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Occurrence of Wheat Curl Mite and Mite-Vectored Viruses of Wheat in Colorado and Insights Into the Wheat Virome

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Abstract

The wheat curl mite (WCM) is a vector of three important wheat viruses in the U.S. Great Plains: wheat streak mosaic virus (WSMV), triticum mosaic virus (TriMV), and High Plains wheat mosaic virus (HPWMoV). This study was conducted to determine the current profile of WCM and WCM-transmitted viruses of wheat and their occurrence in Colorado, including novel wheat viruses via virome analysis. There was a high rate of virus incidence in symptomatic wheat samples collected in 2019 (95%) and 2020 (77%). Single infection of WSMV was most common in both years, followed by coinfection with WSMV + TriMV and WSMV + HPWMoV. Both type 1 and type 2 mite genotypes were found in Colorado. There was high genetic diversity of WSMV and HPWMoV isolates, whereas TriMV isolates showed minimal sequence variation. Analysis of WSMV isolates revealed novel virus variants, including one isolate from a variety trial, where severe disease symptoms were observed on wheat varieties carrying *Wsm2*, a known virus resistance locus. Virome analysis identified two to four sequence variants of all eight RNA segments of HPWMoV, which suggests co-occurrence of multiple genotypes within host populations and presence of a variant of HPWMoV. A possible novel virus in the family Tombusviridae and several mycoviruses were identified. Overall, the data presented here highlight the need to define the effect of novel WCM-transmitted virus variants on disease severity and the role of novel viruses.

Keywords: High Plains wheat mosaic virus, triticum mosaic virus, virome, wheat curl mite, wheat streak mosaic virus

Wheat (*Triticum aestivum* L.) is considered the most important crop in the 21st century because it is used as a source of calories in the human diet worldwide (Arzani and Ashraf 2017; Curtis and Halford 2014). In the United States, wheat ranks third among field crops in planted acreage, production, and gross farm receipts, behind corn and soybeans (USDA-ERS 2020). The wheat curl mite (WCM), *Aceria tosichella* Keifer (Acari: Eriophyidae), is a globally important pest affecting wheat production in the Americas, Europe, Australia, and Asia (reviewed in Singh et al. 2018; Skoracka et al. 2018; Tatineni and Hein 2018). The mite causes direct damage by feeding, reducing cereal yield (Harvey et al. 2000). More importantly, WCM-transmitted viruses including the species *Wheat streak mosaic virus* (WSMV; genus *Tritimovirus*, family Potyviridae) (Slykhuis 1955), *Triticum mosaic virus* (TriMV; *Poacevirus*, Potyviridae) (Seifers et al. 2009), and *High Plains wheat mosaic emaravirus* (HPWMoV; *Emaravirus*, Fimoviridae) (Seifers et al. 1997) are among the most significant viruses in U.S. agriculture, responsible for yield losses in wheat, barley, oats, and rye (Burrows et al. 2009; Navia et al. 2013). Because of their common mode of transmission by the WCM and the difficulty in distinguishing symptoms, the disease caused by these viruses is commonly known as the wheat streak mosaic (WSM) complex. Average yield losses from the WCM-WSM complex range from 2 to 3% in the U.S. Great Plains, but 100% yield losses may occur in severely affected fields (Appel et al. 2015).

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Worldwide, WCM is a diverse species complex with numerous genetic lineages (Skoracka et al. 2018; Szydło et al. 2015). In North America, only two genetically distinct genotypes of WCM have been characterized based on partial sequences of the ribosomal internal transcribed spacer 1/2 (ITS1/2) and mitochondrial cytochrome oxidase I/II partial sequences. The type 1 genotype was initially identified from South Dakota, Kansas, Montana, Nebraska, and Texas, and type 2 was reported only from Nebraska (Hein et al. 2012). More recently, both genotypes have been found to occur in mixed populations in wheat-producing areas of the U.S. Great Plains (Khalaf et al. 2020). The two distinct WCM genotypes demonstrate different responses to curl mite colonization (*Cmc*) genes in wheat (Dhakal et al. 2017; Harvey et al. 1999) and differential virus transmission efficiencies (Hein et al. 2012; McMechan et al. 2014; Seifers et al. 2002; Wosula et al. 2016). The type 1 mites are avirulent to the *Cmc3* gene, whereas type 2 mites are virulent against *Cmc3* (Harvey et al. 1999). Additionally, type 2 mites transmit WSMV at a higher efficiency than type 1 mites (Wosula et al. 2016). More recently, additional WCM genotypes have been identified in the Great Plains, but their response to wheat curl mite genes and virus transmission efficiency is unknown (Khalaf et al. 2020).

The WSMV populations consist of >78 known isolates (Choi et al. 2001; Robinson and Murray 2013; Schubert et al. 2015; Stenger and French 2009). Based on whole-genome sequencing, these isolates can be separated into three clades, A, B, and D (Schubert et al. 2015). Within clade B isolates, there is sequence diversity between isolates infecting crop (wheat) and noncrop (other grass) hosts; therefore, a subtype of grass-associated isolates, B1, was proposed (Singh and Kundu 2017). There is also genetic diversity in HPWMoV populations across the United States, with two distinct groups of isolates (Stewart 2016). In contrast, there is limited sequence variability in field isolates of TriMV from the Great Plains (Fuentes-Bueno et al. 2011).

The management of WCM and the WSM disease complex has focused on an integrated pest management approach that combines controlling alternative hosts such as volunteer wheat, corn, and wild grassy weeds, delayed planting to avoid any overlap between maturing summer crops and newly emerging winter wheat seedlings, and use of mite- and virus-resistant varieties (reviewed in Nachappa et al. 2020; Singh et al. 2018; Skoracka et al. 2018; Tatineni and Hein

2018). Currently, four sources of host plant resistance to WCM (*Cmc1*, *Cmc2*, *Cmc3*, and *Cmc4*) inhibit the reproductive potential of mites, thereby reducing the spread of WSMV (Harvey et al. 1999). Four genes confer resistance to WSMV: *Wsm1*, *Wsm2*, *Wsm3*, and *c2652* (Friebe et al. 2009; Haber et al. 2006; Haley et al. 2002; Liu et al. 2011). The *Wsm1* and *Wsm3* genes also confer resistance to TriMV (Liu et al. 2011). However, some of these resistance alleles are temperature sensitive and do not prevent virus infection and replication at >24°C (Fahim et al. 2012). More recently, a novel quantitative trait locus was identified on wheat chromosome 6DS from the wheat variety TAM 112, which provides partial WCM resistance and moderate WSMV resistance (Dhakal et al. 2018). Genes for resistance to HPWMoV have not been identified.

There is limited information on the WCM–virus pathosystem in Colorado. The last field surveys of WCM-transmitted viruses in Colorado were conducted more than a decade ago (Burrows et al. 2009; Byamukama et al. 2013; Seifers et al. 2013). Since then, much has changed about the landscape, including commonly grown wheat varieties. Widespread use of mite and virus-resistant varieties can impose selection pressure on field populations of mites and viruses, with potential to overcome currently deployed genetic resistance in cultivated wheat varieties. For example, extensive deployment of the variety TAM 107 resulted in mite populations adapting to its inbred WCM resistance gene (Harvey et al. 1997). In 2019, several varieties known to carry WSMV resistance loci were found to have higher than expected WSM disease symptoms in an irrigated variety trial conducted by Colorado State University in Kit Carson County, Colorado. It was not immediately known whether the breakdown in resistance to WSMV was caused by a new WSMV variant, co-occurrence of multiple viruses, or other pathogens. The knowledge of mite and virus genotypes occurring in a given area is critical because these genetic differences can determine host responses at the phenotypic level (Hein et al. 2012). Next-generation sequencing (NGS) is a powerful tool that allows researchers to detect and characterize novel viruses and explore their genetic diversity in crops (Villamor et al. 2019) by revealing viromes that potentially contribute to disease. More recently, third-generation sequencing technology (Oxford Nanopore Technology, Oxford, UK) was used to identify novel WSMV variants in Kansas collected from wheat varieties carrying the *Wsm2* virus resistance gene (Fellers et al. 2019).

The current study was conducted to provide information about the status of WCM and WCM-transmitted virus genotypes and their occurrence in Colorado, including emerging or novel wheat viruses, via shotgun metagenomic sequencing. Furthermore, we analyzed the response of 24 wheat varieties to WSMV and TriMV in an irrigated variety trial, where higher-than-expected WSM disease symptoms were observed, to determine the possibility of new WMSV variants.

Materials and Methods

Sample collection. All leaf samples analyzed in the current study were collected from the field by researchers or submitted by extension specialists and wheat producers for diagnosis. A total of 75 symptomatic wheat samples were obtained from the major wheat-producing counties in eastern Colorado in 2019 and 2020. In 2020, 35 plant samples were collected from 12 counties, and in 2019, 40 samples were collected from 14 counties. In addition, one sample was collected from volunteer wheat in Larimer County in 2018 (Supplementary Table S1), totaling 76 samples. For most samples, a subset of fresh leaf tissue (between 0 and 3 days after collection) was stored at –80°C for virus detection, and the remaining tissue was stored at 4°C for examination of WCMs. Most samples included collection date, location, host species, variety, and symptoms (if noted) (Supplementary Table S1).

Wheat curl mite colony maintenance. Symptomatic wheat leaf tissues from a subset of the field samples described previously were examined under a dissecting microscope for the presence of WCMs. If mites were found, they were transferred to healthy plants of susceptible wheat varieties Pronghorn or Hatcher at the four-leaf stage. Plants were grown in half-gallon pots with two or three plants per pot in Promix HP growing medium. We transferred ≥10 mites either singly with a fine camel-hair brush or by placing a small section of the WCM-infested wheat leaf onto healthy plants. Mite colonies were maintained on wheat plants in 45 × 45 × 76 cm insect cages with No-thrips insect screen (BioQuip, Compton, CA) under a 16:8 h (light:dark) cycle at approximately 23°C under laboratory conditions. The colonies were maintained in separate rooms under similar conditions to avoid cross-contamination. All colonies were routinely tested for the presence of WSMV and TriMV. In addition, we routinely tested mite colonies to make sure they were of the designated genotype.

Virus detection and quantification. Total RNA was isolated from about 40 mg leaf tissue per sample with a Direct-zol RNA Purification Kit or Quick-RNA Miniprep Kit (Zymo Research, Irvine, CA), according to the manufacturer's recommendations. The Direct-zol RNA Purification Kit was used to extract RNA from the 2019 and NGS samples, whereas the Quick-RNA Miniprep kit was used to extract RNA from the 2020 samples. The RNA was quantified with a NanoDrop One spectrophotometer (Thermo Fisher Scientific, Waltham, MA) and stored at –80°C. To detect and quantify WSMV and TriMV, we used about 50 ng of RNA, as in a previously published quantitative real-time PCR duplex assay (Price et al. 2010) with a TaqMan RNA-to-Ct 1-Step kit (Applied Biosystems, Thermo Fisher Scientific) on a QuantStudio3 Real-Time PCR system (Applied Biosystems). Reaction conditions were set to incubate for reverse transcription at 48°C for 30 min, initial denaturation at 95°C for 10 min, and 40 cycles of denaturation at 95°C for 15 s and anneal/extension at 60°C for 1 min. Field samples with quantification cycle (C_Q) values below the lower detection limit, C_Q <34.89 for WSMV and C_Q <35.07 for TriMV, as defined by the standard curves described below, were considered to be positive for the specified virus. To quantify WSMV and TriMV RNA in samples from the wheat variety trial, we generated a standard curve by using a 319-bp amplicon of the nuclear inclusion B (NIb) (putative polymerase) of WSMV and a 677-bp amplicon of the coat protein (CP) of TriMV, both containing the respective reverse transcription quantitative PCR (RT-qPCR) target. Primers used to produce each amplicon are listed in Table 1. Tenfold serial dilutions of each target amplicon ranging from $5E^{-13}$ g to $5E^{-18}$ g of DNA were used to generate a standard curve relating C_Q values to the estimated copy number corresponding to each concentration of target DNA according to the method of Keough et al. (2016).

To detect HPWMoV, we synthesized complementary DNA (cDNA) from approximately 1 µg of total RNA by using the Verso cDNA Synthesis Kit (Thermo Fisher Scientific) or First Strand cDNA Synthesis Kit (Gold Biotechnology). A 290-bp sequence encoding the partial nucleoprotein (NP) of HPWMoV was amplified with specific primers (Table 1) from twofold diluted cDNA with GoTaq Flexi DNA polymerase (Promega, Madison, WI) with the following reaction conditions: initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 1 min, and final extension at 72°C for 5 min. Products were visualized on a 1% agarose gel with positive and negative controls to determine the presence of HPWMoV in each sample.

Mite and virus genotyping. WCMs collected from five locations (Larimer, Adams, Kit Carson, Phillips, and Sedgwick counties) in 2019 and one location in 2018 (Larimer County) (Supplementary Table S1) were subjected to genotyping with AtITS_Fs and AtITS_Rs primers targeting a 398-bp segment of the ribosomal ITS1 (Table 1). Because both genotypes can be found colonizing the same area and even the same plant, it is important to perform individual mite DNA extraction (Khalaf et al. 2020). DNA was extracted from single mites and groups of five mites, with three biological replicates for each location, with a MyTaqExtract-PCR Kit (Bioline Meridian Bioscience, London, UK) according to the manufacturer's recommendations.

To identify virus isolates in wheat leaves collected from field samples, we amplified partial sequences of 319 bp encoding the WSMV NIb protein (one county in 2018, 10 counties in 2019, 11 counties in 2020) with WSMV_NIb_Fq and WSMV_NIb_Rseq

primers, 677 bp encoding the CP of TriMV (one county in 2019) with TriMV_CP_Fseq and TriMV_CP_Rseq primers, and 290 bp encoding the NP of HPWMoV with HPWMoV_NP_F and HPWMoV_NP_R primers (one county in 2019, two counties in 2020) from wheat sample cDNA with GoTaq Flexi DNA polymerase (Promega, Madison, WI), as described previously, with the primers listed in Table 1. The resulting amplicons were purified with the DNA Clean & Concentrator kit (Zymo Research, Irvine, CA) and submitted for Sanger sequencing (GeneWiz, South Plainfield, NJ).

Wheat virome analysis. RNA extracts from leaf samples of symptomatic plants collected during field sampling in 2019 were subjected to shotgun metagenomic analysis. To focus on samples that would probably yield recovery of multiple complete viral genome sequences, we analyzed four wheat samples that tested positive for one or more WCM-transmitted viruses. These samples were from four locations, including Larimer and Phillips (northeastern), Kit Carson (central, variety trial location), and Bent (southern), which span the wheat-producing region of Colorado (Supplementary Table S1). Each sample was a composite of one to three plants obtained from a single wheat field at each location. Total RNA was extracted with a Direct-zol RNA Purification Kit (Zymo Research, Irvine, CA) and quantified with a Qubit 3.0 fluorometer (Thermo Fisher Scientific). Approximately 2 µg of RNA was submitted to the Colorado State University Next Generation Sequencing Facility, where library preparation, quality measurements, and sequencing were performed. Briefly, RNA quality was confirmed with an Agilent TapeStation instrument. Shotgun RNA libraries were constructed with a Kapa Biosystems RNA HyperPrep kit (Roche, Indianapolis, IN) according to the manufacturer's instructions. Pooled libraries from four composite RNA samples were sequenced on an Illumina NextSeq 500 instrument to produce single-end 150-nucleotide reads.

Bioinformatic analyses. Virus and virus-like sequences were identified as previously described (Cross et al. 2018). Analysis scripts are available at https://github.com/stenglein-lab/taxonomy_pipeline/. Low-quality and adapter sequences were removed with CutAdapt software (Martin 2011). Duplicate reads were collapsed with CD-HIT (Li and Godzik 2006). Host (wheat)-derived reads were removed by Bowtie 2 alignment (Langmead and Salzberg 2012) to the *T. aestivum* reference genome (assembly accession GCA_900519105.1) (Appels et al. 2018). The remaining nonhost reads were assembled into contigs with the Spades assembler (Bankevich et al. 2012). Contigs and nonassembled reads were taxonomically categorized first by nucleotide-level alignment to the National Center for Biotechnology Information (NCBI) nucleotide database with BLASTN and then by protein-level alignment to the NCBI protein database with the DIAMOND aligner (Altschul et al. 1990; Buchfink et al. 2015). The identity of the most closely related sequences in GenBank and the percentage identity of BLAST alignments were tabulated to produce a comprehensive metagenomic classification of all nonhost reads. Candidate virus sequences were validated by aligning reads to draft genome assemblies with Bowtie 2 and manually inspecting alignments in Geneious software (Geneious Prime version 2020.2.2). The average depth of coverage from alignments was recorded.

Phylogenetic analysis. Sequences from both amplicon sequencing and NGS were used to generate phylogenetic trees. The sequences from the current study were aligned with corresponding sequences in GenBank via ClustalW in MEGA X (Kumar et al. 2018). Phylogenetic analyses were based on the maximum likelihood method and Tamura–Nei model (Tamura and Nei 1993) with 1,000 iterations. The Tamura–Nei model measures substitution rates to estimate genetic distance, and current versions account for heterogeneous substitution patterns. Furthermore, we ran phylogenetic analyses with both the Tamura–Nei model and the Heseqawa–Kishino–Yano model (Hasegawa et al. 1985), resulting in an almost identical tree topology. The trees were drawn to scale, with branch lengths representing the number of substitutions per site. The tree with the highest log-likelihood is shown, and bootstrap values are shown next to the branches.

Wheat variety response to virus infection. A natural infection of WSMV was observed in the Colorado State University Irrigated Variety Performance Trial at Kit Carson County, Colorado, in 2019. The trial included 24 different host genotypes (released varieties and experimental lines), several of which contained mite and virus resistance genes. The presence of resistance genes (*Wsm2*, Cmc_{TAM112}) in the 24 lines was derived based on the whole genome association analysis (Dhakal et al. 2018) and inferring the resistant and susceptible haplotypes at the two loci (*Wsm2*, Cmc_{TAM112}) based on checks with known status for carrying the two respective genes. Information on the presence of *Wsm2* or Cmc_{TAM112} in each host genotype is listed in Supplementary Table S3. The experimental design was a randomized complete block design with three replications. Each replication or block had all 24 varieties and was seven rows wide, 10.7 m long, with interrow row spacing of 0.23 m. The trial was planted on 3 October 2018 at a seeding rate of approximately 2.97 million seeds/ha. Symptoms of WSMV infection were observed on 15 May 2019, shortly after the heading growth stage (Zadoks 50-60), and visual observations of symptom expression were recorded on 14 June 2019. Symptom expression was recorded on a modified scale according to the method of Dhakal et al. (2017), where 1 = negligible stunting and leaf mosaic symptoms and 9 = severe stunting and leaf mosaic symptoms.

The WSMV and TriMV RNA in each of the 24 varieties were quantified via RT-qPCR analysis. Samples were collected on 21 June 2019, about a month before harvest (17 July 2019). Ten flag leaf samples were collected from each variety from each of the three replicates; therefore, there were 30 leaf tissue samples representing each variety. Samples were collected randomly in a diagonal line extending between opposite corners of each plot. We pooled all 10 leaf samples from each plot (replicate) by stacking the leaves, and then a small section of tissue was cut from all 10 leaves around the midleaf area and subjected to RNA extraction and virus quantification as described previously. The difference in log copy number of viral RNA among wheat varieties was analyzed via two-way ANOVA

Table 1. Primers used for detection and for Sanger sequencing

Primer	Sequence (5′–3′)	Reference
Mite primers		
AtITS1_F	TTGATTACGTCCCTGCCCTTT	Cherry et al. (1997)
AtITS1_R	ACGAGCCGAGTGATCCACCG	Cherry et al. (1997)
Virus primers		
WSMV_ NIb_Fq	CAAAGCTGTGGTTGATGAGTTCA	Price et al. (2010)
WSMV_ NIb_Rq	TTGATTCCGACAGTCCATG	Price et al. (2010)
TriMV_CP_Fq	CATGCACATTGGAGCAATTTG	Price et al. (2010)
TriMV_CP_Rq	GCATGCTCAATCCAAGTCCAT	Price et al. (2010)
HPWMoV_CP_F	TGCTATGTCATTGTTCAGGTGGTC	Stewart et al. (2013)
HPWMoV_CP_R	TTAGGCAGTCCTTGATTGTGCTG	Stewart et al. (2013)
WSMV_NIb_Rseq	TCGAAACTTCTGCACAATCG	This study
TriMV_CP_Fseq	CAAGTGGGTTTCTTATGCTC	This study
TriMV_CP_Rseq	TAGGCTAAAGCTCCAAAGTG	This study

(PROC GLM) with variety and replicate as fixed effects. Treatment comparisons were performed via Tukey's honestly significant difference with family error rate ($P < 0.05$). Spearman's correlation analysis was performed to determine the correlation between WSMV levels and visual rating among varieties.

Results

Occurrence of WCM-transmitted viruses in Colorado. To determine the occurrence of WCM-transmitted viruses in Colorado, we screened symptomatic wheat samples collected during surveys or submitted by extension specialists and growers from across the state of Colorado in 2019 and 2020 by RT-qPCR. All three WCM-transmitted viruses were detected in Colorado in both years (Fig. 1). Of the 40 symptomatic samples collected in 2019 from 14 counties across eastern Colorado, 38 (95%) were positive for one or more WCM-transmitted viruses (Supplementary Table S1). Of the 35 samples collected in 2020 from 12 counties across eastern Colorado, 27 (77%) were positive for at least one WCM-transmitted viruses (Fig. 1; Supplementary Table S1). Infection by WSMV alone was most common in both years, occurring in nine out of 14 counties in 2019 and seven out of 12 counties sampled in 2020 (Fig. 1A and B). Mixed infection with WSMV and TriMV was detected in samples from Bent (1/1; 100%), Kiowa (3/4; 75%), Kit Carson (2/5; 40%), and Weld (1/2; 50%) counties in 2019 and three samples from Prowers (2/4; 50%) and Kiowa (1/3; 33%) counties in 2020 (Fig. 1A and B). Mixed infection with WSMV and HPWMoV was also observed in three samples from Phillips County (3/5; 60%) in 2019 and a sample from Washington (1/2; 50%) and Kit Carson (1/3; 33%) counties in 2020 (Fig. 1A and B). Only one sample from Prowers county had an infection by TriMV alone, but other samples from that same location were coinfected with WSMV. No single infection of HPWMoV or coinfection of TriMV and HPWMoV was detected.

Mite genotypes. To investigate the presence of the two designated mite genotypes (type 1 and 2) in Colorado, we analyzed the ribosomal ITS1 sequence from six WCM populations collected in 2018 and 2019 from Colorado, including Larimer, Kit Carson, Adams, Phillips, and Sedgwick counties (MT465683 to MT465687) and representative sequences from different states in the United States and around the world. Overall, there was limited variability among the ITS1 nucleotide sequences. Still, there is a clear distinction between type 1 and type 2 mites, based on comparative phylogenetic analysis that included reference sequences of both genotype groups, EU734729 (type 1) and EU734726 (type 2) (Fig. 2). The WCM populations collected from two counties, Larimer in 2018 and Sedgwick in 2019, shared 99 to 100% nucleotide identity with each other and with corresponding ITS1 sequences of previously described type 1 mites from Texas, Nebraska, Kansas, Montana, and South Dakota. In contrast, mites collected from four counties in 2019, Larimer, Kit Carson, Adams, and Phillips, had ITS1 sequences with 100% nucleotide identity to that of type 2 mites from Nebraska and 97 to 98% nucleotide identity to that of type 1 mites (Fig. 2). Mites collected from volunteer wheat in Larimer County in fall 2018 (MT465682) belong to type 1, and mites collected from the same field in the spring of 2019 (MT465684) belong to type 2.

Virus isolates. To determine the genetic diversity in WSMV isolates collected from wheat field samples, we performed phylogenetic analysis of a portion of the WSMV NIb nucleotide sequence for 22 isolates collected in the 2018, 2019, and 2020 growing seasons (MT465688 to MT465692 and MW081551 to MW081566) from Colorado along with corresponding sequences of isolates of the virus from other states and countries. The NIb protein is conserved among potyviruses and was previously used to define the lineage of WSMV because of its congruence with results obtained with the entire polyprotein sequences (Stenger et al. 1998). None of the 22 isolates from Colorado were sequence haplotypes based on the partial NIb nucleotide segment, but they grouped within clade D along with many other isolates from the Americas (Fig. 3). Two of the isolates from Colorado, MT465688 and MW081555, shared 100% nucleotide identity with the isolates KSHm2014 (MK318278) and KSWal2017 (MK318281), respectively, from Kansas that were collected from wheat varieties carrying the *Wsm2* virus resistance gene (Fellers et al. 2019). Fourteen

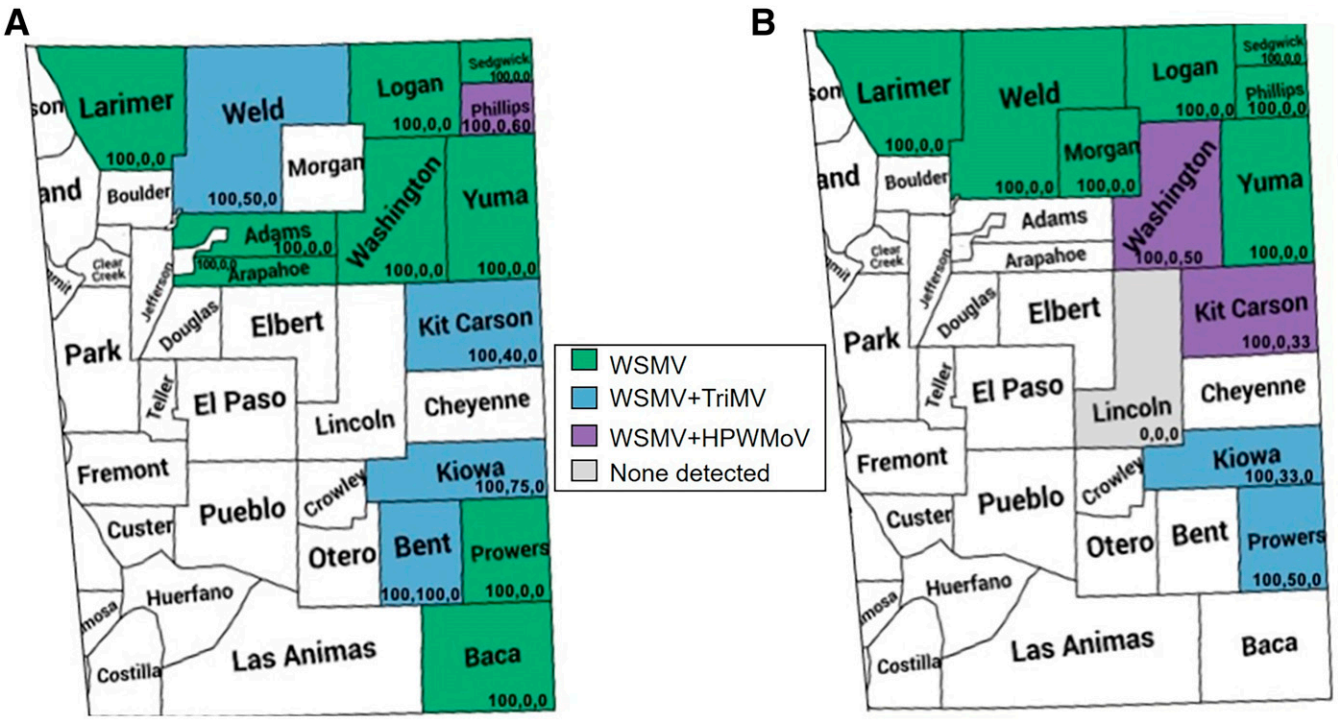

Fig. 1. Maps of occurrence of wheat curl mite-transmitted viruses in eastern Colorado **A,** in 2019 and **B,** in 2020, as determined by quantitative reverse transcription PCR. The 2019 map includes 40 total samples, and the 2020 map includes 35 samples. The three numbers in the lower right corner of each county indicate percentage incidence of positive samples per county for wheat streak mosaic virus (WSMV), triticum mosaic virus (TriMV), and High Plains wheat mosaic virus (HPWMoV), respectively. The white areas indicate counties that were not sampled.

isolates were collected from wheat varieties carrying *Wsm2* or Cmc_{Tam112}. In contrast, there was a high (98 to 99%) nucleotide partial CP sequence identity between the TriMV isolate from Kit Carson County (MT563401), where the variety trial was located, and the other 21 TriMV sequences available in GenBank. Phylogenetic analysis of the HPWMoV is discussed in the virome section because this analysis contains sequences derived from Sanger sequencing and NGS data (Fig. 4).

Wheat virome analysis. Using the Illumina NextSeq 500 platform, we explored the viromes of wheat fields from Kit Carson, Larimer, Bent, and Phillips counties by using some of the field samples collected in 2019 (Supplementary Table S1). The Kit Carson sample was obtained from the variety trial described below. Datasets contained an average of 9.4×10^6 reads. After we removed low-quality and adapter sequences, an average of 8.3×10^6 sequences remained per library (93%). Duplicate reads were collapsed, leaving an average of 1.8×10^6 unique reads per dataset (20%). After all filtering operations, an average of 0.25×10^6 reads (3%) remained per dataset (Supplementary Table S2). WSMV was detected in all four locations, although the Bent County sample had low WSMV reads, and coverage was not sufficient for coding sequences to be assembled. TriMV was detected in two out of four locations. HPWMoV was detected only in the Phillips County sample (Table 2).

We assembled apparently complete genomes of several of the viruses infecting wheat field samples by using the virome data and based on comparisons with published genomes of each virus. We obtained complete coding sequences for the polyprotein of WSMV from two of the four samples from Kit Carson (MT762110) and Phillips counties (MT762109). Six partial sequence contigs that span the WSMV polyprotein were detected in the Larimer County sample (MT822723 to MT822728), but they contained sequencing gaps and could not be assembled into a contiguous sequence. Alignment of the Colorado WSMV polyprotein sequences with six recently published potentially resistance-breaking isolates from Kansas (Fellers et al. 2019), isolate KSMHK (MK318280), strain type AF285169, and Sidney 81 (AF057533), revealed a common amino acid change at position 2235, where Sidney 81 contains threonine and other isolates have valine or methionine. Furthermore, analysis of partial coat protein sequences of the only historical isolate from Colorado

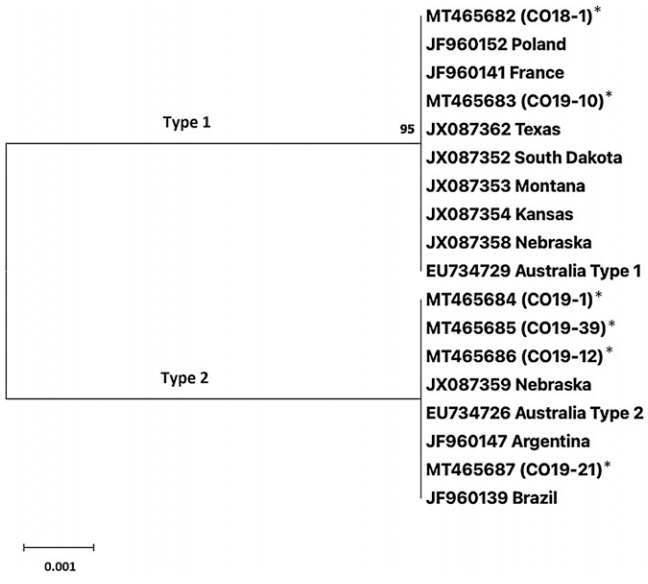

Fig. 2. Phylogenetic tree derived from representative sequences of six wheat curl mite populations collected from Colorado and different states in the United States and around the world using the ribosomal internal transcribed spacer region. Scale bar indicates number of substitutions per site. Phylogenetic analysis by maximum likelihood method and Tamura–Nei model (Tamura and Nei 1993) was based on sequence alignment with ClustalW in MEGAX (Kumar et al. 2018). Asterisks indicate sequences from Colorado. Bootstrap values are indicated on the nodes.

available in GenBank (U54572) with that of the 12 sequences described previously revealed four amino acid changes unique to U54572 in this gene segment. Further studies are needed to verify the true nature of these observed amino acid changes.

We obtained two TriMV sequences: one apparently complete genome from Kit Carson County wheat (MT762125) and one partial sequence from Bent County wheat. Both sequences shared high (99%) nucleotide identity with each other and the other available corresponding TriMV sequences. The TriMV sequences of the CP and RNA silencing suppressor of two samples previously collected from Colorado (Seifers et al. 2013) and the corresponding TriMV gene sequences obtained here shared 98 to 99% nucleotide identity with each other, suggesting that little change has occurred within the genomes of TriMV isolates in Colorado over time.

The metagenomic assembly of HPWMoV reads from the single Phillips County wheat sample produced 19 contigs that generated significant hits to several RNA segment-specific HPWMoV sequences in GenBank (Table 3). Thirteen of these were apparently complete coding sequences and six were partial. Two distinct sequence variants were detected for each of the HPWMoV genomic RNA segments 1, 2, 4, 6, 7, and 8; three variants for RNA3; and four variants for RNA5. These different variants of the HPWMoV segments shared about 50 to 80% pairwise nucleotide identity, indicating that they represented distinct genotypes. Some of the assembled segments were similar to existing HPWMoV sequences in the GenBank database. For instance, one of the four RNA5 segments (MT762115) shared 99.5% pairwise nucleotide identity with an RNA5 segment from Nebraska (KJ939628). Other segments were only distantly related to previously described sequences (Table 3). The identification of two to four variants of each of the eight RNA segments known to make up the HPWMoV genome suggests co-occurrence of at least two genotypes within a single sample (Table 3).

Phylogenetic analysis of sequences encoding the partial HPWMoV-NP (RNA3) from wheat samples collected in three counties (Kit Carson and Washington [Sanger sequencing] and Phillips [NGS]) and other available sequences originating in the United States confirmed two distinct groups of isolates as previously reported (Fig. 4A) (Stewart 2016). One of the RNA3 segments from Colorado isolate MT563400, collected in 2019 from Phillips County, revealed high (98%) nucleotide identity to isolates in the group from Ohio and Texas (group I). Two additional isolates collected from Kit Carson (MW081567) and Washington (MW081568) counties in 2020 had high (99%) nucleotide identity to group II isolates (Fig. 4). The two RNA3 nucleotide sequences obtained through NGS (MT762122 and MT762121) had 74 to 75% nucleotide identity with other sequences and did not cluster with group I or group II isolates of HPWMoV (Fig. 4A). Phylogenetic analysis of the three apparently complete RNA5 nucleotide sequences obtained from NGS data and other available sequences (Fig. 4B) confirmed the presence of novel variant segments of HPWMoV in Colorado, as suggested by analysis of the partial RNA3 nucleotide sequences shown in Figure 4A. The complete coding sequences have been deposited in GenBank under accession numbers MT762111 to MT762124.

The partial sequence (3,021 nt) of a possible novel virus, unclassified Tombusviridae sp. (MW346663), was identified from a wheat sample collected in Phillips County in 2019. In BLASTx analysis, it showed 45% amino acid identity to the RNA-dependent RNA polymerase (RdRP) of Ixeridium yellow mottle virus 2 (YP_009352229) at 52% query coverage (Table 2), 48% identity (45% query coverage) with Carrot mottle virus, and 47% identity (45% query coverage) with Carrot mottle mimic virus. The conserved RdRP domain cd01699 was identified in the 3,021-bp sequence of this possible novel virus. The contribution of these sequences to a functional virus and the biological significance of this putative novel virus, if any, are unknown.

The NGS reads of the wheat sample from Phillips County also contained sequences related to several mycoviruses (Table 2), including Plasmopara viticola lesion associated mononega virus 1 (MT822729; 38% RdRP amino acid identity, 59% query coverage), Plasmopara viticola lesion associated mitovirus 7 (MT822730;

96% nucleotide identity, 90% query coverage), Fusarium poae negative-stranded virus 2 (MT822731; 70% amino acid identity, 97% query coverage), and Coniothyrium diplodiella negative-stranded RNA virus 1 (MT822732; 41% amino acid identity, 78% query coverage). These viruses may represent mycovirus infections of wheat-associated fungi present in the sampled wheat tissue. Furthermore, conserved domains of Mononegavirales RdRP polymerase (pfam00946) and Mononegavirales mRNA-capping region V (pfam14318) were detected in these sequences (data not shown). The raw sequence data have been deposited in the NCBI Sequence Read Archive repository under submission number SUB7870854.

Response of wheat lines to virus infection. We observed unexpectedly high WSM symptoms in the Colorado State University Irrigated Variety Performance Trial at Kit Carson County in 2019. The wheat varieties included some with no specific resistance genes, some with a single resistance gene (either Cmc_{Tam112} for WCM resistance or *Wsm2* for WSMV resistance), and one variety, Guardian (PI 695151), with both resistance genes (Cmc_{Tam112} and *Wsm2*) (Supplementary Table S2). There was significant effect of replicate (F = 4.69, df = 2, P = 0.01) and variety (F = 2.90, df = 23, P = 0.0007) on WSMV RNA copy number (Fig. 5). The variety, Guardian, had the lowest amount of WSMV RNA, albeit not statistically different from Snowmass 2.0, harboring a single resistance gene, and Thunder CL, which does not contain any known resistance genes (Fig. 5). Although TriMV was detected in these samples, there were no significant differences between replicate plots (F = 1.76, df = 2, P = 0.10) and varieties (F = 1.65, df = 23, P = 0.06). We did not detect HPWMoV in any of the varieties. There was a significant difference in visual symptom rating between varieties (F = 16.74, df = 23, P < 0.0001) but not between replicate plots (F = 0.35, df = 2, P = 0.70). There was no correlation between visual symptom ratings and WSMV RNA copy number across varieties (Spearman rho = 0.01, P = 0.928, n = 72; i.e., 24 varieties × 3 replicates).

Discussion

WCM-vectored viruses continue to cause significant yield losses to wheat production in the Great Plains (Appel et al. 2015; Hollandbeck et al. 2019). The current study found a high level of virus incidence in symptomatic wheat samples collected in 2019 (95%) and 2020 (77%) as determined via RT-qPCR analysis. In both years, infection by WSMV alone was most common, followed by coinfection of WSMV + TriMV and WSMV + HPWMoV. These results are similar to previous findings on WCM-transmitted virus occurrence in Colorado (Burrows et al. 2009; Byamukama et al. 2013). Coinfections of WSMV with TriMV and WSMV with HPWMoV occurred less frequently. This knowledge is beneficial to growers because WSMV and TriMV act synergistically in wheat, resulting in more severe symptoms and yield losses compared with single infections (Byamukama et al. 2012; Tatineni et al. 2010). Furthermore, this information can inform breeding activities because *Wsm2* confers resistance to WSMV alone but not to the other two viruses.

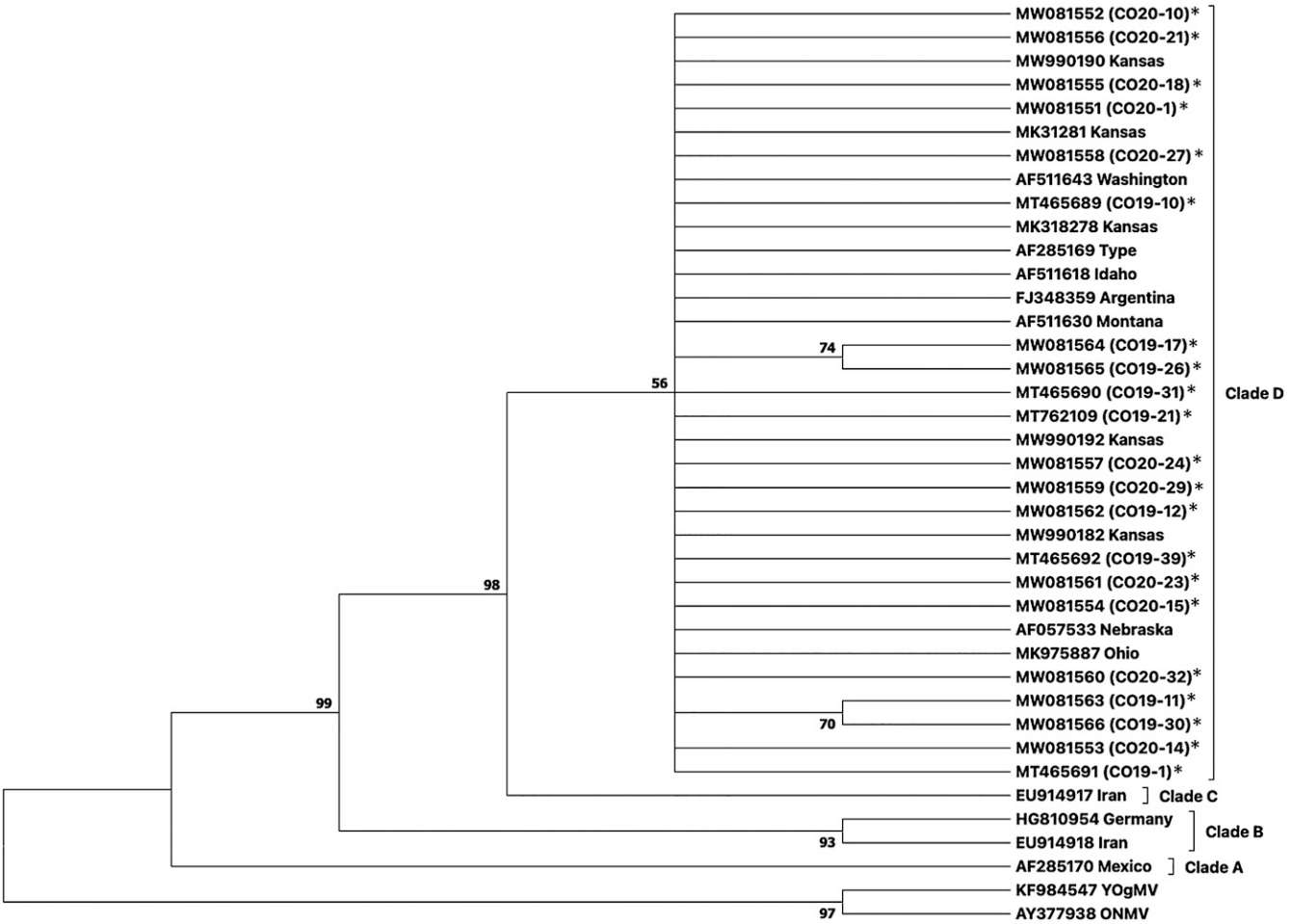

Fig. 3. Phylogenetic tree of wheat streak mosaic virus (WSMV) isolates collected in the 2019 and 2020 field seasons in Colorado and other isolates from around the world with a partial WSMV-NIb region. Oat necrotic mottle virus (ONMV) was used as an outgroup. Asterisks indicate isolates from Colorado. Host sample information for each Colorado isolate is indicated by the sample ID in parentheses after the accession numbers. The NIb sequence from one Colorado isolate (MT762109) was extracted from next-generation sequencing data. All other sequences of Colorado isolates were derived from amplicon sequencing. Phylogenetic analysis by maximum likelihood method and Tamura–Nei model (Tamura and Nei 1993) was based on a sequence alignment with ClustalW in MEGAX (Kumar et al. 2018). Bootstrap values are indicated on the nodes. Branches with bootstrap values <50% were collapsed.

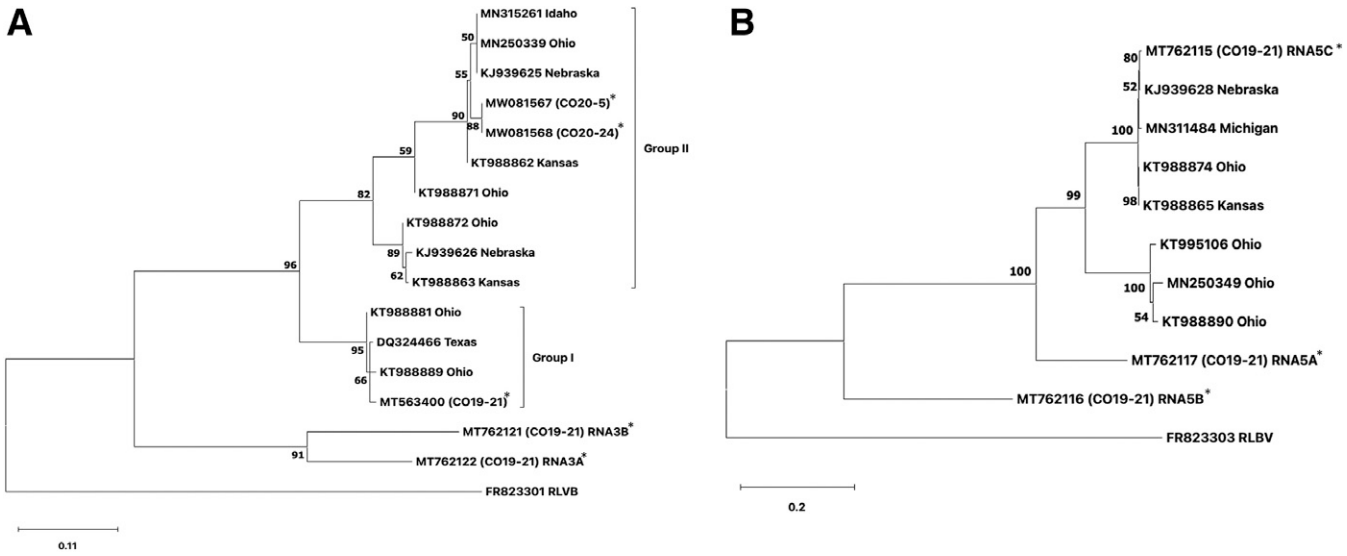

Fig. 4. Phylogenetic tree of High Plains wheat mosaic virus (HPWMoV) isolates collected during the 2019 and 2020 field seasons in Colorado and other isolates from around the United States from **A,** the partial RNA3 segment and **B,** the complete RNA5 segment. The two RNA3 sequences, MT762121 (CO19-21) RNA3B and MT762122 (CO19-21) RNA3A, were extracted from next-generation sequencing (NGS) data. The other three sequences from Colorado isolates were derived from amplicon sequencing. The complete RNA5 segments were extracted from NGS data collected from sample CO19-21. Corresponding segments of Raspberry leaf blotch virus (RLBV) were used as an outgroup. Asterisks indicate isolates from Colorado. Host sample information for each Colorado isolate is indicated by the sample ID in parentheses after accession numbers. Scale bar indicates number of substitutions per site. Phylogenetic analysis by maximum likelihood method and Tamura–Nei model (Tamura and Nei 1993) was based on a sequence alignment with ClustalW in MEGAX (Kumar et al. 2018). Bootstrap values are indicated on the nodes.

Table 2. Summary of wheat viromes from Colorado

Location	Taxon_ID	Nearest GenBank sequence	% Query coverage	% Identity	Average coverage[a]	Region	Accession number	Length (bp)
Phillips; sample ID 19-21	Wheat streak mosaic virus	Wheat streak mosaic virus isolate KSIct2017	100	97 (nt)[b]	408	Complete polyprotein	MT762109	9,371
	Wheat mosaic virus/High Plains wheat mosaic emaravirus[c]	Wheat mosaic virus isolate K1 segment RNA3, complete sequence KT988889.1	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]
	Fusarium poae negative-stranded virus 2-like	*Fusarium poae* negative-stranded virus 2 YP 009272912.1	97	70 (aa)[d]	43	Complete polyprotein	MT822731	6,522
	Coniothyrium diplodiella negative-stranded RNA virus 1	Coniothyrium diplodiella negative-stranded RNA virus 1 QFQ60954.1	78	41 (aa)	43	Complete polyprotein	MT822732	1,321
	Unknown *Tombusviridae* sp.	Ixeridium yellow mottle virus 2 YP 009352229.1	52	45 (aa)	26	Partial genome	MW346663	3,021
	Plasmopara viticola lesion associated mitovirus 7	Plasmopara viticola associated mitovirus 7 isolate DMG-D_DN27174 MN539769.1	90	96 (nt)	8	Complete polyprotein	MT822730	2,377
	Plasmopara viticola lesion associated mononega virus 1-like	Plasmopara viticola associated mononega virus 1 isolate DMG-B_DN53692 MN556996.1	59	38 (aa)	25	Partial polyprotein	MT822729	8,656
Kit Carson; sample ID 19-39	Wheat streak mosaic virus	Wheat streak mosaic virus isolate KSIct2017 MK318279.1	100	99 (nt)	320	Complete polyprotein	MT762110	9,368
	Triticum mosaic virus	Triticum mosaic virus isolate U06-123 FJ263671.1	99	99 (nt)	99	Complete polyprotein	MT762125	10,238
Bent; sample ID 19-19	Triticum mosaic virus	Triticum mosaic virus isolate U06-123 FJ263671.1	75	99 (nt)	3	Partial polyprotein	N/A[e]	N/A[e]
Larimer; sample ID 19-1	Wheat streak mosaic virus	Wheat streak mosaic virus isolate H95S AF511614.2	100	98–99 (nt)	2	Partial polyprotein	MT822723–MT822728	Multiple

[a] Average mapped read coverage across contig as reported in Geneious.
[b] nt, percentage nucleotide identity calculated for genome length sequences.
[c] Obtained all eight segments but at least two different variants of all segments. See Table 3.
[d] aa Percentage amino acid identity calculated for coding sequences.
[e] N/A, partial sequences that were not submitted to GenBank or could not be annotated.

The WCM is a cryptic species complex (i.e., morphologically similar but genetically different individuals), as determined by analysis of mitochondrial and nuclear DNA markers (Szydło et al. 2015). The WCM genotypes, type 1 and 2, are most widespread, having been found in the Middle East, Europe, Australia, and Americas (Skoracka et al. 2014). Hein et al. (2012) identified both genotypes from five populations collected across the Great Plains: type 1 (collected from Kansas, Texas, Montana, and South Dakota) and type 2 (collected from Nebraska). In the current study, we found both type 1 and type 2 genotypes in Colorado; moreover, mites from Larimer County, collected from the same area but during different times of the growing season, belonged to both genotypes. This finding suggests that mixed populations can occur within fields similar to that observed for other wheat-producing regions of the U.S. Great Plains (Siriwetwiwat 2006). We also found WCMs on volunteer wheat coinfected with WSMV and TriMV in the fall of 2018, and at the same location in the spring of 2019, WSMV-positive symptomatic wheat was present shortly after wheat emergence (Supplementary Table S1). This finding highlights the importance of management practices to eliminate volunteer wheat and other summer crops that could serve as green bridges to harbor populations of the WCM before the new wheat crop emerged. A recent study identified additional genotypes in the Great Plains region that appear divergent from the type 1 and 2 sequences; however, their responses to resistance genes and their relative virus transmission efficiencies are unknown (Khalaf et al. 2020). One factor contributing to the increase in WCM diversity may be that selection pressure is being imposed on their populations because of the widespread use of mite-resistant varieties. For example, Harvey et al. (1997) reported that WCM developed resistance to the *Cmc3* gene because of widespread deployment of the variety TAM107 carrying the gene. Therefore, the characterization of WCM diversity is critical to monitor the emergence of virulent mite populations and to elucidate their ability to overcome host resistance.

There is high genetic diversity among WSMV isolates from the United States and around the world, as determined by comparison of CP sequences (Robinson and Murray 2013), and more recently, whole-genome sequences (Schubert et al. 2015). Our analyses based on the WSMV NIb region and NGS data (virome analysis) revealed differences between isolates from Colorado that were collected from wheat fields in 2019 and 2020. A comparative analysis of the WSMV polyprotein sequences from Larimer, Phillips, and Kit Carson counties (this study) and the corresponding sequences of global WSMV isolates suggests similarities between the isolates from Colorado and the potentially resistance-breaking isolates from Kansas (Fellers et al. 2019). It is likely that WCMs are moving between the states via wind currents (Sabelis and Bruin 1996) carrying divergent virus variants. Alternatively, it is possible that these WSMV isolates are evolving separately but with the same mutations. A *Wsm2*-breaking WSMV variant has also been isolated from green foxtail (*Setaria viridis*) (Kumssa et al. 2019). Further analysis to determine whether viral sequence changes are responsible for resistance breakdown is needed. A priority for future research will be to characterize the *Wsm2* gene. To date, no WSMV resistance gene has been cloned, although characterization of the *Wsm1* and *Wsm2* genes revealed that they both prevent the long-distance transport of

Table 3. Summary of High Plains wheat mosaic virus RNA segments obtained from Phillips County sample ID 19-21, GenBank accessions and average coverage

Segment	Nearest GenBank sequence	% Query coverage	% Identity	Average coverage[a]	Coding sequence	Accession number	Length (bp)
RNA1A[b]	Wheat mosaic virus RdRP AML03165.1	96	75 (aa)[c]	11	Complete	MT762124	7,036
RNA1B	High Plains wheat mosaic emaravirus isolate HPVWMoV_NW2 P1 RNA-dependent RNA polymerase gene, complete cds MN250345.1	46	98 (nt)[d]	1	Partial	N/A[e]	N/A
RNA2A[b]	Wheat mosaic virus glycoprotein precursor AML03208	87	69 (aa)	14	Complete	MT762123	2,200
RNA2B	Wheat mosaic virus isolate W1 segment RNA2, complete sequence KT970500.1	97	97 (nt)	4	Partial	N/A	N/A
RNA3A[b]	Wheat mosaic virus nucleoprotein AML03167.1	47	60 (aa)	42	Complete	MT762122	1,838
RNA3B[b]	Wheat mosaic virus nucleoprotein AML03193.1	46	62 (aa)	24	Complete	MT762121	1,840
RNA3C	Wheat mosaic virus isolate K1 segment RNA3, complete sequence KT988889.1	100	98 (nt)	9	Complete	MT762120	1,478
RNA4A	Wheat mosaic virus isolate KS7 segment RNA4 KT988864.1	58	80 (nt)	23	Complete	MT762119	1,676
RNA4B	Wheat mosaic virus isolate W1 segment RNA4, complete sequence KT970502.1	100	98 (nt)	3	Partial	MT762118	1,113
RNA5A[b]	High Plains wheat mosaic virus isolate Nebraska segment RNA 5 KJ939628.1	88	76 (nt)	22	Complete	MT762117	1,662
RNA5B[b]	Wheat mosaic virus segment RNA5 hypothetical protein gene KT995106.1	56	63 (nt)	9	Complete	MT762116	1,610
RNA5C	High Plains wheat mosaic virus isolate Nebraska segment RNA 5 KJ939628.1	100	99.5 (nt)	5	Complete	MT762115	1,410
RNA5D	Wheat mosaic virus segment RNA5 hypothetical protein gene, complete cds KT995106.1	55	92 (nt)	1	Partial	N/A	N/A
RNA6A	Wheat mosaic virus isolate W1 segment RNA6 KT970503.1	69	69 (nt)	56	Complete	MT762114	1,748
RNA6B	High Plains wheat mosaic emaravirus isolate HPVWMoV_NW2 P6 hypothetical protein MN250350.1	100	98 (nt)	5	Complete	MT762113	1,619
RNA7A	High Plains wheat mosaic emaravirus isolate HPWMoV_MI MN311485.1	34	73 (nt)	10	Complete	MT76112	1,624
RNA7B	High Plains wheat mosaic emaravirus isolate HPVWMoV_NW2 P7 RNA silencing suppressor gene, complete cds MN250351.1	100	94 (nt)	6	Partial	N/A	N/A
RNA8A	High Plains wheat mosaic emaravirus isolate HPVWMoV_NW2 P8 N250352.1	16	72 (nt)	25	Complete	MT762111	1,335
RNA8B	Wheat mosaic virus isolate H1 segment RNA8, complete sequence KT988886.1	96	92 (nt)	4	Partial	N/A	N/A

[a] Average mapped read coverage across contig as reported in Geneious.
[b] Variant RNA segments.
[c] aa, percentage amino acid identity calculated for coding sequences.
[d] nt, percentage nucleotide identity calculated for genome length sequences.
[e] N/A, partial sequences that were not submitted to GenBank or could not be annotated.

WSMV within the infected wheat plant (Tatineni et al. 2016), providing clues to the identity of the candidate gene. A better understanding of the host–virus protein interactions could reveal insights into the underlying mechanism causing breakdown of resistance and help direct breeding efforts in the long term. The increasing acreage of *Wsm2*-containing varieties may further accelerate selection of WSMV isolates that can overcome *Wsm2*-mediated host resistance. We found that wheat varieties carrying the WSMV resistance gene, *Wsm2*, had similar WSMV copy numbers compared with varieties that did not contain any resistance gene. The WSMV variant from the variety trial was most similar to an isolate reported from *Wsm2*-containing lines in Kansas (MK318276) (Fellers et al. 2019). However, we could not establish a correlation between virus isolate and host plant resistance because the samples were coinfected with TriMV, which may have exacerbated symptom severity in the resistant varieties. Future research should be directed toward screening varieties with these novel WSMV variants to determine the potential for resistance breakdown.

Based on phylogenetic analysis, three HPWMoV RNA3 segments obtained from wheat samples clustered with the known HPWMoV grouping (Stewart 2016). Still other RNA3 segments identified in the sample collected from Phillips County appear to be divergent and did not group with known sequences. Species in this genus are demarcated based on the amino acid sequence of relevant gene products of RNA1 (RdRP), RNA2 (glycoprotein, GP), and RNA3 (nucleocapsid protein, NP), differing by >25% according to the International Committee on Taxonomy of Viruses criteria. The *Emaravirus* variant RNA segments have 75% amino acid identity with the nearest RdRp of HPWMoV, 73% amino acid identity with the nearest GP of HPWMoV, and 62 and 61% amino acid identity with the nearest HPWMoV NP (Table 3). Further characterization of these sequences is challenging because this wheat sample had mixed infection of at least two *Emaravirus* isolates. In contrast, there was limited sequence variability in isolates of TriMV in Colorado and from the Great Plains, indicating that the populations are largely homogeneous (Fuentes-Bueno et al. 2011). Overall, these data indicate diversity among WSMV and HPWMoV isolated in Colorado, which may make breeding for durable resistance difficult if there are differential host responses of resistance genes to different isolates.

To date, a handful of studies have detected viruses in wheat via NGS (Redila et al. 2021a, b; Singh et al. 2020; Zhang et al. 2017). In the current study, we identified 10 virus-like sequences in the wheat virome, including the three WCM-transmitted viruses, WSMV, TriMV and HPWMoV. In addition, sequences encoding a putative RdRP of a possible novel virus, unclassified Tombusviridae sp., were identified. In the current study, consensus sequences were well supported by individual mapped reads. Subconsensus variation (variants present at ≤50% frequency) was detectable in most datasets. This diversity could be derived from infection in one or more hosts from one or more closely related viruses. Indeed, the sequenced RNA samples were derived from one to three plants per location, so the virus-specific sequences captured from a specific RNA sample might be from one or more plants. Further work is needed to confirm the presence of these viruses and obtain their complete genomes to facilitate taxonomic assignments of the novel or low-identity viruses. Lastly, the sequences of several mycoviruses or fungus-infecting viruses were identified in the Phillips County sample, including those related to Plasmopara viticola lesion associated mononega virus 1, Plasmopara viticola lesion associated mitovirus 7, Fusarium poae negative-stranded virus 2-like, and Coniothyrium diplodiella negative-stranded RNA virus 1. Whereas the fungi *Plasmopara viticola* and *Coniothyrium diplodiella* are both disease causal agents of grapes, *Fusarium poae* is pathogenic on wheat, being the causal agent of Fusarium head blight (Schmale and Bergstrom 2010). F. poae negative-stranded virus 2 has been isolated from *F. poae* strain SX63 (Wang et al. 2016). Interestingly, *Fusarium* sp. was the most abundant nonhost taxon identified in the metagenome dataset, suggesting mycovirus sequences may have derived from infection of fungi associated with this wheat sample. The sample was collected from a field with continuous wheat, which can increase the likelihood of infection by fungal pathogens such as *Fusarium* sp. However, the sample was collected early in the season, which may be one reason why we did not observe symptoms caused by *Fusarium* sp. Future research may be aimed at elucidating the diversity and dynamics of WCM-transmitted wheat viruses and mycovirus–host interactions.

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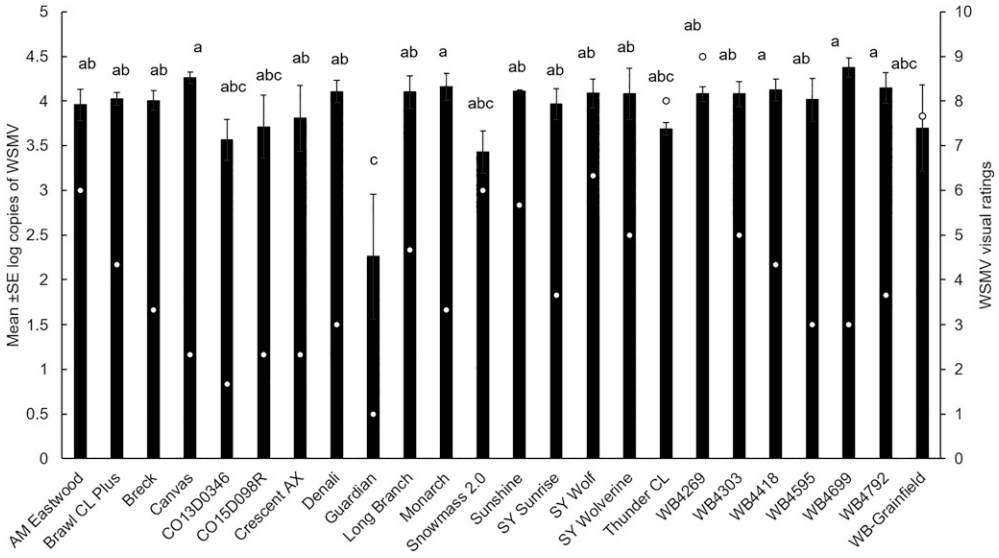

Fig. 5. Response of wheat varieties and Colorado State University advanced breeding lines to natural infection of wheat streak mosaic virus (WSMV) in an irrigated variety trial. Bars indicate mean of three biological replicates ± SE log copies of WSMV RNA per variety. Circles indicate average WSMV visual rating on a 1 to 9 scale. Different letters indicate significant differences between varieties at $P < 0.05$.

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