 to 22), and (iv) early spring WSMV inoculation (four- to five-tiller stage; Zadoks stage 24 to 30). Each main plot was 18 by 4.5 m and, within the main plots, four split plots were each 0.6 by 0.6 m (four rows with spacing of 20 cm). Split plots were spaced approximately 3 m apart in the middle four rows of each main plot. Plants surrounding the 0.6-by-0.6-m experimental plots functioned as guard rows, with no measurements taken.

The study was conducted during two growing seasons (2013–14 and 2014–15). Both main-plot treatment cultivars were seeded using a tractor-mounted planter at a rate of 20 seeds per 30-cm row. Plots were planted into tilled ground previously rotated to oat (2013) and soybean (2014). Plots were maintained using conventional agronomic practices for the area. In the first cropping season (2013–14), plots were seeded 25 September 2013 and were inoculated with WSMV (mechanical and mite) on 9 October 2013 (early fall), 8 November 2013 (late fall), and 9 April 2014 (early spring). In the second cropping season (2014–15), plots were seeded on 23 September 2014 and were inoculated with WSMV (mechanical and mite) on 16 October 2014 (early fall), 6 November 2014 (late fall), and 10 April 2015 (early spring).

Mechanical inoculation process. Wheat cultivar Settler CL was sown in 10 pots (15 cm in diameter) filled with standard greenhouse soil. Pots were sown with 25 seeds each, placed on greenhouse benches under natural lighting, and watered as needed. Greenhouse temperatures ranged between 18 and 28°C. After 14 days, plants were mechanically inoculated with WSMV isolate Sidney 81. WSMV isolate Sidney 81 was 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). Inoculum was prepared by grinding infected wheat tissue in sterile distilled water (1:10 [wt/vol]) using a mortar and pestle. Plants were lightly dusted with carborundum and inoculated by gently rubbing the inoculum onto upper leaf surfaces using a pestle. WSMV-infected plants showing symptoms were harvested after 4 to 5 weeks and bulked from 10 pots. Inoculum for field inoculation was prepared by grinding leaf tissue in sterile, ice-cold, distilled water (1:10 [wt/vol]) in a blender. The inoculum was kept on ice until used for field inoculation on the same day of preparation.

Plots designated for mechanical inoculation were inoculated using a spray gun (Binks, Glendale Heights, IL) attached to a 0.5-liter inoculum reservoir that was connected to an air compressor (Bobcat, West Fargo, ND) maintained at a pressure of 96 psi. Each plot was inoculated with 50 ml of inoculum mixed with 0.5 g of carborundum. During the 2014–15 growing season, Overland plants (approximately 10 plants) from guard rows from each of the six replicates were mechanically inoculated with WSMV and then transplanted into pots. These potted plants were held in the greenhouse (22 to 27°C) and tested for WSMV after 3 weeks. This was conducted after each inoculation to determine effectiveness of the mechanical method used to inoculate WSMV into wheat. HOBO data loggers (MicroDAQ.com, Ltd., Contoocook, NH) were placed in the field in three replicates to record temperature for 7 days after WSMV inoculation.

Infestation process for viruliferous mite inoculation. WCM used for inoculations were the type 2 mite genotype, designated as Nebraska from a colony established two decades ago (Hein et al. 2012). This colony has been used in studies involving host resistance in wheat, virus transmission, and genotype characterization (Harvey et al. 1999; Hein et al. 2012; McMechan et al. 2014; Oliveira-Hofman et al. 2015; Seifers et al. 2002; Wosula et al. 2016). Wheat cultivar Settler CL was sown in 20 pots (15 cm in diameter, 25 seeds/pot). After 14 days, wheat plants were moved to a separate greenhouse and thinned to 20 seedlings/pot, then infested with WCM. Leaf pieces (1 cm long, with approximately 10 to 30 mites) were prepared from a previously established colony (3 weeks old). Leaf pieces were placed in the axil of the second leaf of each plant in the pot. The mites were allowed to establish for 1 week, after which the plants were mechanically inoculated with WSMV isolate Sidney 81. The mites were allowed to reproduce for 4 weeks, after which WSMV-infected plants showing symptoms were harvested and bulked from the 20 pots. The harvested plants were observed under a stereomicroscope to confirm the presence of mites, and those with mites were cut into 1-cm leaf pieces (approximately 200 pieces/bag and a total of 12 bags) used to inoculate field plots. Plots designated for mite infestation and subsequent virus inoculation were infested with a single 1-cm leaf piece (with an average of approximately 100 mites) placed in the axil of each plant within the plot. Plots infested with mites were covered with BugDorm cages (0.6 by 0.6 by 0.6 m; MegaView Science Co., Ltd., Taichung, Taiwan) for 2 weeks immediately following infestation to allow for mite establishment. Cages were placed over all four mite infestation treatments for 2 weeks after each inoculation to maintain consistency across treatments.

Disease assessment. Relative chlorophyll content was measured on flag leaves on 11 June 2014 and 01 June 2015 in the 2013–14 and 2014–15 cropping seasons, respectively. Relative chlorophyll content was measured nondestructively (Richardson et al. 2002) by using a soil plant analytical development (SPAD) meter (model 502 Plus; Konica Minolta Sensing, Inc., Osaka, Japan) (Guinta et al. 2002). Higher SPAD readings indicate higher intensity of greenness of leaf tissue (more healthy) and lower SPAD readings indicate lower chlorophyll levels (yellowing) and more severe virus symptoms (Byamukama et al. 2014). In each plot, SPAD readings were taken on the newest fully emerged leaf from 10 randomly selected plants and averages were recorded for each plot. On each leaf, SPAD readings were consistently taken at about one-third the length of the leaf from the leaf base. To avoid interference of foliar fungal diseases on yield and chlorophyll content, a single fungicide spray (Prosaro [prothioconazole + tebuconazole]; Bayer Corp., Research Triangle Park, NC) was applied at a rate of 0.507 ml/liter when wheat was at Zadoks growth stage 50 (Zadoks et al. 1974). To assess efficiency of virus inoculation in plots, 10 randomly-selected flag leaves were harvested from each plot (Zadoks growth stage 39) and tested for WSMV presence using enzyme-linked immunosorbent assay (ELISA), as described by Wosula et al. (2016). Samples also were tested for TriMV and HPWMoV.

Harvesting of the 0.6-by-0.6-m plots was done by hand. Each year, all plants from within each plot were cut and placed in individual paper bags. The number of spikes from each plot was counted, and later threshed by using a head thresher (Wintersteiger Inc., Salt Lake City, NE). Total kernel number for each plot was determined using a seed counter (Agriculex Inc., Guelph, ON, Canada). Spikes per square meter, number of kernels per spike, grain yield (= yield kg/ha), and 1,000-kernel weight were determined.

Data analysis. SPAD readings, WSMV incidence, spikes per square meter, number of kernels per spike, 1,000-kernel weight, and yield were subjected to analysis of variance using PROC GLIMMIX (SAS version 9.4; SAS Institute, Cary, NC). Treatment effects or interactions were considered significant at $P \leq 0.05$. 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. Fixed effects were cultivar, inoculation method, and time of inoculation, with replication included as a random effect. Treatment means and standard errors were obtained using the PROC MEANS statement. Data from the 2 years were subjected to a Bartlett's test (Gomez and Gomez 1984) for homogeneity of error variances. Tests for homogeneity of variance for yield revealed unequal variances between the 2 years ($\chi^2 = 4.12$, $P = 0.042$); hence, data for each year were analyzed separately.

Results

In the 2013–14 season, temperatures during the 7 days after WSMV inoculation were as follows: early fall, maximum = 30.7°C, minimum = 0.6°C, average = 14.6°C; late fall, maximum = 17.9°C, minimum = −13.0°C, average = 1.8°C; and early spring, maximum = 32.2°C, minimum = −7.5°C, average = 10.8°C. In the 2014–15 season, temperatures for the 7 days after WSMV inoculation were as follows: early fall, maximum = 28.4°C, minimum = 0.7°C, average = 13.3°C; late fall, maximum = 19.9°C, minimum = −12.7°C, average = 1.0°C; and early spring, maximum = 28.4°C, minimum = −1.9°C, average = 13.8°C.

WSMV symptoms. Overland wheat inoculated with WSMV in the early fall (mite and mechanical) showed mild mottling within 4 weeks after inoculation. Overland plants inoculated in the late fall did not show symptoms during the fall. Mace wheat inoculated in the early fall and late fall did not show visible WSMV symptoms during the fall. After temperatures warmed in the spring, Overland plants inoculated (mite and mechanical) in the early fall had typical WSMV symptoms (mosaic, mottling, and streaking) and were severely stunted, with tillers spraddled (projecting about 45° from the ground) in 80 to 100% of the plants. Overland plants that were inoculated in the late fall and early spring (mite) had mild mosaic and streaking in 40 to 80% of the plants but height was comparable with that of plants in the noninoculated control. Overland plants mechanically inoculated in the late fall and early spring had mild symptoms in 5 to 10% of the plants in 2013–14, whereas those of the 2014–15 season had about 10 and 75% mild symptoms in late fall and early spring inoculations, respectively. None of the Mace plants in any treatments expressed obvious WSMV symptoms in the spring. No virus symptoms were observed in the control (noninoculated) treatments throughout the growing season.

WSMV infection rates. The percentage of WSMV-infected plants in the 2013–14 growing season was significantly affected by cultivar, method of inoculation, and timing of inoculation ($P \leq 0.0006$). A significant three-way interaction occurred between cultivar, method of inoculation, and timing of inoculation ($P = 0.0002$). The highest percentage of WSMV-infected samples was in the Overland mite plots infested in early fall (91.7%), which was significantly greater ($P = 0.05$) than in late fall (65%) or early spring (55%) (Table 1). WSMV infection in Overland mechanically inoculated in early fall (66.7%) was significantly greater ($P = 0.05$) than spring (28.3%) and late fall (5%) inoculations. WSMV infections in Mace with mite and mechanical inoculations were not different from controls. All control (noninoculated) samples tested negative for WSMV (Table 1).

The percentage of WSMV-infected samples in the 2014–15 growing season was significantly affected by cultivar, method of inoculation, timing of inoculation, and interactions between cultivar, method of inoculation, and timing of inoculation ($P \leq 0.009$). The highest percentage of WSMV infections occurred in mechanically inoculated Overland in the early fall (96.7%), and it was significantly greater ($P = 0.05$) than late fall (13.3%) and early spring (8.4%) inoculations (Table 2). Overland mite inoculation had WSMV in 78.3 and 83.3% of the samples in the early fall and early spring, respectively, and these were significantly greater ($P = 0.05$) than late fall inoculations (35%) (Table 2). WSMV infection in mite-inoculated Mace in the early fall (28.3%) was significantly greater ($P = 0.05$) than in late fall (6.7%) or early spring (0%). However, there were no differences in infection rates among mechanical inoculation treatments in Mace, which were all less than 5% (Table 2).

Samples from all control plots were negative for WSMV, indicating that distance separating treatment plots was effective in preventing WSMV spread between plots. All control and treatment samples tested negative for TriMV and HPWMoV. In 2014–15, Overland plants that were mechanically inoculated with WSMV and transplanted into pots showed typical symptoms after 3 weeks and tested positive in all 30 plants sampled from the six pots, each representing the early fall and late fall inoculation, and 29 of 30 plants in the early spring inoculation.

SPAD readings. SPAD readings during the 2013–14 growing seasons were significantly affected by cultivar, method of inoculation, and timing of inoculation ($P \leq 0.026$). SPAD readings for Overland mite inoculation in the early fall (34.5), late fall (38.5), and early spring (38.6) treatments were significantly lower ($P = 0.05$) than the control (43.4) (Table 1). However, for the mechanical inoculation of Overland, SPAD reading in the early fall inoculation (36.9) was significantly lower ($P = 0.05$) than the control, while those of late fall and spring inoculations were not different. SPAD readings in all Mace mite and mechanical inoculations were not different than the control (Table 1).

SPAD readings for the 2014–15 growing season were significantly affected by timing of inoculation and interactions between method and timing of inoculation and cultivar and timing of inoculation ($P \leq 0.002$). SPAD readings for Overland mite inoculation in the early fall (33.4) and early spring (37.4) were significantly lower ($P = 0.05$) than the control (43.5). For the mechanical inoculation of Overland, SPAD readings in the early fall inoculation (34.1) were significantly lower ($P = 0.05$) but no differences were observed in the late fall and early spring inoculations compared with the control (Table 2). Similar to the 2013–14 growing season, method and timing of inoculation did not affect SPAD readings in the Mace treatments (Table 2).

Yield components. Yield (kilograms per hectare) during the 2013–14 growing season was significantly affected by cultivar, method

Table 1. Effect of cultivar, inoculation method and time of inoculation on impact of *Wheat streak mosaic virus* (WSMV) on percent virus infection, soil plant analytical development (SPAD) units, and yield components during the 2013–14 growing season (means)[z]

Treatment	WSMV infection (%)	SPAD units	Yield (kg/ha)	Spikes/m^2	Kernel/spike	1,000-kernel weight (g)
Mace mechanical						
Control	0.0 d	47.7 a	5,843.7 abc	521.9 ab	26.3 a	24.1 abc
Early fall	1.7 d	47.7 a	6,033.2 ab	537.5 ab	26.3 a	25.2 abc
Late fall	1.7 d	46.6 ab	5,351.1 abc	466.4 ab	25.5 a	25.2 abc
Early spring	3.3 d	46.6 ab	5,910.2 abc	509.3 ab	26.2 a	24.6 abc
Overland mechanical						
Control	0.0 c	42.7 abcd	6,925.3 a	573.6 a	25.5 a	26.6 a
Early fall	66.7 b	36.9 ef	4,048.6 c	378.4 bc	23.8 a	24.0 abc
Late fall	5.0 d	41.6 bcde	5,786.7 abc	528.5 ab	24.0 a	25.9 ab
Early spring	28.3 c	40.8 cde	5,535.4 abc	453.3 ab	25.3 a	27.1 a
Mace mite						
Control	0.0 d	46.8 a	5,350.7 abc	479.0 ab	25.9 a	24.1 abc
Early fall	10.0 dc	45.5 abc	4,991.4 abc	505.0 ab	22.4 a	24.9 abc
Late fall	5.0 d	45.6 abc	4,701.6 bc	488.3 ab	22.2 a	24.8 abc
Early spring	10.0 dc	46.1 ab	5,479.0 abc	513.7 ab	25.5 a	23.7 abc
Overland mite						
Control	0.0 d	43.4 abc	6,353.9 ab	529.0 ab	25.3 a	26.8 a
Early fall	91.7 a	34.5 f	642.7 d	223.3 c	9.8 b	15.2 d
Late fall	65.0 b	38.5 def	4,020.4 c	485.5 ab	21.9 a	21.3 bc
Early spring	55.0 b	38.6 def	4,018.1 c	470.3 ab	22.4 a	21.4 bc

[z] Means with same letters within column are not significantly different ($P = 0.05$, Tukey-Kramer test).

of inoculation, timing of inoculation, and interactions between cultivar and method of inoculation, method and timing of inoculation, and cultivar and timing of inoculation ($P \leq 0.024$). There were no differences in yield in any Mace treatments compared with the control (Table 1). However, yields in mite-inoculated Overland treatments in the early fall, late fall, and early spring treatments (642.7, 4,020.4, and 4,018.1 kg/ha, respectively) were significantly lower ($P = 0.05$) than the control (6,353.9 kg/ha). This represented 90, 37, and 37% yield reduction, respectively. Mechanical inoculation of Overland in the early fall yielded 4,048.6 kg/ha, representing 41% yield reduction compared with the control (6,925.3 kg/ha), but the late fall and early spring inoculation treatments were not different from the control (Table 1).

Yield during the 2014–15 growing season was reduced to about half that of the previous year. This was due to multiple factors that included lodging, bacterial streak infections, and Fusarium head blight (FHB) that developed to severe epidemic levels in all FHB-prone wheat-growing areas in Nebraska, including the southeast, where this experiment was conducted. Main-plot treatment effects for cultivar were significant ($P = 0.002$), with Mace yielding higher across treatments compared with Overland. Mite inoculation led to increased severity of WSMV that caused significant yield reduction across cultivars compared with mechanical inoculation ($P < 0.0001$).

Timing of inoculation was significant, as were the interactions between cultivar and timing of inoculation; method and timing of inoculation; and cultivar, method, and timing of inoculation ($P \leq 0.038$). Yields in mite inoculation of Overland in the early fall, late fall, and early spring treatments (105.2, 722, and 852 kg/ha, respectively) were significantly lower ($P = 0.05$) than the control (3,298.4 kg/ha) (Table 2). This represented yield reductions of 97, 78, and 74%, respectively (Table 2). Mechanical inoculation of Overland in the early fall yielded 299.1 kg/ha, which was significantly lower than the control (2,660.7 kg/ha), resulting in an 89% yield reduction. However, yield in the late fall and early spring inoculation treatments did not differ from the control (Table 2). Yield in mite-inoculated Mace was significantly reduced ($P = 0.05$) by 73 and 69% in the early fall (771.5 kg/ha) and late fall (911.9 kg/ha) inoculation treatments, respectively, but not in the early spring treatment compared with the control treatment (2,910.7 kg/ha) (Table 2).

The number of spikes per square meter during the 2013–14 growing season was significantly affected by cultivar ($P = 0.017$), with lower spike numbers for Overland. Mite and mechanical inoculation of Overland in the early fall reduced the number of spikes per square meter by 58 and 34%, respectively, compared with the control; however, spikes per square meter for all other treatments across cultivar and inoculation timings did not differ from the controls (Table 1). The number of spikes per square meter during the 2014–15 growing season was significantly affected by cultivar ($P = 0.004$), with Mace producing a greater number of spikes. Mite inoculation treatments also had significantly fewer spikes compared with mechanical inoculation ($P = 0.0004$). Spikes per square meter for Mace was consistent across both inoculation method and timings. However, mite inoculation of Overland in the early fall and early spring resulted in a 69 and 38% reduction in number of spikes per square meter, respectively, compared with the control. Mechanical inoculation of Overland in the early fall also resulted in a reduction in the number of spikes per square meter (49%) compared with the control (Table 2).

The number of kernels per spike during the 2013–14 growing season was significantly affected by cultivar and method of inoculation ($P < 0.0001$), with Overland having fewer kernels compared with Mace. Timing of inoculation and interactions between cultivar and method of inoculation; cultivar and timing of inoculation; method and timing of inoculation; and cultivar, method, and timing of inoculation also were significant ($P \leq 0.02$). Overland mite inoculation in the early fall resulted in fewer kernels per spike compared with late fall, early spring, and control treatments (Table 1). Overland mechanical inoculation, and Mace mite and mechanical inoculations had no effect on the number of kernels per spike (Table 1). The number of kernels per spike during the 2014–15 growing season was significantly affected by cultivar ($P < 0.0001$), with Mace producing a greater number of kernels compared with Overland. Mite inoculation also resulted in significantly fewer kernels compared with mechanical inoculation ($P = 0.0002$) but there was no cultivar–inoculation method interaction. The effect of timing of inoculation was significant and so were interactions between cultivar and timing of inoculation; method and timing of inoculation; and cultivar, method, and timing of inoculation ($P \leq 0.007$). Overland mite inoculation in early fall, late fall, and early spring resulted in a significant 87, 66, and 52% reduction in kernels per spike, respectively, compared with control (Table 2). Overland mechanical inoculation in early fall resulted in a 75% reduction in kernels per spike compared with the control. No effect on kernels per spike was seen for mechanical inoculation of Mace but, for mite-inoculated Mace, early fall and late fall

Table 2. Effect of cultivar, inoculation method and time of inoculation on impact of *Wheat streak mosaic virus* (WSMV) on percent virus infection, soil plant analytical development (SPAD) units, and yield components during the 2014–15 growing season (means)[z]

Treatment	WSMV infection (%)	SPAD units	Yield (kg/ha)	Spikes/m^2	Kernel/spike	1,000-kernel weight (g)
Mace mechanical						
Control	0.0 d	40.7 abcd	3,789.3 a	704.6 a	11.2 ab	24.6 ab
Early fall	5.0 d	38.9 bcd	2,554.9 ab	610.5 abc	9.6 ab	23.1 ab
Late fall	0.0 d	39.4 bcd	2,963.3 ab	604.0 abcd	10.5 ab	24.2 ab
Early spring	1.7 d	40.7 abcd	2,540.5 abc	576.6 abcd	9.8 ab	23.7 ab
Overland mechanical						
Control	0.0 d	43.2 a	2,660.7 ab	598.7 abcd	8.9 ab	26.4 a
Early fall	96.7 a	34.1 ef	299.1 ef	304.3 fe	2.2 d	23.6 ab
Late fall	13.3 cd	41.7 abc	2,660.1 ab	570.9 abcd	8.9 ab	26.3 a
Early spring	8.4 cd	42.7 ab	2,026.9 bcd	586.2 abcd	7.3 bc	23.2 ab
Mace mite						
Control	0.0 d	40.7 abcd	2,910.7 ab	528.2 abcd	11.8 a	24.2 ab
Early fall	28.3 bc	38.5 cd	771.5 def	410.6 dec	4.0 cd	22.7 ab
Late fall	6.7 d	38.5 cd	911.9 cdef	488.3 bcde	4.6 cd	21.4 ab
Early spring	0.0 d	39.4 bcd	1,872.1 bcde	518.1 abcd	10.3 ab	19.7 b
Overland mite						
Control	0.0 d	43.5 a	3,298.4 ab	636.5 abc	10.3 ab	26.3 a
Early fall	78.3 a	33.4 f	105.2 f	198.8 f	1.3 d	21.6 ab
Late fall	35.0 b	41.5 abc	722.0 def	443.2 dec	3.5 cd	23.5 ab
Early spring	83.3 a	37.4 de	852.0 def	396.7 de	4.9 cd	22.2 ab

[z] Means with same letters within column are not significantly different ($P = 0.05$, Tukey-Kramer test).

inoculations resulted in a significant 66 and 61% reduction in kernels per spike, respectively, compared with the control (Table 2).

The 1,000-kernel weight (in grams) during the 2013–14 growing season was significantly affected by method of inoculation ($P <$ 0.0001), with mite-inoculated treatments having lower weight. Timing of inoculation was significant and so were interactions between cultivar and method of inoculation; cultivar and timing of inoculation; method and timing of inoculation; and cultivar, method, and timing of inoculation ($P \leq 0.004$) (Table 1). However, Overland mite inoculation in early fall, late fall, and early spring resulted in a reduction of 43, 21, and 20% in 1,000-kernel weight, respectively, compared with the control. The 1,000-kernel weight during the 2014–15 growing season was significantly affected by method of inoculation ($P = 0.018$), with mite inoculation treatments having lower weight compared with mechanical inoculation. The early fall inoculation treatment also had significantly lower weight ($P = 0.004$) compared with late fall and early spring treatments (Table 2).

Discussion

Knowledge regarding the impact of cultivar, cultural practices, and prevailing conditions on disease severity is essential for development of control and management strategies. The findings from this study indicate that both method of inoculation and wheat cultivar influenced severity of WSM; however, timing of inoculation had the most dramatic influence.

Mechanical inoculation worked very well in early fall, when temperatures following inoculation were favorable, but this method worked poorly in late fall and early spring, despite the fact that, in 2014–15, sample plants inoculated at the same time in the field and brought back to the greenhouse quickly developed symptoms and tested positive for WSMV. Because the samples were held at warmer temperatures in the greenhouse, the low incidence of WSMV in the mechanically inoculated late fall and early spring treatments could be attributed to inhibited replication and titer increase resulting from cool temperatures that prevailed following inoculation. However, temperature does not fully explain differences seen in this study because, in 2014–15, temperatures following inoculation in the spring were comparable with those after the early fall inoculation (13.8°C for spring versus 13.3°C for fall). Another factor possibly influencing this is the more advanced growth stage of the plants in the spring. Therefore, mechanical inoculation is not an adequate method when temperatures are not ideal and, thus, too variable for seasonal studies like this one that use field inoculation to investigate cultivar resistance and the impact of WSMV on yield (Byamukama et al. 2014; Hunger et al. 1992; Miller et al. 2014).

Mite inoculation was more consistent during the three inoculation timings. This could be attributed to the presence of viruliferous mites continuously exposing plants to WSMV beyond the date of infestation. Therefore, this method is more representative of natural field conditions as opposed to mechanical inoculation. The mite infestation techniques, using leaf pieces with multiple mites potentially infesting each plant, were developed to provide a high probability of infection resulting in every plant.

The results from this study indicate that WSMV incidence was higher in the early-fall-inoculated Overland compared with late fall and early spring inoculations, in mite-inoculated compared with mechanically inoculated plants, and in Overland compared with Mace. In Oklahoma, Hunger et al. (1992) reported that samples from winter wheat plants mechanically inoculated in the fall consistently tested positive for WSMV, whereas test results for plants inoculated in the spring were inconsistent. However, in Montana, Miller et al. (2014) reported WSMV incidence of 5 to 7% and 45 to 57% in fall and spring, respectively, in mechanically inoculated winter wheat. As observed in this study, some of these differences could be attributed to unfavorable temperatures following inoculation, because fall temperatures in the southern and central Great Plains are warmer than those in the northern region (Miller et al. 2014). Despite Miller et al. (2014) reporting higher WSMV incidence in spring compared with fall inoculation, the infection rate was comparable with what was observed in this study in the spring mite inoculation, although much higher compared with the mechanical inoculation. Other studies have also reported WSMV incidences ranging between 67 and 100% in mechanically inoculated susceptible cultivars, and between 0 and 29% in resistant cultivars (Fahim et al. 2012; Lehnhoff et al. 2015). The higher inoculation rate in the spring in Montana was likely affected by inoculation at an earlier growth stage because of limited plant development in the fall. This phenomenon was noted by Hunger et al. (1992), where later fall planting dates showed greater infection rates and virus impact when infected in the spring.

Wheat plants infected with WSMV before tillering were highly likely to be severely damaged, while those inoculated after tillering were affected less and responded more erratically. In the central and southern Great Plains, exposure of wheat to WSMV during early fall when temperatures are warmer usually resulted in severe symptoms, whereas the effects of spring exposure are relatively mild and inconsistent (Hunger et al. 1992; Sill 1953). However, these effects likely result from the combined effect of favorable temperatures and plant growth stage at the time of inoculation, with younger plants showing greater impact.

Yield in the 2014–15 season was lower than that of the 2013–14 season by up to approximately 50%. This is attributed to contributing factors that were not present in the 2013–14 season, including bacterial streak and FHB epidemics, and lodging due to extreme plant growth. However, the impact of late fall and early spring mite inoculations was greater than expected in some instances, especially for Mace treatments that had low or no WSMV presence or symptoms. Some of this variance in yield likely resulted from considerable increases in mite populations causing extreme leaf curling and subsequent reduction in leaf area, thus reducing yield potential. This phenomenon has been observed in other studies with Mace where minimal virus symptoms develop, considerable mite buildup occurs on healthy plants, and considerable leaf curling occurs to affect the otherwise healthy plants (G. L. Hein, unpublished). Typically, on susceptible plants, by the time mite populations reach this level, the virus has progressed extensively and induced severe symptoms.

Yield losses due to early fall inoculation in Overland are comparable with the 70 to 84% average that Hunger et al. (1992) reported in seven susceptible cultivars mechanically inoculated in the early fall. Hunger et al. (1992) reported a nonsignificant yield reduction (3 to 23%) in spring inoculation, a trend that was similar to our mechanical inoculation of Overland (13%), but these incidences are much lower compared with the 55% yield loss observed in this study with mite inoculation. Ito et al. (2012) reported higher incidence of WSMV in barley (88%) with mite inoculation, while Lehnhoff et al. (2015) reported very poor infection (average 13%) when barley was mechanically inoculated with WSMV under field conditions. The average yield losses due to mite inoculation in this study are generally greater but those due to mechanical inoculation are within the 32 to 74% reported in other studies that used mechanical inoculation in susceptible cultivars (Byamukama et al. 2014; Fahim et al. 2012; Miller et al. 2014; Sharp et al. 2002).

Price et al. (2014) reported a decline in WSMV titer across cultivars when plants previously held at high temperatures were moved to low temperatures. A laboratory study using a green fluorescent protein (GFP)-tagged WSMV construct (WSMV-GFP) (Tatineni et al. 2011) to monitor the effect of temperature on virus replication and movement indicates that low temperatures inhibit replication and titer increase even in the susceptible cultivar Tomahawk. WSMV-GFP was observed only at the point of inoculation or as dispersed lesions when plants were held at 10 and 15°C and some of those plants tested negative for WSMV in ELISA (Wosula et al. 2017). The failure to detect WSMV or low incidence of it in field-inoculated plants using ELISA does not suggest that all plants that test negative are WSMV free because the virus could be present in low titers or in portions that were not sampled for testing given that only a small amount of tissue (0.15 g) is used in ELISA. Thus, in this study, infection rates for some treatments could be greater.

Knowledge regarding the seasonal dynamics of WSMV and its impact on the wheat crop is essential in assessing disease risk and determination of management practices that minimize disease severity and the associated yield loss. The findings from this study and others indicate that cooler fall temperatures could limit WCM populations and mite movement, and also reduce virus transmission and infection. Our findings indicate that WSMV incidence and impact on yield could be higher and more representative of what is likely to happen under natural field inoculations when mite inoculation is used as opposed to mechanical inoculation in field experiments. To our knowledge, this is the first study documenting the impact of timing (early fall, late fall, and early spring) of WSMV infection using both mite and mechanical methods on disease severity and yield in winter wheat.

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