An Experimental Host Range for Triticum mosaic virus

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ABSTRACT

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Triticum mosaic virus (TriMV) is a newly discovered virus isolated from wheat (*Triticum aestivum*). This study was conducted to determine an experimental host range for TriMV and identify species that could serve as differential hosts for isolating TriMV from *Wheat streak mosaic virus* (WSMV). Plants tested were mechanically inoculated with the 06-123 isolate of TriMV or the Sidney 81 isolate of WSMV. Some plants were analyzed by enzyme-linked immunosorbent assay (ELISA) using antibodies of TriMV and WSMV. Plants infected with TriMV always produced mosaic symptoms and only extracts of symptomatic plants reacted with antibodies of TriMV. Maize is not a host for TriMV but barley, oat, rye, and triticale are hosts of TriMV. Certain barley and triticale accessions are hosts for TriMV but not WSMV. These plants can be used in combination with maize to separate WSMV and TriMV in plants infected by both viruses. We also showed that 8 wild grass species were susceptible to TriMV and 25 were not. All of the grasses susceptible to infection with TriMV have been reported as susceptible to infection with WSMV. Because of their growth habits, these plant species would be less desirable for use as differential hosts than maize, barley, and triticale.

Triticum mosaic virus (TriMV) was first identified infecting wheat (*Triticum aestivum* L.) in Kansas in 2006 (16). TriMV has since been identified from infected wheat in: Colorado, Nebraska, Oklahoma, South Dakota, Texas, and Wyoming (2). The virus is mechanically transmissible, and associated with a 35-kDa coat protein when analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Antiserum raised to the coat protein preferentially reacted only to TriMV in an enzyme-linked immunosorbent assay (ELISA) and a Western blot assay. Symptomatic plants were shown to be associated with flexuous rods when analyzed by electron microscopy.

The sequence of the coat protein of TriMV was determined by time-of-flight mass spectroscopy and from DNA sequenced of cloned, reverse-transcribed viral RNA. TriMV was found to be distantly related to *Sugarcane streak mosaic virus* (16). The complete nucleotide sequences of the Kansas 06-123 isolate (5) and a Nebraska isolate (25) of TriMV have been determined.

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Wheat curl mites (WCM) (*Aceria tosichella* Keifer) were observed on plants originally infected with TriMV (16). Subsequent studies using WCM obtained from Ellis County, KS demonstrated that these WCM could transmit TriMV both singly and in combination with *Wheat streak mosaic virus* (WSMV; 15). The WCM also transmits WSMV (22) and the High Plains virus (HPV) (12).

Previously, only wheat and barley have been confirmed as hosts of TriMV (16); therefore, we tested a greater range of plants for susceptibility to mechanical infection with TriMV to determine an experimental host range for this virus and identify plant species that might serve as differential hosts for TriMV and WSMV.

MATERIALS AND METHODS

Virus isolates. The 06-123 isolate of TriMV was isolated from KS06HW79 wheat in 2006 at the Kansas Agricultural Research Center–Hays (KSU-ARCH) at Hays, KS (16). The Sidney 81 isolate of WSMV was collected at Sidney, NE in 1981 and was obtained from W. G. Langenberg (17). Systemically infected wheat was used as the inoculum source for propagation of these isolates using the leaf-rub technique described previously (14). Following inoculation, the plants were held in a greenhouse under natural lighting.

Sources of antiserum. Anti-TriMV serum was used as a whole serum at a 1:4,000 (vol/vol) dilution and was prepared as described previously (16), and antiserum prepared to WSMV was used at

a 1:1,000 (vol/vol) dilution (10). Working dilutions were made from stocks adjusted to protein at 1 mg/ml.

Indirect ELISA. The ELISA procedure was conducted as described previously (16). Antiserum concentrations were used as described above. The goat anti-rabbit antibody/alkaline phosphatase conjugate was used at a dilution of 1:3,000 vol/vol (Southern Biotechnology Associates, Birmingham, AL). Absorbance was measured at 405 nm using a Titertek Multiscan plate reader (Flow Laboratories, Inc., McLean, VA). Absorbance values were arbitrarily considered positive if they were twice those of the equivalent mock-inoculated control.

Host range. The sources of the seed for these plant species tested are provided in Table 1. The maize, barley, oat, rye, pearl millet, sorghum, and triticale seed were planted into metal (21- by 31-cm) flats filled with a Harney clay loam soil (fine montmorillonitic, mesic type Argiustoll) having 22 rows per flat so that two sets of each seed source were planted. However, one set of each seed source of pearl millet and sorghum were planted. Each species was planted in separate experiments. At the two-leaf stage, one set of plants was mechanically inoculated with a 1:10 (wt/vol) extract of TriMV-infected wheat and the other set with an extract from wheat infected with WSMV. The inoculum for each virus was prepared from Tomahawk wheat inoculated 14 to 21 days previously with either TriMV or WSMV. Sorghum and pearl millet plants were inoculated only with TriMV. Following inoculation, the plants were held in a greenhouse under natural lighting. After 21 days in the greenhouse, the plants were rated for symptoms and, in the first experiment, either the third or fourth leaf was harvested and analyzed in ELISA using antibodies prepared to either TriMV or WSMV. Unless otherwise stated, the experiments were repeated three times.

Wild grasses. Seed sources of wild grasses used are listed in Table 1. Seed of these grasses were planted into soil-filled “plant cells” (Stuewe & Sons, Corvallis, OR) and inoculated at the two- to three-leaf stage with TriMV. Following inoculation, the plants were held in a greenhouse under natural lighting. After 21 days in the greenhouse, the plants were rated for symptoms. A plant species was tested in two experiments if it was infected with TriMV; a species symptomless following

Table 1. Names and seed sources of plants used in host range experiments

Species	Common name	Source ^a
<i>Aegilops cylindrica</i> Host	Jointed goatgrass	3
<i>Andropogon gerardi</i> Vitman	Big bluestem	3
<i>Avena fatua</i> L.	Wild oats	2
<i>A. sativa</i> L.	'Armor'	13
<i>A. sativa</i> L.	'Brawn'	13
<i>A. sativa</i> L.	'Chairman'	13
<i>A. sativa</i> L.	'Lodi'	13
<i>A. sativa</i> L.	'Rio Grande'	13
<i>Bouteloua curtipendula</i> (Michx.) Torr.	Sideoats grama	3
<i>B. gracilis</i> (Willd. ex Kunth) Lag. ex Griffiths	Blue grama 'El Reno'	3
<i>Bromus arvensis</i> L. = (<i>Bromus japonicas</i>)	Field brome	1
<i>B. inermis</i> Leyss.	Smooth brome	3
<i>B. secalinus</i> L.	Rye brome	1
<i>B. tectorum</i> L.	Cheatgrass	1
<i>Cenchrus longispinus</i> (Hack.) Fernald	Mat sandbur	1
<i>Chloris gayana</i> Kunth	Rhodes grass	8
<i>C. ventricosa</i> R., Br.	Australian Windmill grass	8
<i>Dactylis glomerata</i> L.	Orchardgrass	2
<i>Digitaria sanguinalis</i> (L.) Scop.	Hairy crabgrass	1
<i>Echinochloa crus-galli</i> (L.) P. Beauv.	Barnyard grass	1
<i>Elymus repens</i> (L.) Gould = (<i>Agropyron repens</i>)	Quackgrass	2
<i>Eragrostis cilianensis</i> (All.) Link ex Vign.	Stinkgrass	1
<i>Eriochloa acuminata</i> (J. Presl) Kunth	Tapertip cupgrass	1
<i>E. contracta</i> Hitchc.	Prairie cupgrass	1
<i>Hordeum vulgare</i> L.	'Baronesse'	7
<i>H. vulgare</i> L.	BZ-489-74	14
<i>H. vulgare</i> L.	'Black hullless'	14
<i>H. vulgare</i> L.	'Gallatin'	7
<i>H. vulgare</i> L.	'Horseford Hay'	7
<i>H. vulgare</i> L.	'Westford'	7
<i>H. vulgare</i> L.	'Lewis'	7
<i>H. vulgare</i> L.	'Summit'	7
<i>H. vulgare</i> L.	'Westbred medallion'	7
<i>H. vulgare</i> L.	'Westford'	7
<i>Lolium perenne</i> L. subsp. <i>multiflorum</i> (Lam.) Husnot = (<i>Lolium multiflorum</i>)	Italian ryegrass	2
<i>L. perenne</i> L. subsp. <i>perenne</i>	Perennial ryegrass	2
<i>Panicum capillare</i> L.	Witchgrass	1
<i>P. dichotomiflorum</i> Michx.	Fall panicgrass	2
<i>P. maximum</i> Jacq. R. Webster	Guineagrass	2
<i>P. miliaceum</i> L.	Broomcorn millet	1
<i>P. virgatum</i> L.	Switchgrass	2
<i>Pascopyrum smithii</i> (Rydb.) A. Love = (<i>Agropyron smithii</i>)	Western wheatgrass	3
<i>Pennisetum glaucum</i> (L.) R. Br.	Pearl millet (94-6667)	1
<i>Poa pratensis</i> L.	Kentucky bluegrass	3
<i>Schizachyrium scoparium</i> (Michx.) Nash = (<i>Andropogon scoparius</i>)	Little bluestem	3
<i>Secale cereale</i> L.	'Bonel',	1
<i>S. cereale</i> L.	'Mayton'	6
<i>Setaria pumila</i> (Poir.) Roem. & Schult subsp. <i>pumila</i> = (<i>Setaria glauca</i>)	Yellow foxtail	1
<i>S. viridis</i> (L.) P. Beauv.	Green bristlegrass	1
<i>Sorghum bicolor</i> (L.) Moench.	IS1056	1
<i>S. bicolor</i> (L.) Moench.	KS56A	1
<i>S. bicolor</i> (L.) Moench.	PI550610	1
<i>S. bicolor</i> (L.) Moench.	TX626	1
<i>S. bicolor</i> (L.) Moench.	TX 632	1
<i>S. bicolor</i> (L.) Moench.	TX 636	1
<i>S. bicolor</i> (L.) Moench.	TX 2567	1
<i>S. halapense</i> (L.) Pers.	Johnsongrass	2
<i>Thinopyrum intermedium</i> (Host) Barkw & D.R. Dewey subsp. <i>intermedium</i> = (<i>Agropyron intermedium</i>)	Intermediate wheatgrass	3
<i>T. ponticum</i> (Podp.) Z.-W. Liu & R.-C Wang = (<i>A. elongatum</i>)	Tall wheatgrass	3
<i>Tripsacum dactyloides</i> (L.) L.	Eastern gamagrass	3
<i>Triticale</i> (× <i>Triticosecale rimpani</i> Wittmack)	'Arcia'	9
<i>Triticale</i>	FL010-G7	9
<i>Triticale</i>	FL98122-H3	9
<i>Triticale</i>	FL98178-H4	9
<i>Triticale</i>	NCPT01-1433	9
<i>Triticale</i>	NCPT04-2151	9
<i>Triticale</i>	NCT021619	9

(continued on next page)

^a Seed source: 1 = Kansas State University Agricultural Experiment Station–Hays; 2 = Valley Seed Service, Fresno, CA 93791; 3 = Sharp Bros. Seeds, Healy, KS 67850; 4 = Ohio Agricultural Research and Development Center, Wooster 44691; 5 = Dr. David Baltensperger, University of Nebraska, Scottsbluff 69361; 6 = National Small Grains Collection, United States Department of Agriculture–Agricultural Research Service, Aberdeen, ID 83210; 7 = Townsend Seeds, Inc., Townsend, MT 59644; 8 = Regional Plant Introduction Station, Griffin, GA 30223-1797; 9 = Triticale seed obtained from P. S. Baenziger, University of Nebraska–Lincoln, Lincoln 68583; 10 = Commercial cultivar; 11 = North Central Regional Plant Station, Ames, IA 50011; 12 = Robson Seed Farms, Hall, NY 14463; 13 = Dr. Kraig Roozeboom, Manhattan, KS 66506; and 14 = Western Plant Breeders, Bozeman, MT 59715.

Table 1. (continued from preceding page)

Species	Common name	Source ^a
<i>Triticale</i>	NCT99-821	9
<i>Triticale</i>	NE03T407	9
<i>Triticale</i>	NE03T449	9
<i>Triticale</i>	NT01435	9
<i>Triticale</i>	NT01451	9
<i>Triticale</i>	NT02458	9
<i>Triticale</i>	'Sunland'	9
<i>Triticale</i>	'Trical 342'	9
<i>Triticum aestivum</i> L.	Tomahawk wheat	1
<i>Zea mays</i> L.	H84Ht (PI587128)	4
<i>Z. mays</i> L.	B73Ht (PI550473)	11
<i>Z. mays</i> L.	'Candy Corner'	10
<i>Z. mays</i> L.	'Chubby Checkers'	10
<i>Z. mays</i> L.	'Country Gentleman' (Ames 14238)	11
<i>Z. mays</i> L.	'Early & Often'	10
<i>Z. mays</i> L.	'Early Golden Bantam'	10
<i>Z. mays</i> L.	'Early Sunglow'	10
<i>Z. mays</i> L.	'Falconer' (PI213781)	11
<i>Z. mays</i> L.	'Midland' (PI221877)	11
<i>Z. mays</i> L.	N28Ht (Ames 27138)	11
<i>Z. mays</i> L.	Pa405 (Ames 22752)	11
<i>Z. mays</i> L.	'Peaches & Cream'	10
<i>Z. mays</i> L.	'Seneca Chief'	12
<i>Z. mays</i> L.	'Silver Choice'	10
<i>Z. mays</i> L.	'Silver Queen'	10
<i>Z. mays</i> L.	'Sugar Dots'	10
<i>Z. mays</i> L.	'Sweet Sunshine'	10

Table 2. Numbers of maize and wheat (Tomahawk) plants with mosaic symptoms 21 days following mechanical inoculation with Triticum mosaic virus (TriMV) or Wheat streak mosaic virus (WSMV) and enzyme-linked immunosorbent assay (ELISA) values from symptomatic (S) or nonsymptomatic (NS) plants^a

Plant sample	TriMV ^c	WSMV ^c	Antiserum (absorbance value) ^b			
			TriMV		WSMV	
			S	NS	S	NS
Country Gentleman	0/20	4/19	NA	0.006	0.269	0.018
Pa405	0/22	0/24	NA	0.006	NA	0.026
N28Ht	0/19	15/19	NA	0.005	0.286	0.014
Falconer	0/23	7/22	NA	0.006	0.286	0.020
Midland	0/21	10/21	NA	0.006	0.323	0.030
B73Ht	0/23	0/22	NA	0.006	NA	0.027
H84Ht	0/20	0/20	NA	0.006	NA	0.039
Tomahawk	30/30	29/29	0.357	NA	0.394	NA
WSMV virus control	NA	NA	0.004	NA	0.187	NA
TriMV virus control	NA	NA	0.181	NA	0.013	NA
Wheat healthy control	NA	NA	NA	0.015	NA	0.010
Maize healthy control	NA	NA	NA	0.018	NA	0.021

^a Inoculation viruses were the 06-123 isolate of TriMV and the Sidney 81 isolate of WSMV.

^b Mean absorbance values were from one experiment. Means were determined from the numbers of symptomatic or nonsymptomatic plants in a treatment (e.g., the 0.269 value for Country Gentleman was the mean of the ELISA values of the four symptomatic plants). Because only symptomatic plant extracts reacted with antibodies to the homologous virus with which the plants were inoculated and not symptomless plants, the other experiments were not analyzed (NA) by ELISA.

^c The numerator represents the number of symptomatic plants and the denominator the total number of plants inoculated. Temperature range during the experiments was 18 to 34°C.

inoculation with TriMV was tested in three experiments. In a given test, a species with only symptomless plants had the leaves bulked within a row and these leaves were then tested in ELISA using antibodies prepared to TriMV. A single symptomatic plant (if present) from a given line was also randomly sampled as an internal control along with the Tomahawk plants inoculated with TriMV. The temperature ranged from 18 to 30°C during these experiments. In Table 1, the scientific names of several grasses have changed; however, the older names have been provided as well, to provide conti-

nunity with the host range information with other viruses (13).

RESULTS

The first set of maize hybrids and lines initially tested by inoculation with TriMV were not infected (Table 2). The 'Tomahawk' wheat susceptible control plants were infected, indicating that the TriMV inoculum was infective. When the second set of plants of these lines were inoculated with WSMV, 'Country Gentleman', N28Ht, 'Falconer', and 'Midland' had systemically infected plants with mosaic symptoms similar to the Tomahawk wheat

control plants. All symptomatic plants reacted only to the antiserum made to the respective inoculation virus. Symptomless plants inoculated with TriMV or WSMV did not react to antibodies prepared to these viruses in ELISA. We analyzed only the first experiment of this set of plants by ELISA because only extracts from symptomatic plants reacted with a respective antibody, indicating that none of the plants represented symptomless infections.

Additional maize hybrids were tested for infection with TriMV but none were susceptible (Table 3). In contrast, the maize hybrids 'Chubby Checkers', 'Early

Golden Bantam', 'Early & Often', 'Early Sunglow', 'Sugar Dots', 'Silver Queen', Country Gentleman, and 'Seneca Chief' had plants symptomatic for WSMV in the three experiments. These plants were not analyzed by ELISA because we showed that only symptomatic plants reacted to antibodies of TriMV and WSMV in the first set of experiments (Table 2).

Analysis of nine barley sources inoculated with TriMV showed that all had plants that were susceptible (mosaic symptoms) to infection (Table 4). In contrast, only the 'Black Hulless', 'Lewis', 'Sum-

mit', 'Westbred Medallion', and 'Westford' barley sources had plants that were susceptible to infection with WSMV. Symptomless barley plants inoculated with TriMV or WSMV did not react to antibodies prepared to WSMV or TriMV.

The oat cultivars inoculated with TriMV or WSMV all had plants that were susceptible (mosaic symptoms) to infection with both viruses (Table 5). 'Lodi' and 'Rio Grande' had the least number of plants infected with TriMV and 'Brawn' the least number infected with WSMV. ELISA values for TriMV in oat cultivars were lower than those for WSMV in oats.

All rye cultivars analyzed under TriMV or WSMV pressure were susceptible to infection with both viruses (Table 6). Both cultivars had high numbers of plants infected with TriMV but low numbers of plants infected with WSMV. ELISA values were lower for all plants infected with TriMV than for WSMV but this was also observed for the TriMV control compared with the WSMV control.

Fifteen triticales sources were tested for susceptibility to infection with either TriMV or WSMV (Table 7). All lines had plants that were susceptible (mosaic symptoms) to infection with TriMV. Testing with WSMV showed that only FL98178-H4, NCPT01-1433, and 'Arcia' were resistant to infection with WSMV.

Wild grasses, pearl millet, and sorghum lines were tested for susceptibility to infection with TriMV (Table 8). Species susceptible to infection with TriMV were jointed goatgrass, wild oats, rye brome, cheatgrass (downy brome), field brome, prairie cupgrass, tapertip cupgrass, and green bristlegrass.

Many plant species tested were not infected by TriMV in our studies (Table 8):

barnyardgrass, mat sandbur, orchardgrass, hairy crabgrass, stinkgrass, perennial ryegrass, fall panicgrass, broomcorn millet, switchgrass, Kentucky bluegrass, blue grama, big bluestem, little bluestem, eastern gamagrass, johnsongrass, quackgrass, sideoats grama, intermediate wheatgrass, pearl millet, tall wheatgrass, western wheatgrass, rhodes grass, Australian windmill grass, smooth brome, yellow foxtail, and the sorghum lines IS1056, KS56A, PI550610, TX626, TX632, TX636, and TX2567. Only extracts from symptomatic plants reacted with TriMV antibodies when analyzed by ELISA, and only the symptomatic Tomahawk wheat control plants infected with TriMV reacted to antibodies of TriMV (*data not shown*).

DISCUSSION

We observed that all maize lines or hybrids were not infected with TriMV following mechanical inoculation (Tables 2 and 3). However, in our first maize experiments, we found that Country Gentleman, N28Ht, Falconer, and Midland maize were susceptible to infection with WSMV and the other maize lines were not susceptible (Table 2). The susceptibility of N28Ht to infection with WSMV (9) and Falconer and Midland (19) are in keeping with previous results. The Pa405 (8), B73 (8), and H84 (9) lines have been reported as being immune to infection with WSMV. However, we infected 4 of 19 Country Gentleman plants with WSMV, in contrast to previous work that reported this cultivar as immune (21). The four infected Country Gentleman plants occurred in the first experiment only. However, in our second set of maize experiments, we found that Country Gentleman had 2 of 17 plants infected with WSMV, each plant in a dif-

Table 3. Number of plants with mosaic symptoms after mechanical inoculation with either Triticum mosaic virus (TriMV) or *Wheat streak mosaic virus* (WSMV)

Plant source ^a	TriMV ^b	WSMV ^b
B73	0/19	0/19
Candy Corner	0/16	0/19
Chubby Checkers	0/16	1/18
Country Gentleman	0/17	2/17
Early & Often	0/22	1/23
Early Golden Bantam	0/8	3/14
Early Sunglow	0/22	4/21
Peaches & Cream	0/23	0/24
Seneca Chief	0/23	1/23
Silver Choice	0/10	0/12
Silver Queen	0/22	5/22
Sugar Dots	0/24	2/25
Sweet Sunshine	0/23	0/23
Tomahawk	22/22	27/27

^a Tomahawk was a wheat cultivar and all other entries were maize hybrids. Maize hybrids were obtained from a commercial source.

^b Inoculation viruses were the 06-123 isolate of TriMV and the Sidney 81 isolate of WSMV. The numerator represents the number of symptomatic plants and the denominator the total number of plants inoculated. The temperature ranged from 17 to 26°C during the experiments.

Table 4. Numbers of barley plants with mosaic symptoms 21 days following mechanical inoculation with Triticum mosaic virus (TriMV) or *Wheat streak mosaic virus* (WSMV) and enzyme-linked immunosorbent assay (ELISA) values from symptomatic (S) or nonsymptomatic (NS) plants^a

Plant source ^c	TriMV ^d	WSMV ^d	Antiserum (absorbance value) ^b			
			TriMV		WSMV	
			S	NS	S	NS
Baronesse	8/21	0/17	0.333	0.009	NA	0.018
Black Hulless	12/14	10/12	0.410	0.011	0.318	0.014
BZ-489-74	13/18	0/10	0.385	0.003	NA	0.010
Gallatin	21/21	0/16	0.435	NA	NA	0.002
Horsford Hay	16/17	0/17	0.312	0.031	NA	0.019
Lewis	16/16	6/16	0.387	NA	0.212	0.017
Summit	16/20	1/20	0.412	0.007	0.081	0.021
Westbred Medallion	20/20	18/20	0.366	NA	0.340	0.015
Westford	20/20	18/20	0.336	NA	0.218	0.014
Tomahawk wheat	20/20	15/15	0.337	NA	0.225	NA
TriMV ELISA control (EC)	NA	NA	0.283	NA	0.025	NA
WSMV EC	NA	NA	0.010	NA	0.176	NA
Healthy barley EC	NA	NA	NA	0.009	NA	0.015

^a Inoculation viruses were the 06-123 isolate of TriMV and the Sidney 81 isolate of WSMV.

^b Mean absorbance values are from one experiment. Means were determined from the numbers of symptomatic or nonsymptomatic plants in a treatment (e.g., the 0.333 value for Baronesse barley was the mean of the ELISA values of the eight symptomatic plants). Because only symptomatic plant extracts reacted with antibodies to the homologous virus with which the plants were inoculated and not symptomless plants, the plants in the other experiment were not analyzed (NA) by ELISA.

^c Tomahawk was a wheat cultivar and all other entries were barley.

^d The numerator represents the number of systemically infected plants with mosaic symptoms and the denominator the total number of plants inoculated. Plants were from two experiments. The temperature ranged from 16 to 26°C during the experiments.

ferent experiment. This difference may result from off-types that may have been in our seed source. It is also a possibility that the seed of cv. Country Gentleman were genetically uniform but the plants were not easily infected by mechanical inoculation. We have observed this with wheat cv. Ike that is susceptible to infection by WSMV in the field but is difficult to infect by mechanical inoculation (D. L. Seifers, *unpublished*). No information is available concerning the reaction of the Chubby Checkers, Early Golden Bantam, Early & Often, Early Sunglow, Sugar Dots, and Silver Queen to inoculation with WSMV. However, Seneca Chief has been reported to be susceptible to infection with WSMV (9). These maize lines provide a means to obtain WSMV free of TriMV from a source infected by both viruses. In our analyses (with one TriMV isolate), we could not mechanically infect maize lines or hybrids, indicating that TriMV may not pose a threat to maize production.

All barley accessions were susceptible to infection with TriMV (Table 4). We reported in an earlier study (16) natural

infection of barley by TriMV, in which eight barley lines had a few symptomatic plants, four lines each had a single infected plant, two lines had a single plant infected with WSMV only, and two lines had a single plant infected with both TriMV and WSMV. Barley is listed as a host for WSMV (20); however, we observed that not all barley accessions ('Baronesse', 'Gallatin', 'Horsford Hay', and BZ-489-74) tested were susceptible to infection with WSMV following mechanical inoculation. Thus, these barley accessions could serve as a set of differential hosts for TriMV and WSMV. Using Gallatin barley and N28Ht maize, we were able to isolate TriMV free of WSMV, and then used that isolate in studies identifying that the WCM was the vector of TriMV (15). Other work has shown that not all barley sources are susceptible to infection with WSMV (1). In that study, cv. Rodeo was immune to both the PV 57 and PV 106 isolates of WSMV but six other cultivars were susceptible to both virus isolates.

The results of our experiments with oat (Table 5) and rye (Table 6) accessions

showed that they all had plants that were susceptible to infection with either TriMV or WSMV. Oat and rye have been shown previously to be a host for WSMV (20).

The 15 triticale lines or cultivars tested all had plants that were susceptible to infection with TriMV (Table 7). Interestingly, we observed that, when inoculated with WSMV, only the lines FL98178-H4 and NCPT01-1433 and cv. Arcia had plants that were not infected. As with barley, triticale can provide a differential host for TriMV and WSMV. Previous work has shown that triticale lines are susceptible to infection with WSMV (6). In that study, an unidentified isolate of WSMV was used to mechanically inoculate 33 triticale sources; 3 were considered immune and the remainder had levels of infection ranging from 4 to 93%.

Several grass species were susceptible to infection with TriMV (Table 8). These species, also reported as hosts of WSMV, included jointed goatgrass (24), wild oats (24), cheatgrass (downy brome; 20,24), field brome (20,24), prairie cupgrass (3), tapertip cupgrass (D. L. Seifers, *unpub-*

Table 5. Numbers of oat plants with mosaic symptoms following mechanical inoculation with Triticum mosaic virus (TriMV) or *Wheat streak mosaic virus* (WSMV) and enzyme-linked immunosorbent assay (ELISA) values from symptomatic (S) or nonsymptomatic (NS) plants^a

Plant source ^c	TriMV ^d	WSMV ^d	Antiserum (absorbance value) ^b			
			TriMV		WSMV	
			S	NS	S	NS
Armor	23/26	30/30	0.143	0.009	1.051	NA
Brawn	24/25	14/25	0.197	0.001	0.764	0.009
Chairman	24/31	27/29	0.231	0.007	0.961	0.008
Lodi	6/20	23/24	0.152	0.005	0.994	0.012
Rio Grande	9/27	28/29	0.150	0.004	1.111	0.001
Tomahawk wheat	31/31	27/27	0.206	NA	0.999	NA
TriMV ELISA control (EC)	NA	NA	0.181	NA	0.036	NA
WSMV EC	NA	NA	0.023	NA	0.676	NA
Healthy wheat EC	NA	NA	NA	0.010	NA	0.021

^a Inoculation viruses were the 06-123 isolate of TriMV and the Sidney 81 isolate of WSMV.

^b Mean absorbance values were from one experiment. Means were determined from the numbers of symptomatic or nonsymptomatic plants in a treatment (e.g., the 0.143 value for Armor oat was the mean of the ELISA values of the 23 symptomatic plants). Because only symptomatic plant extracts reacted with antibodies to the homologous virus with which the plants were inoculated and not symptomless plants, the plants in the other experiments were not analyzed by ELISA.

^c Tomahawk was a wheat cultivar all other entries are oat. Temperature range was 18 to 29°C during the experiments.

^d The numerator represents the number of systemically infected plants with mosaic symptoms and the denominator the total number of plants inoculated.

Table 6. Numbers of rye plants with mosaic symptoms following mechanical inoculation with Triticum mosaic virus (TriMV) or *Wheat streak mosaic virus* (WSMV) and enzyme-linked immunosorbent assay (ELISA) values from symptomatic (S) or nonsymptomatic (NS) plants^a

Plant source ^c	TriMV ^d	WSMV ^d	Antiserum (absorbance value) ^b			
			TriMV		WSMV	
			S	NS	S	NS
Bonel	19/25	9/25	0.225	0.001	0.658	NA
Mayton	15/29	6/29	0.313	0.013	0.408	0.012
Tomahawk wheat	32/32	34/34	0.455	NA	1.129	NA
TriMV ELISA control (EC)	NA	NA	0.217	NA	0.032	NA
WSMV EC	NA	NA	0.006	NA	1.263	NA
Healthy wheat EC	NA	NA	NA	0.015	NA	0.023

^a Inoculation viruses were the 06-123 isolate of TriMV and the Sidney 81 isolate of WSMV.

^b Mean absorbance values are from the one experiment. Means were determined from the numbers of symptomatic or nonsymptomatic plants in a treatment (e.g., the 0.225 value for Bonel rye was the mean of the ELISA values of the 19 symptomatic plants). Because only symptomatic plant extracts reacted with antibodies to the homologous virus with which the plants were inoculated and not symptomless plants, the plants in the other experiments were not analyzed by ELISA.

^c Tomahawk was a wheat cultivar and all other entries were rye. Temperature range was 18 to 30°C during the experiments.

^d The numerator represents the number of systemically infected plants with mosaic symptoms and the denominator the total number of plants inoculated.

Table 7. Numbers of triticale plants with mosaic symptoms following mechanical inoculation with Triticum mosaic virus (TriMV) or *Wheat streak mosaic virus* (WSMV) and enzyme-linked immunosorbent assay (ELISA) values from symptomatic (S) or nonsymptomatic (NS) plants^a

Plant source ^c	TriMV ^d	WSMV ^d	Antiserum (absorbance value) ^b			
			TriMV		WSMV	
			S	NS	S	NS
ARCIA	26/26	0/34	0.373	NA	NA	0.004
FL010-G7	26/36	5/27	0.307	0.008	0.166	0.010
FL98122-H3	37/38	33/33	0.293	0.015	0.537	NA
FL98178-H4	26/31	0/35	0.328	0.030	NA	0.024
NCPT01-1433	25/25	0/32	0.417	NA	NA	0.034
NCPT04-2151	17/23	7/23	0.307	0.012	0.412	0.012
NCT021619	29/29	15/23	0.238	NA	0.552	0.017
NCT99-821	19/19	4/17	0.177	NA	0.349	0.012
NE03T407	21/25	15/29	0.572	0.007	0.402	0.006
NE03T449	23/24	16/23	0.467	0.	0.487	0.017
NT01435	29/29	26/29	0.466	NA	0.583	0.027
NT01451	27/27	12/30	0.686	NA	0.455	0.011
NT02458	22/22	21/21	0.403	NA	0.521	NA
Sunland	32/32	3/36	0.504	NA	0.282	0.015
Trical 342	28/28	28/28	0.392	NA	0.459	NA
Tomahawk wheat	37/37	34/34	0.267	NA	0.463	NA
TriMV ELISA control (EC)	NA	NA	1.404	NA	0.068	NA
WSMV EC	NA	NA	0.041	NA	1.149	NA
Healthy wheat EC	NA	NA	NA	0.017	NA	0.053

^a Inoculation viruses were the 06-123 isolate of TriMV and the Sidney 81 isolate of WSMV.

^b Mean absorbance values are from one experiment. Means were determined from the numbers of symptomatic or nonsymptomatic plants in a treatment (e.g., the 0.373 value for Arcia triticale was the mean of the ELISA values of the 26 symptomatic plants). Because only symptomatic plant extracts reacted with antibodies to the homologous virus with which the plants were inoculated and not symptomless plants, the plants in the other experiments were not analyzed by ELISA.

^c Tomahawk was a wheat cultivar and all other entries were triticale. Temperature range was 16 to 31°C during the experiments.

^d The numerator represents the number of systemically infected plants with mosaic symptoms and the denominator, the total number of plants inoculated. Plants were from two experiments.

lished), and green bristlegrass (3,4,20,24). There were also many of the grasses we tested that were not hosts for TriMV but have been reported as hosts of WSMV (Table 8). These species that are not susceptible to infection with WSMV included barnyardgrass (3,20,24), pearl millet (11), mat sandbur (3,4,20,24), hairy crabgrass (24), stinkgrass (4,20), fall panicgrass (20), broomcorn millet (19), and the sorghum lines IS1056, KS56A, PI550610, TX626, TX632, TX636, and TX2567 (11). The sorghum lines tested may not be ideal hosts to separate WSMV from TriMV because not all WSMV isolates will infect these sorghum lines (11). In the current study, pearl millet plants were not susceptible to infection with TriMV but this species has been reported as a host of WSMV (11). However, it has been demonstrated that not all WSMV isolates can infect pearl millet (11).

We showed that many grasses are not hosts for TriMV (Table 8). These grasses that are not hosts of WSMV include blue grama (24,26), big bluestem (20), little bluestem (20), eastern gamagrass (20), johnsongrass, (19,20,24), orchardgrass (18,19,24), perennial ryegrass (23), switchgrass (19,20,24), quackgrass (17,20,24), sideoats grama (24), intermediate wheatgrass (7,18,20), tall wheatgrass (20,24), western wheatgrass (19,20,24), yellow foxtail (13,20,24), and possibly Australian windmill grass and rhodes grass, but no information is available if they are susceptible to WSMV infection.

In summary, we have identified an experimental host range using mechanical inoculation with the 06-123 isolate of TriMV and the Sidney 81 isolate of WSMV. We have shown that maize in combination with barley and triticale accessions can be used to separate WSMV and TriMV in plants infected by both viruses. Because these species are easy to grow, they would be the desired species to use for this purpose. We also showed that several grass species were susceptible to TriMV and not WSMV but, due to their slow growth habits, they would be less desirable for use as differential hosts. In addition, we showed that many grasses were not susceptible to infection with TriMV, and these grasses are also reported not to be hosts of WSMV. Additional work of importance would be analysis of these differential hosts using a large set of TriMV isolates from a wide geographic region.

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Table 8. Numbers of plants with mosaic symptoms following mechanical inoculation with Triticum mosaic virus (TriMV)

Plant species	Common name or variety	NSP ^a	Host for WSMV ^b
<i>Aegilops cylindrica</i>	Jointed goatgrass	42/52	S (20,24)
<i>Andropogon gerardii</i>	Big bluestem	0/48	I (20)
<i>Avena fatua</i>	Wild oat	29/66	S (24)
<i>Bouteloua curtipendula</i>	Sideoats grama	0/39	I (24)
<i>B. gracilis</i>	Blue grama 'El Reno'	0/19	I (26)
<i>Bromus arvensis</i>	Field brome	24/49	S (20,24)
<i>B. inermis</i>	Smooth brome	0/34	I (18,19)
<i>B. secalinus</i>	Rye brome	5/8	S (20,24)
<i>B. tectorum</i>	Cheatgrass	15/22	S (20,24)
<i>Cenchrus longispinus</i>	Mat sandbur	0/18	S (3,4,20,24)
<i>Chloris gayana</i>	Rhodes grass	0/34	No information
<i>C. ventricosa</i>	Australian windmill grass	0/46	No information
<i>Dactylis glomerata</i>	Orchardgrass	0/17	I (18,19,24)
<i>Digitaria sanguinalis</i>	Hairy crabgrass	0/63	S (24)
<i>Echinochloa crusgalli</i>	Barnyardgrass	0/27	S (3,20,24)
<i>Elymus repens</i>	Quackgrass	0/60	I (7,10,20,24)
<i>Eragrostis cilianensis</i>	Stinkgrass	0/47	S (4,20)
<i>Eriochloa acuminata</i>	Tapertip cupgrass	4/20	S (D. L. Seifers, unpublished)
<i>E. contracta</i>	Prairie cupgrass	2/20	S (3)
<i>Lolium perenne</i> ssp. <i>perenne</i>	Perennial ryegrass	0/43	I (23)
<i>Panicum dichotomiflorum</i>	Fall panicgrass	0/51	S (20)
<i>P. miliaceum</i>	Broomcorn millet	0/61	S (19)
<i>P. virgatum</i>	Switchgrass	0/32	I (19,20,24)
<i>Pascopyrum smithii</i>	Western wheatgrass	0/14	I (19,20,24)
<i>Pennisetum glaucum</i>	Pearl millet	0/67	S (11)
<i>Poa pratensis</i>	Kentucky bluegrass 'Glade'	0/58	I (20,24)
<i>Schizachyrium scorparium</i>	Little bluestem	0/29	I (20)
<i>Setaria glauca</i>	Yellow foxtail	0/53	I (20,24)
<i>S. viridis</i>	Green bristlegrass	4/25	S (3,4,20,24)
<i>Sorghum bicolor</i>	IS 1056	0/30	S (11)
<i>S. bicolor</i>	KS56A	0/30	S (11)
<i>S. bicolor</i>	PI 550610	0/53	S (11)
<i>S. bicolor</i>	TX 626	0/18	S (11)
<i>S. bicolor</i>	TX 632	0/42	S (11)
<i>S. bicolor</i>	TX 636	0/43	S (11)
<i>S. bicolor</i>	TX 2567	0/39	S (11)
<i>S. halepense</i>	Johnsongrass	0/37	I (19,20,24)
<i>Thinopyrum intermedium</i>	Intermediate wheatgrass	0/80	I (19,20,24)
<i>T. ponticum</i>	Tall wheatgrass	0/54	I (20,24)
<i>Tripsacum dactyloides</i>	Eastern gamagrass	0/16	I (24)

^a NSP = numbers of symptomatic plants following mechanical inoculation with the 06-123 isolate of TriMV. The numerator represents the number of symptomatic plants and the denominator the total number of plants mechanically inoculated. Experiments were repeated two times for species developing symptoms and three times for species that remained symptomless following inoculation.

^b Susceptible (S) or immune (I) rating of a plant species is followed by the reference number in parentheses that established that response to inoculation with *Wheat streak mosaic virus* (WSMV).

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