Natural Infection of Pearl Millet and Sorghum by Wheat Streak Mosaic Virus in Kansas

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ABSTRACT

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Pearl millet (*Pennisetum glaucum*) and sorghum (*Sorghum bicolor*) plants at Hays, Kansas, were observed to have viruslike symptoms. Symptomatic plants were tested by enzyme-linked immunosorbent assay (ELISA) against wheat streak mosaic virus (WSMV), maize dwarf mosaic, sugarcane mosaic virus strain MDMV-B, and johnsongrass mosaic virus. Positive reactions were obtained only with WSMV antisera. Pearl millet and sorghum plants (of genotypes naturally infected in the field) mechanically inoculated in the greenhouse developed symptoms and were positive in ELISA for WSMV. The virus was vectored by wheat curl mites (*Aceria tosichella*) from pearl millet and sorghum to the host from which it was obtained and to wheat. Mechanical inoculation of several sorghum lines showed that the WSMV isolates differed in ability to infect sorghum, and the type specimen could not infect any of the sorghum lines. These results indicated that WSMV occurring at Hays can infect sorghum and pearl millet, crop plants not reported previously as susceptible to WSMV.

Wheat streak mosaic (WSM), caused by wheat streak mosaic virus (WSMV), is an important pathogen of wheat (*Triticum aestivum* L.) in western Kansas. Estimated losses from WSM in Kansas from 1976 to 1987 were as high as 13% of the value of the crop, with an average loss of 2.5% each year (16,25,29). The virus is vectored by the wheat curl mite (*Aceria tosichella* Keifer). Viruliferous wheat curl mites can infect barley (*Hordeum vulgare* L.) (26,27), corn (*Zea mays* L.) (8,26,27), oats (*Avena sativa* L.) (26), and rye (*Secale cereale* L.) (26,27) with WSMV.

The specific epithet *tosichella* is used here because *tulipae* has been stated to apply only to mites developing on tulips and other *Liliaceae* (22). In 1970, Shevtchenko described a new species, *Aceria tritici,* for mites occurring on wheat; however, the identical mite was apparently described by Keifer in 1969 (11) from wheat in Yugoslavia and named *Aceria tosichella,* which should hold priority (2).

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During the summer of 1994, pearl millet (*Pennisetum glaucum* L.) and sorghum (*Sorghum bicolor* (L.) Moench) plants growing in the field were observed with systemic mosaic symptoms characteristic of virus-infected plants. Field and greenhouse data confirm that the symptomatic pearl millet and sorghum were infected by WSMV and that the pearl millet and sorghum WSMV isolates differ from the type specimen with respect to host range. This is the first report of natural infection of pearl millet and sorghum with WSMV, extending its host range among cereal crops.

MATERIALS AND METHODS

WSMV virus isolates. Isolates PV57 and PV106 were obtained from the American Type Culture Collection (Rockville, MD 20852). The H81 and H88 isolates were collected from wheat at Hays, Kansas, in 1981 and 1988, respectively; and the pearl millet (H94PM) and sorghum (H94S) isolates were collected from those hosts at Hays in 1994. The H94S and H94PM isolates were maintained in PI550610 sorghum and a pearl millet F1 hybrid (94-3810), respectively. All other isolates were maintained in wheat cultivar Tomahawk. Isolate purity was verified using antiserum to other viruses and inoculation of indicator plants (20). Inoculum preparation and inoculation procedures were as previously described (17).

Antiserum. Antiserum was prepared to WSMV (20), maize dwarf mosaic virus (MDMV) (21), sugarcane mosaic virus strain MDMV-B (21), and Agropyron mosaic virus (AMV) (20) using previously described procedures. Antiserum to brome mosaic virus (PVAS178) was obtained from the American Type Culture Collection.

Indirect enzyme-linked immunosorbent assay (ELISA). Leaf tissue was ground in 0.05 M carbonate buffer, pH 9.6 (6). Samples were absorbed to ELISA plates (Immulon 1, Dynatech Laboratories, Inc., Chantilly, VA) for 1 h at 37°C. Plates were rinsed and then incubated with a 5-µg/ml dilution of antivirus antibody in blocking buffer (6) for 1 h at 37°C. Plates were rinsed and blocked with a solution containing 5% (wt/vol) nonfat dry milk and 0.01% (vol/vol) antifoam A (Sigma A-5758, Sigma Chemical Co., St. Louis, MO 63178) made in phosphate buffered saline, pH 7.0, for 1 h at 37°C. Following rinsing, antirabbit antibody:alkaline phosphatase conjugate (Sigma) in blocking buffer (1:1,000, vol/vol dilution) was added, and the plates were incubated for 1 h at 37°C. The plates were rinsed, substrate (*p*-nitrophenyl phosphate) in substrate buffer (6) was added at 0.714 mg/ml, and plates were incubated for 30 min at room temperature. Absorbance (405 nm) values were considered positive if they were twice those of the equivalent mock-inoculated control.

Infectivity assays. Ten days following planting (in 30 × 50 cm soil-filled metal flats), Tomahawk wheat, Lodi oats, foxtail millet (*Setaria italica* L.), proso millet (*Panicum miliaceum* L.), Westford barley, N28 Ht corn, and Golden Acres TE76 and PI550610 sorghum plants were inoculated mechanically on the second leaf as previously described (20). Inoculated plants were maintained in a greenhouse for 3 weeks under natural light conditions with a temperature range of 18 to 27°C. All infectivity assays were repeated three times.

Minipurification, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), and Western blotting of viral proteins. The extraction and partial purification of virus protein were done according to Lane (14). The pearl millet and sorghum leaf tissues (1 g for each symptomatic sorghum and pearl millet plant with each plant obtained from a different row for each of the three tests) were ground (1:5, wt/vol dilution) with a mortar and pestle in 0.1 M ammonium citrate buffer, pH 6.5, containing 0.25% mercaptoethanol, 0.1% sodium diethyldithiocar-

bamate, and 1% polyvinylpyrrolidone. The extract was filtered through cheesecloth and centrifuged for 10 min at 5,900 × *g*. The supernatant was mixed with 2 ml of 33% Triton X-100 and centrifuged through a 1-cm layer of 0.988 M sucrose (made in ammonium citrate buffer) for 2 h at 85,000 × *g*. The pellet was resuspended in 200 µl of SDS treatment buffer (0.125 M Tris-Cl, pH 6.8, 4% SDS, 20% glycerol, and 10% 2-mercaptoethanol), heated for 4 min at 100°C, reduced, and alkylated (13); then 25 µl was loaded into a well of a 10% gel. Electrophoresis was conducted using the procedure of Laemmli (12). The gels were stained with Coomassie Blue R-250. Gels were scanned to determine molecular masses of separated proteins using a Hoefer GS300 densitometer, and data were collected and processed using Hoefer GS-365 data analysis software, which calculated values for the proteins using a linear regression fit to protein standards used on the gel.

All electrophoretic analyses were repeated three times with extracts from different plants. Healthy pearl millet, sorghum, and wheat were treated identically as controls for all experiments.

For Western blotting, both healthy and infected sorghum and pearl millet were extracted and separated on SDS-PAGE as described above. Two 10% gels were loaded identically, one for Coomassie Blue R-250 staining and the other for Western blotting. At the end of SDS-PAGE, proteins were transferred onto wetted nitrocellulose (NitroCell 0.45 µm, Hoefer, San Francisco, CA) membranes for 1 h with a power setting of 100 V at 400 mA in a TE-52 Transphor electrophoresis cell (Hoefer) or a Mini-Transblot cell (BioRad, Hercules, CA) using a transfer buffer consisting of 39 mM glycine, 40 mM Tris-Cl, pH 8.3, 0.037% SDS, and 20%, vol/vol methanol (28). Western blotting was done using alkaline phosphatase antirabbit goat antibody as described by Bollig and Edelstein (4).

Field rating and testing of symptomatic plants from pearl millet and sorghum nurseries. Rows (11 to 30 plants per row) in the nurseries were rated for the presence of plants with mosaic symptoms, and estimates of percent infection were recorded. Each row having a plant or plants with symptoms had a leaf from one plant tested by ELISA against antiserum to WSMV, MDMV, sugarcane mosaic virus strain MDMV-B, and johnsongrass mosaic virus. These antisera were used in an attempt to determine if the plants were infected with other viruses.

Inoculation of pearl millet and sorghum lines with different WSMV isolates. Seed of 40 pearl millet and 71 sorghum lines were planted in 30 × 50 cm soil-filled metal flats in a greenhouse. Seven days following planting, the seedlings (10 plants for each experiment) were inoculated on the primary leaf with a given WSMV isolate as previously described (17). Symptom notes were taken on a weekly basis for 6 weeks following inoculation. After the last symptom rating, symptomatic plants were tested individually and nonsymptomatic plants were bulked and tested separately by ELISA for WSMV. The experiment was repeated twice using all 40 pearl millet and 71 sorghum lines. A third experiment was conducted using only pearl millet or sorghum lines that developed symptoms to any of the virus isolates in the first two experiments. The 40 pearl millet lines were selected from lines in the field having estimates of 1 to 100% natural infection and having plants in these lines testing positive by ELISA to WSMV. Some sorghum lines used were infected naturally in the field and others were selected because of their use in sorghum breeding programs.

Wheat curl mite vectoring experiments. Wheat curl mites (WCM) were raised from eggs in a growth chamber on healthy wheat plants, then adults or second instar nymphs were transferred manually to WSMV-infected sorghum or pearl millet and given a 24-h acquisition access period. The WCM were removed manually from the WSMV source plants, and five WCM were placed on each sorghum, pearl millet, or wheat plant. No attempt was made to remove the WCM following infestation of the assay plants. The plants were incubated in a growth chamber (Warren/Sherer Model CEL39-15) with 12 h of fluorescent light (approximately 500 $\mu E \cdot s^{-1} \cdot m^{-2}$) for 21 days at 25°C. Controls consisted of plants infested with WCM not having fed on WSMV-infected pearl millet or sorghum tissue.

Inoculation of WSMV-immune grasses. The grasses and origin of the seed for each species used in host range studies are listed in Table 1. The grasses were planted in soil-filled 2.5 × 16.5 cm pine cells (Stuewe and Sons, Inc., Corvallis, OR 97333) and were inoculated (10 plants for each virus isolate per experiment) at the two- to three-leaf stage (all leaves) with a given WSMV isolate (HS94, HPM94, H88, H81, PV106, and PV57) as previously described (17). Following inoculation, the plants were grown in a greenhouse under natural lighting with a temperature range of 18 to 29°C. The plants were rated for symptoms at 28 days following inoculation. Immediately after that symptom rating, the plants were tested by ELISA against WSMV antiserum as described above. Symptomatic plants were tested individually, and nonsymptomatic plants were bulked. Mock-inoculated plants were treated identically to controls.

Symptom severity and dry weight of sorghum infected with different WSMV isolates. Seed of PI550610 sorghum was

Table 1. Grasses previously reported to be immune to wheat streak mosaic virus (WSMV) and inoculated with six strains of WSMV and determined to be infected (+) or not infected (–) by WSMV

Grass host	Common name	Seed source[z]	Reaction to WSMV isolate
Agropyron elongatum (Host) P. Beauv. = (*Thinopyrom ponticum* (Podp.) Barkw. & D.R. Dewey)	Tall wheatgrass	3	–
A. intermedium (Host.) P. Beauv. = (*Thinopyrom intermedium* (Host) Barkw. & D.R. Dewey subsp. *intermedium*)	Intermediate wheatgrass	3	–
A. repens (L.) P. Beauv. = (*Elytrigia repens* (L.) Nevski)	Quackgrass	2	–
A. smithii Rydb. = (*Pascopyrum smithii* (Rydb.) A. Love)	Western wheatgrass	3	–
Andropogon gerardii Muhl.	Big bluestem	3	–
A. scoparius Michx.	Little bluestem	3	–
Avena fatua L.	Wild oats	2	+
Bouteloua curtipendula (Michx.) Torr.	Sideoats grama	3	–
B. gracilis (H.B.K.) Lag. ex Steud.	Blue grama	3	–
Bromus inermis Leyss.	Smooth brome	3	–
Dactylis glomerata L.	Orchardgrass	2	–
Elymus junceus Fisch. = (*Psathyrostachys juncea* (Fischer) Nevski)	Russian wild rye	3	–
Panicum maximum Jacq.	Guineagrass	2	–
P. virgatum L.	Switchgrass	2	–
Setaria italica (L.) P. Beauv.	Italian or foxtail millet	5	+
S. glauca (L.) P. Beauv.	Yellow foxtail	1	–
Sorghastrum nutans (L.) Nash.	Indiangrass	2	–
Sorghum halepense (L.) Pers.	Johnsongrass	2	–
Tripsacum dactyloides (L.) L.	Eastern gammagrass	3	–
Zea mays L.	H84Ht	4	–

[z] 1 = Kansas State University Agricultural Experiment Station-Hays. Plant identity was established using (9). 2 = Valley Seed Service, Fresno, CA. 3 = Sharp Bros. Seeds, Healy, KS. 4 = Ohio Agricultural Research and Development Center, Wooster. 5 = David Baltensperger, University of Nebraska, Scottsbluff.

sieved to uniform size (8/64 round sieve) and planted at 20 seeds per row in 30 × 50 cm soil-filled (9.988 kg of soil) metal flats. At 7 days after planting (two-leaf stage), plants were inoculated on the first leaf with a 1:10 (wt/vol) dilution of inoculum prepared from source plants H88 and PV106 in Tomahawk wheat inoculated 14 days previously, H94PM in pearl millet hybrid 94-3810 inoculated 28 days previously, and the H94S isolate in PI550610 sorghum inoculated 21 days previously as described (17). Inoculated rows were randomized within the flat (two rows of sorghum for each virus isolate, one mock-

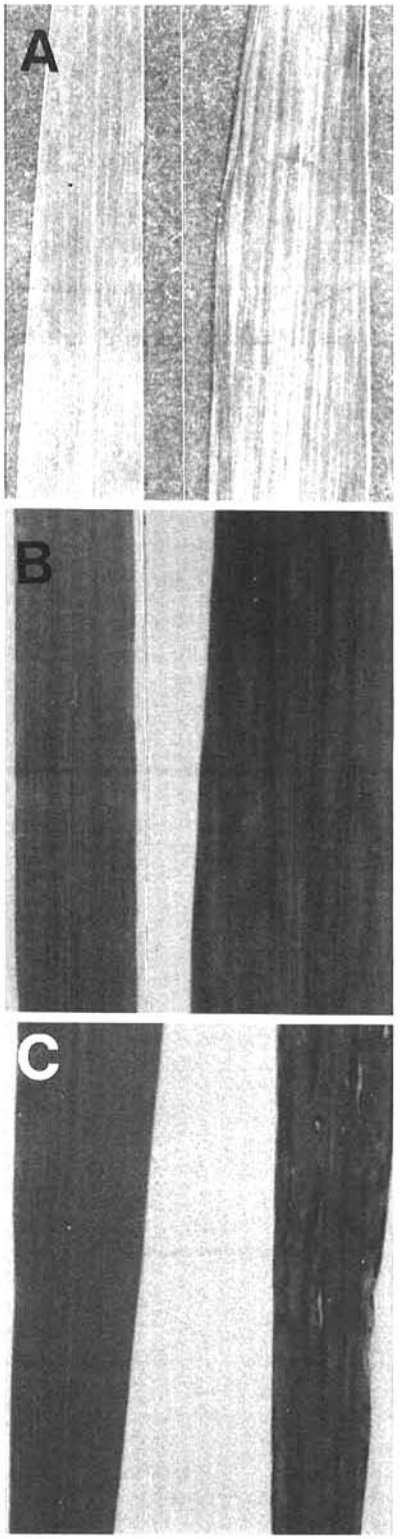

Fig. 1. Healthy (left) and symptomatic wheat streak mosaic virus-infected leaves (right) of **(A)** pearl millet, **(B)** IS1056 sorghum, and **(C)** PI550610 sorghum.

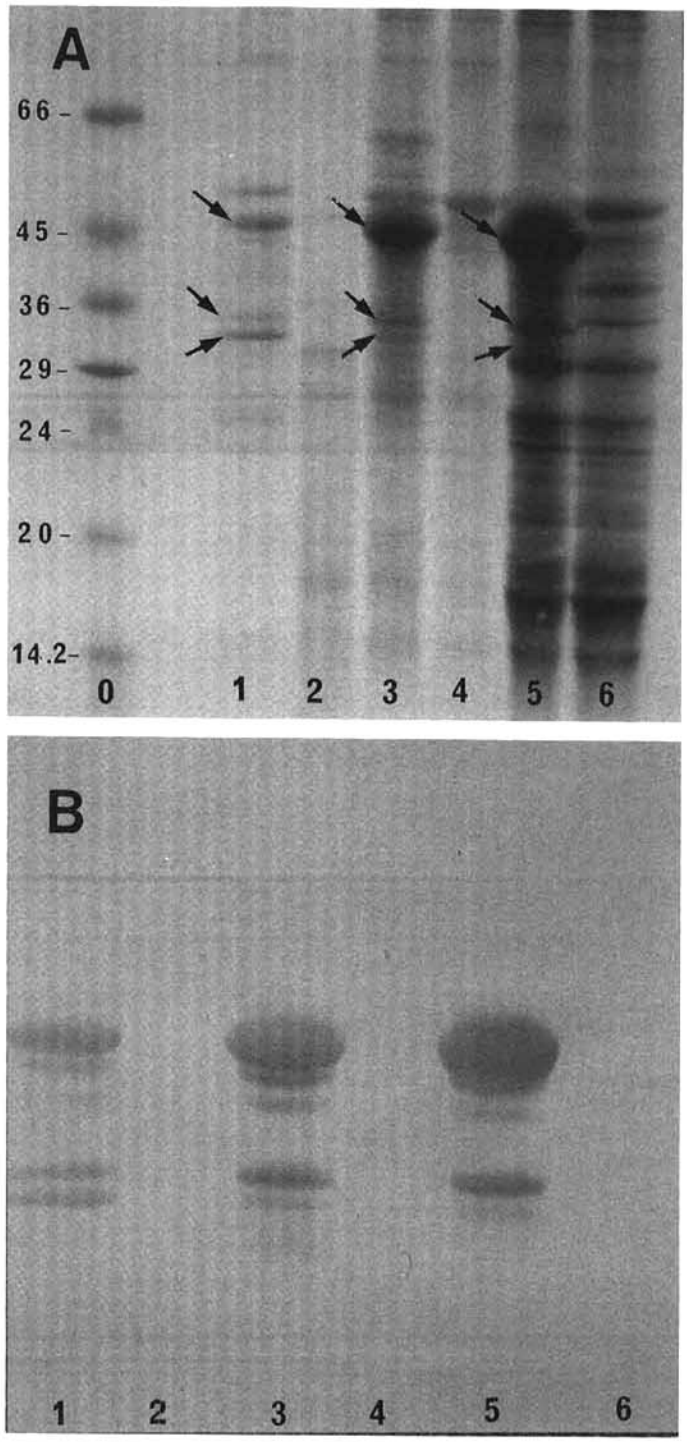

Fig. 2. Analysis of wheat streak mosaic virus (WSMV) proteins concentrated from pearl millet, sorghum, and wheat by minipurification and separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) **(A)** and probed with antibodies to WSMV (1:600 dilution) and anti-rabbit goat antibody:alkaline phosphatase conjugate (1:4,000 dilution) in a Western blot **(B). (A)** SDS-PAGE counterpart gel to gel used for Western blot. Lane 0, numbers (in kilodaltons) left of lane 0 indicate position of molecular weight markers: 66 = bovine serum albumin, 45 = chicken egg ovalbumin, 36 = rabbit muscle glyceraldehyde-3-phosphate, 29 = bovine erythrocyte carbonic anhydrase, 24 = bovine pancreas trypsinogen, 20 = soybean trypsin inhibitor, and 14.2 = bovine milk *b*-lactoalbumin. Lane 1, WSMV-infected pearl millet; lane 2, healthy pearl millet; lane 3, WSMV-infected PI550610 sorghum; lane 4, healthy PI550610 sorghum; lane 5, WSMV-infected Tomahawk wheat; and lane 6, healthy Tomahawk wheat. **(B)** Western blot. Treatments in lanes 1 to 6 are as described for **(A)**. Bands reacting to WSMV antiserum in the Western blot are equivalent to those marked by arrows in **(A)**.

inoculated and the other inoculated with WSMV), thus allowing all test plants to be surrounded by rows of PI550610 sorghum and all WSMV isolates in the same sorghum flat. After inoculation, flats containing the sorghum were randomized (block) and maintained in a greenhouse under natural lighting. At 21 days following inoculation, sorghum plants were thinned to equal numbers of infected plants, and each flat was watered with 100 ml of nutrient solution (11.7 g of a 20:20:20 N-P-K mix in 1 liter of distilled water). Sorghum was rated for symptom severity (by one rater who did not know the identity of the cultivars and virus isolates) at 42 days following inoculation. Sorghum was harvested immediately after the 42-day rating by cutting the plants off at the soil line; plants were bulked within a treatment, placed in paper bags, and dried to a constant weight in a forced-air oven at 65°C. Control plants were treated in a like manner. Temperature range for the three experiments was 18 to 37°C. The experiment was conducted three times with four replicates per experiment. Data were analyzed by ANOVA in a randomized complete block, and differences among means were compared with the Waller-Duncan multiple range test (19).

RESULTS AND DISCUSSION

Many rows of pearl millet and sorghum in nurseries at Hays had plants with systemic viruslike symptoms (Fig. 1). In the initial testing of symptomatic plants (three genotypes of one pearl millet [six samples] and 21 genotypes of sorghum [21 samples]) by ELISA, all samples were positive, but only against WSMV antiserum. Extracts from the six pearl millet and 10 of the 21 sorghum samples, when used to inoculate back-assay plants, induced symptoms in N28ht corn, Westford barley, foxtail millet, proso millet, Lodi oats, Bonel rye, and Tomahawk wheat. These assay plants tested positive by ELISA only to WSMV antiserum. Of the original samples, three pearl millet and five sorghum samples had ELISA values between 2 and 3 times that of the healthy control, and the others ranged from 4 to 9 times that of the healthy control. The extracts from the plants having ELISA values between 2 and 3 times the healthy values all infected wheat. When tested by ELISA, such infected wheat had ELISA values greater than 5 times the healthy control. Therefore, the value twice that of the healthy control was used as a positive threshold in ELISA in all studies.

We found foxtail millet to be susceptible to the H94PM, H94S, and H88 isolates used in the infectivity assays. This species has been reported as immune (24) and susceptible (23) to WSMV.

Concentration of WSMV proteins from symptomatic pearl millet, sorghum, and control wheat by minipurification and SDS-PAGE did not reveal bands different from those of the WSMV control (Fig. 2A). Gels were scanned by a densitometer in two experiments, and the relative MWs (in Daltons) of the capsid for the PM94, S94, and PV106 isolates were 48,789 and 47,826, 48,672 and 47,459, and 47,542 and 46,474, respectively. These results are within previously determined limits for WSMV (15,20). Because the values obtained for PV106 were similar to those obtained in previous work in our lab for both minipurified and highly purified samples (D. L. Seifers, *unpublished*), we did not further purify the pearl millet and sorghum WSMV. Results from Western blotting of these extracts also revealed identical banding patterns (Fig. 2B). Except for the report by Harvey and Seifers (10), who found one asymptomatic WSMV-infected sorghum line, sorghum has been reported as immune to WSMV (5,23,24,27), as has pearl millet (24). The sorghum plants observed in the field had distinct symptoms, in contrast to the previous report by Harvey and Seifers (10) that sorghum was asymptomatic. This prompted us to rate five sorghum and two pearl millet nurseries for incidence of WSMV along with concomitant testing of symptomatic plants by ELISA against WSMV, maize dwarf mosaic virus, sugarcane mosaic virus-MDMV-B, and johnsongrass mosaic virus antiserum. Thus, information was obtained on what lines were susceptible to natural infection by WSMV and any other viruses present.

The pearl millet and sorghum nurseries had various levels of infection (Table 2). All symptomatic pearl millet and sorghum samples were positive in ELISA only to WSMV antiserum. However, two samples of sorghum taken later in the season were positive in ELISA to both WSMV and

Table 2. Numbers of rows in pearl millet and sorghum nurseries having symptomatic plants testing positive by enzyme-linked immunosorbent assay (ELISA) to wheat streak mosaic virus, and number of rows with plants having different symptoms

Nursery	Rows rated	Symptomatic	Positive by ELISA	Rows with different symptoms		
				1[v]	2[w]	3[x]
Pearl millet[y]						
1	532	159	159	138	21	0
2	119	16	16	9	5	2
Sorghum[z]						
1	800	113	113	25	88	0
2	1,008	12	12	5	7	0
3	480	22	22	3	19	1
4	672	23	23	3	19	1
5	1,600	30	30	7	21	2

[v] Mosaic only.
[w] Mosaic with interveinal red or brown coloration.
[x] Mosaic with interveinal red or brown coloration and necrosis within the red and brown pigmented areas.
[y] Pearl millet nursery 1 planted on 2 June 1994 and rated on 3 and 4 August 1994; nursery 2 was planted on 7 June 1994 and rated on 22 August 1994.
[z] Sorghum nurseries 1 to 5 rated on 22, 22, 23, 23, and 24 July, respectively. Planting dates in 1994 of sorghum nurseries: 1 = 3 June, 2 = 3 June, 3 = 3 June, 4 = 7 June, 5 = 8 June.

Table 3. Number of experiments out of three in which a pearl millet (PM) line[y] had at least one plant with symptoms after mechanical inoculation with different wheat streak mosaic virus (WSMV) isolates[z] and incubation in a greenhouse for 42 days

PM line	Field infection (%)	WSMV isolate					
		H94S	H94PM	H88	H81	PV106	PV57
92-4938	1	0	2	1	1	0	1
93-4070	50	0	0	0	1	0	0
94-6743	25	0	0	0	1	0	0
92-1089	50	0	2	1	0	0	1
93-1229	5	0	1	0	0	0	1
94-6413	50	0	0	0	1	1	0
94-6547	90	1	0	0	0	1	0
94-6644	25	1	1	1	2	1	0
90-1126	1	0	0	1	0	0	0
90-1113	1	0	1	0	0	0	0
94-6608	1	0	0	1	1	2	1
92-1087	50	0	2	0	0	1	1
94-6425	90	0	0	1	1	0	1
93-4018	100	1	1	1	0	0	0

[y] Pearl millet line selected from those evaluated in the field as having 1 to 100 percent natural infection.
[z] H94PM and H94S isolated from pearl millet and sorghum, respectively, in 1994, and the H81 and H88 isolated from wheat in 1981 and 1988, respectively, at Hays, Kansas. The type specimen (PV57) and the PV106 isolate were obtained from the American Type Culture Collection.

johnsongrass mosaic virus antiserum. Randomly sampled nonsymptomatic pearl millet and sorghum tissues were always negative in ELISA. Infection estimates in rows with symptomatic plants ranged from 1 to 100%. For both pearl millet and sorghum, symptoms ranged from mosaic with interveinal reddening to mosaic with interveinal reddening and necrosis within the red areas. In some rows, all symptom types were observed, and each tested positive in ELISA against only WSMV antiserum. A possible explanation of the symptom diversity is that these rows contained segregating plants and thus were genetically variable. It is also possible that an additional virus or viruses were present and not identified but contributed to the different symptoms. However, the two sorghum plants reacting to both WSMV and johnsongrass mosaic virus antiserum had only mosaic symptoms and displayed neither discoloration nor necrosis. As the season progressed, plants in most of the sorghum lines had flag leaves that were symptomless. However, some lines had plants with systemic symptoms in the flag leaf. One of these lines (PI550610) had a 100% infection rate. This is an important sorghum line, because it is a source for resistance to Biotype I greenbugs (3) and is being used in many sorghum breeding programs. To determine if the WSMV isolates from sorghum and pearl millet were different from other WSMV isolates in their ability to infect these hosts, different pearl millet and sorghum lines were planted and inoculated mechanically with six different WSMV isolates with collection dates ranging from 1932 to 1994.

Of the 40 pearl millet lines that had been naturally infected in the field, only 14 became infected when mechanically inoculated. Not all were infected by the same isolate, but all isolates infected some of the lines at least once, although not in all experiments (Table 3). The success of mechanical infection of pearl millet was not any greater for lines 100% naturally infected than for lines with a 1% natural infection. In all three experiments, symptoms did not appear until 21 days following inoculation, with other plants developing symptoms up to 35 days following inoculation. Time of first symptom appearance for a given isolate varied among experiments. Tomahawk wheat controls developed symptoms by 7 days following inoculation. The fact that Sill and Agusiobo (23) were unable to infect this host may reflect the difficulty we observed in mechanically infecting pearl millet. Many pearl millet lines that were infected naturally could not be infected mechanically, indicating that wheat curl mites are more efficient than mechanical inoculation in infecting pearl millet.

Symptoms developed on plants of 18 of 71 sorghum lines inoculated mechanically with the WSMV isolates. The H94S isolate infected all 18; H94PM, 16; H88, 13; H81, 14; PV106, 2; and PV57 did not infect any sorghum lines (Table 4). The lines IS1056, PI550610, and TX626A were the only lines for which all plants developed symptoms, and often only one to a few plants developed symptoms in an experiment with the other lines. At 7 days following inoculation, no symptoms were observed in any sorghum line, but all Tomahawk wheat controls inoculated with each isolate had plants with symptoms. At 14 days following inoculation, PI550610 and TX626A were the only sorghums with symptoms. The H94S isolate caused symptoms in both, whereas the H94PM isolate caused symptoms only in PI550610, and the remaining WSMV isolates caused no symptom expression in any sorghum. At 21 through 28 days following inoculation, all isolates except PV57 caused symptoms in some sorghum lines. Symptomless plants were negative in ELISA. The type specimen (PV57), collected in Kansas in 1932 (1), differs from all other isolates used in these experiments, and the PV106 isolate collected in 1962 (1) was not characteristic of the WSMV H81, H88, H94PM, and H94S isolates collected at Hays in that it could infect only two sorghum lines. The lines IS1056, PI550610, and TX626A provided a means of dependably separating isolates, whereas the remaining lines were erratic with respect to reproducible infection between experiments. These aforementioned lines should provide an assay method for separating isolates for further testing and thus replace more subjective symptom assays. The line Sarvasi developed symptoms by 21 days following inoculation, but the virus did not spread systemically past leaf four as it did in the other lines with symptomatic plants. The delayed symptom expression explains our earlier results of not observing symptoms but detecting virus by ELISA, because those assays were terminated prior to 21 days following inoculation (10). Of the 71 sorghum lines tested, IS809, KS65A, Martin B, TX430R, TX635B,

Table 4. Number of sorghum lines[v] in experiments 1, 2, and 3 with symptomatic plants after incubation in a greenhouse for 42 days after mechanical inoculation with different wheat streak mosaic virus (WSMV) isolates[w]

Sorghum line	WSMV isolate																	
	H94S			H94PM			H88			H81			PV106			PV57		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
IS1056	+	+	+[x]	+	+	+	+	+	+	+	+	+	–	–	–[y]	–	–	–
KS65A	–	–	+	+	+	+	–	–	+	+	+	–	–	–	–	–	–	–
KS65B	+	+	+	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–
KS93R	–	–	+	–	–	–	–	+	–	+	–	–	–	–	–	–	–	–
KS95R	+	NP	NP[z]	NP	–	–	–	–	–	–	NP	–	NP	–	–	NP	NP	–
PI550610	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–
Saravasi	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–
TX428A	–	+	+	+	–	–	+	–	–	+	–	–	–	–	–	–	–	–
TX428B	+	–	–	+	–	–	+	–	–	+	–	+	–	–	–	–	–	–
TX431R	+	+	+	+	+	–	+	–	+	+	–	–	–	–	–	–	–	–
TX432R	–	–	–	+	NP	–	–	–	–	–	–	–	–	–	–	–	–	–
TX626A	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–
TX626B	+	+	+	+	–	+	+	–	–	+	+	+	–	–	–	–	–	–
TX636A	+	+	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–
TX636B	+	+	+	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–
TX2567R	+	+	+	–	+	+	–	+	–	–	+	+	–	–	–	–	–	–
TX2568	+	+	+	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–
TX2752B	+	–	+	NP	–	–	–	–	–	–	–	+	–	–	–	–	–	–
TX2816	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–

[v] Five to 10 plants were inoculated with a given isolate in each experiment.
[w] Collected from the following sources: H94S, sorghum at Hays, Kansas, in 1994; H94PM, pearl millet at Hays, 1994; H88, from wheat at Hays, 1988; H81, from wheat at Hays, 1981; and PV106 and PV57 from the American Type Culture Collection (Rockville, MD).
[x] At least one symptomatic and ELISA positive (WSMV) plants in the experiment.
[y] No symptomatic or ELISA positive (WSMV) plants in the experiment.
[z] NP = No plants grew after planting seed.

Table 5. Number of pearl millet (PM), sorghum (S), and Tomahawk wheat (TW) plants with symptoms 21 days following infestation with five wheat curl mites per plant that had previously fed on symptomatic sorghum or pearl millet testing positive by enzyme-linked immunosorbent assay (ELISA)[y] to wheat streak mosaic virus

Experiment number	From PM to		From S to	
	PM	TW	S	TW
1	4/20[z]	10/10	2/27	6/9
2	6/20	10/10	10/20	8/20
3	1/20	10/10	7/10	6/9

[y] All pearl millet, sorghum, and wheat plants infested with wheat curl mites were tested by ELISA against WSMV antiserum, all symptomatic plants were positive, and all symptomless plants were negative.

[z] Numerator represents the number of plants with symptoms, and denominator represents the total number of plants infested with wheat curl mites.

Table 6. Average[x] symptom rating[y] of leaves 3, 4, 5, and 6 of PI550610 sorghum plants inoculated 42 days previously with different wheat streak mosaic virus (WSMV) isolates

Leaf[z] number	WSMV isolate		
	H94PM	H94S	PV106
6	1.33	2.41	1.00
5	3.88	5.23	2.70
4	4.59	5.62	3.25
3	1.95	3.37	1.49

[x] The average values for the symptom ratings for each leaf were from a total of 68 PI550610 sorghum plants for each WSMV isolate from the three experiments.

[y] Symptom ratings are as follows: 1 = no symptoms; 2 = faint mosaic (FM) (faint yellow dashes forming elongated streaks covering only small areas of the leaf); 3 = mosaic (M) (prominent yellow dashes covering leaf); 4 = severe mosaic (SM) (severe yellow dashes forming large yellow mosaic areas over leaf); 5 = FM plus red necrotic streaks within mosaic areas; 6 = M plus red necrotic streaks within mosaic areas; 7 = SM plus red necrotic streaks within mosaic areas.

[z] Leaf 6 is youngest leaf, and leaf 3 is the second leaf above the primary leaf.

Table 7. Dry weights from symptomatic PI550610[y] sorghum mechanically inoculated 42 days previously with various wheat streak mosaic virus (WSMV) isolates

Treatment	Dry wt in mg
Healthy PI550610	926 a[w]
PI550610 × PV106[x]	466 b
PI550610 × H94PM[y]	415 b
PI550610 × H94S[z]	318 c
LSD (0.05)	94

[y] PI550610 sorghum used as host because of its importance as a source for Biotype I greenbug resistance (2).

[w] The values are averages from three experiments with four replicates per experiment. Means not followed by the same letter are significantly different ($P = 0.05$) according to the Waller-Duncan multiple range test (17). Dry weights were from a total of 68 PI550610 sorghum plants for each WSMV isolate from the three experiments and compared to an equal number of mock-inoculated PI550610 sorghum plants.

[x] PV106 = WSMV isolate obtained from American Type Culture Collection, Rockville, MD.

[y] H94PM = WSMV isolated from pearl millet at Hays, KS, in 1994.

[z] H94S = WSMV isolated from sorghum at Hays in 1994.

TX2536R, and TX7000 were found naturally infected in the field but could not be infected mechanically. As with pearl millet, wheat curl mites are able to infect more sorghum lines than can be done mechanically. To determine if wheat curl mites could acquire WSMV from pearl millet and sorghum, we conducted studies using these hosts as acquisition sources.

Wheat curl mites were able to acquire WSMV from mechanically inoculated symptomatic pearl millet or sorghum and cause infection of the host from which the virus was acquired and also of wheat in each of the three experiments (Table 5). Wheat curl mites rarely could be found on the pearl millet or sorghum plants after 24 h. Seedling sorghum has been reported as a host (poor to very good depending on the cultivar) for wheat curl mites (7,10). Mature sorghum was a poor host, with wheat curl mites confined mainly to the leaf whorl. Field studies of sorghum as a wheat curl mite host are lacking. Wheat curl mites have been reported to occur on field-grown pearl millet (27), but no information was available about wheat curl mite numbers and duration of colonization, nor was information provided about seedling pearl millet as a wheat curl mite host.

With the exception of foxtail millet (shown in original back-assays to be infected by H94PM, H94S, and H88), all the grasses listed in Table 1 were mechanically inoculated in two different experiments with the H94S, HPM94, H88, H81, PV106, and PV57 WSMV isolates. Only wild oat (*Avena fatua* L.) plants were infected and by all isolates. This grass has been reported as immune (24) and susceptible (23) to WSMV. Because all isolates infected this grass and caused symptoms, the difference in results reported in the literature may have been due to different seed sources used. All grasses inoculated, with and without symptoms, were tested by ELISA, but only symptomatic plants tested positive. The maize line H84 also was included in these tests because it was reported to be susceptible to some, but not all, Kansas WSMV isolates (18). We could not infect this maize line with any of the six isolates, indicating that the isolates were different than those used in the earlier study that infected H84 maize. The isolates reported to infect H84 maize were no longer available for comparison.

Symptom severity rating on a single leaf of PI550610 sorghum showed that the H94S isolate caused more severe symptoms in each leaf than did any of the other isolates (Table 6). The H94PM isolate caused slightly more severe symptoms than the PV106 isolate. Both of these isolates caused either faint or no symptoms in leaf six, whereas the H94S isolate caused some prominent symptoms in leaf six.

When the symptomatic sorghum was harvested and dried, significant reductions in dry weight were noted for plants inoculated with each WSMV isolate when compared to the mock-inoculated controls. The H94S isolate caused the most severe reduction in dry weight (Table 7). Data analysis showed the experiment by treatment (virus isolate) variation to have an F value of 0.69, indicating no interaction, so the results of one experiment could predict those of the other experiments.

This study has shown that pearl millet and sorghum plants having prominent viruslike symptoms in the field were infected with WSMV. This is the first report of natural infection of these hosts, which hitherto have been considered immune to WSMV. The sorghum and pearl millet WSMV isolates were vectored by wheat curl mites from sorghum and pearl millet back to those hosts and to wheat from either host. Comparison of these WSMV isolates with other isolates collected at different times demonstrated that the type specimen (PV57) could not infect sorghum, whereas the other isolates could, but at different levels. The virus isolates caused differences in symptom severity in sorghum and in amount of dry weight reduction in the assay host PI550610, a sorghum resistant to Biotype I greenbug but very susceptible to WSMV. Sorghum may have a limited role in spread of the virus back into wheat, depending on whether wheat curl mites can colonize WSMV-infected sorghum at the time of wheat emergence. A problem that could arise is the creation of susceptible sorghums by inadvertently making crosses with susceptible but important sorghum lines, such as PI550610. If this were to occur, sorghum could suffer serious losses following WSMV infection when viruliferous wheat curl mites moved from ripening wheat into the newly emerged sorghum.

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