Occurrence and Symptom Expression of American Wheat Striate Mosaic Virus in Wheat in Kansas

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ABSTRACT

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Extracts of winter wheat with viruslike symptoms from Comanche County, Kans., did not react with antisera to viruses previously found infecting wheat in the state, nor was the virus mechanically transmissible to wheat, maize, or sorghum. Leaves of infected wheat developed thin, chlorotic striations that were more severe on the abaxial than adaxial surface. The virus reacted with American wheat striate mosaic virus (AWSMV) antiserum and was vectored by the painted leafhopper (*Endria inimica*), confirming the virus as AWSMV. A protein of 59 kDa, consistently detected in extracts of infected leaves, reacted with antiserum to AWSMV in Western blots. This is the first report of AWSMV in the Southern Great Plains region. Experiments showed that, on some cultivars, AWSMV caused brown necrotic streaking of culms and glumes, a symptom not described previously for wheat infected by this virus. Hard red winter wheat cultivars Ike, Karl, and TAM 107 had positive enzyme-linked immunosorbent assay values, but exhibited few or no symptoms.

American wheat striate mosaic virus (AWSMV) was first reported in the United States in 1953 (12). The main vector of AWSMV is the painted leafhopper *Endria inimica* (14). The virus has been reported in the north central United States and Canada (3,4,12–14,16). Infection of wheat (*Triticum aestivum* L.) causes a progression of symptoms starting with thin, parallel, white or chlorotic streaks that are more severe on the abaxial leaf surface. Streaks may spread over the entire leaf and may become necrotic (19).

In 1992, an unidentified virus was found infecting wheat in Kansas. The virus did not react to antisera of viruses previously found infecting wheat or sorghum in the state. We report experiments verifying the unknown virus to be AWSMV, which has not been reported previously in Kansas, and show that infection of wheat by the virus occasionally induced necrotic symptoms on culms and glumes that had not been previously described for ASWMV-infected wheat. In addition, significant differences in cultivar reaction to infection were observed.

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MATERIALS AND METHODS

Virus isolates. AWSMV isolate PV126 was obtained from the American Type Culture Collection (Rockville, Md.) and was used as the positive control in enzyme-linked immunosorbent assays (ELISA).

Antiserum. Antiserum to the following viruses was prepared using previously described procedures: wheat streak mosaic virus (WSMV) (8), maize dwarf mosaic virus (MDMV) (9), sugarcane mosaic virus strain MDMV-B (SCMV-MDMVB) (9), and Agropyron mosaic virus (AMV) (8). Antiserum to AWSMV was obtained from R. C. Sinha (Ottawa, Ontario, Canada).

Indirect ELISA. Leaf tissue was ground in 0.05 M carbonate buffer, pH 9.6, (2). Samples (200-µl aliquots) were placed in wells of ELISA plates (Immulon 1, Dynatech Laboratories, Inc., Chantilly, Va.) for 1 h at 37°C. Wells were rinsed and charged with antibody (5 µg/ml) in blocking buffer (2) for 1 h at 37°C to allow antibody to bind to the antigens. Plates were rinsed and wells were blocked with a solution containing 5% (wt/vol) nonfat dry milk and 0.01% (vol/vol) antifoam A made in phosphate buffered saline, pH 7.0, for 1 h at 37°C. Then anti-rabbit/antibody/alkaline phosphatase conjugate (Sigma Chemical Co., St. Louis, Mo.) in blocking buffer (1: 1,000, vol/vol dilution) was added to the wells and the plates were incubated for 1 h at 37°C. The plates were rinsed, substrate (p-nitrophenyl phosphate, 0.714 mg/ml) in substrate buffer (2) was added and the plates were incubated for 30 min at room temperature. Absorbance at (405_{nm}) was measured using a Titertek Mulitskan micro ELISA plate reader (Flow Laboratories, Inc., Huntsville, Ala.) and values were considered positive if they were twice those of the equivalent mock-inoculated control.

Field sampling of naturally infected wheat. The original Mesa wheat plants with viruslike symptoms observed in the fall of 1992 were dug up, potted, and moved into a growth chamber (Warren/Sherer Model CEL39-15) maintained at 25°C and a 12-h photoperiod of fluorescent light (approximately 500 µmol · sec^{-1} m^{-2}).

Additional wheat plants with symptoms were obtained from the field in the spring of 1993 and transported to the laboratory. All plants were tested by indirect ELISA for AWSMV and WSMV, and AWSMV-infected plants free of WSMV. Plants not doubly infected with wheat streak mosaic virus were used for further experiments.

Infectivity assays. Ten days after planting, wheat cv. Tomahawk, N28 Ht corn (*Zea mays* L.), and Golden Acres TE76 sorghum (*Sorghum bicolor* (L.) Moench) plants were inoculated manually as previously described (8). Inoculated plants were maintained in a greenhouse under natural light conditions at temperatures of 18 to 27°C.

Electron microscopy procedures. Leaf tissue collected from naturally infected wheat plants in Comanche County was fixed in 0.5% PIPES [piperazine-*N*-*N*′-bis(2-ethanesulfonic acid)], pH 8.0 buffer made to 5% with glutaraldehyde and post-fixed in 2% osmium tetroxide in 0.18 M PIPES, pH 6.8 buffer (10). Thin sections obtained by ultramicrotomy were stained with 5% uranyl acetate in 50% ethyl alcohol (EtOH) followed by Reynold's lead citrate and then viewed with a Philips EM 201 transmission electron microscope at 60 and 80 KV.

Minipurification, SDS-PAGE, and Western blotting of viral proteins. The extraction and partial purification of virus protein were done according to Lane (7). Leaf tissue (1 g) was ground (1:5 wt/vol) in extraction buffer (0.1 M ammonium citrate buffer, pH 6.5, containing 0.25% mercaptoethanol, 0.1% sodium diethyldithiocarbamate, and 1% polyvinylpyrrolidone) using a mortar and pestle. 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 the mixture was centrifuged through a 1-cm layer of 0.988 M sucrose (in ammonium citrate buffer) for 2 h at 85,000 *g*. The pellet was resuspended in 200 µl of SDS (sodium dodecyl sulfate) treatment buffer (0.125 M Tris-Cl, pH 6.8, 4% SDS, 20% glycerol, and 10% 2-mercaptoethanol); the mixture was heated for 4 min at 100 C; reduced; and alkylated (6); then 25 µl was loaded into a well of a 10% gel. SDS-PAGE (polyacrylamide gel electrophoresis) was conducted using the procedure of Laemmli (5). The gels were stained with Coomassie blue R-250 and scanned using a Hoefer GS300 densitometer. The data were processed using Hoefer GS-365 data analysis software that calculated values for the molecular masses of separated proteins using a linear regression fit to protein standards.

All electrophoretic analyses were conducted three times with extracts from different plants. Healthy wheat was treated identically for all determinations.

For Western blotting, extractions and separations on SDS-PAGE were done as described above for both healthy and AWSMV-infected wheat. 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 membranes (Nitro-Cell 0.45 µm, Hoefer, San Francisco, Calif.) 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, Calif.) using a transfer buffer consisting of 39 mM glycine, 40 mM Tris-Cl, pH 8.3, 0.037% SDS, and 20% (vol/vol) methanol (17). The immunoblotting was done using alkaline phosphatase anti-rabbit goat antibody as described by Bollig (1).

Virus transmission. The painted leafhopper *E. inimica* (the reported vector of AWSMV) (14) and *Macrosteles quadrilineatus* (Forbes) (nonvector of AWSMV) (14) were used in vector experiments. The leafhoppers were collected from bermudagrass in a field at Hays, Kans., and their identities confirmed by Derrick Blocker (Kansas State University, Manhattan). The original leafhoppers were tested for presence of AWSMV by infesting wheat cv. Arkan (two-leaf stage planted into "plant cells" [2.4 × 16.5 cm plastic planting cones; Stuewe & Sons, Corvallis, Oreg.]) for 7 days. The plants were covered with vented, transparent, plastic, tubular cages (2.3 × 30.5 cm) to contain the leafhoppers and prevent possible contamination by wheat curl mites (*Eriophyes tulipae* (Keifer)). After 4 days, the leafhoppers were moved onto other Arkan or Mesa wheat and caged as described above. The original plants then were incubated for 14 more days in the greenhouse, after which symptoms were recorded and the plants tested for AWSMV and WSMV by indirect ELISA. Leafhoppers free of AWSMV intended for vectoring studies were obtained from the wheat infested with leafhoppers from the original source that did not cause AWSMV-infection of wheat.

To test vectoring ability, the leafhoppers shown to be free of AWSMV were allowed a 4-day acquisition period on AWSMV-infected or healthy wheat and then transferred to Mesa and TAM 107 wheat (two- to three-leaf stage) for 7 days. Then the leafhoppers were removed by fumigation (3 h exposure in a sealed box containing a "Vapona" strip; active ingredient 2,2-Dichlorovinyl dimethyl phosphate). Following removal of the leafhoppers, the Mesa and TAM 107 wheat plants were incubated for 3 weeks in a greenhouse (20 to 29°C), rated for symptoms, and tested for AWSMV and WSMV by ELISA. The experiment was conducted three times.

Culm and glume symptoms. Seeds of wheat (Ike, Karl, Mesa, TAM 107, Thunderbird, and Tomahawk) were planted into plant cells as previously described, and 10 seedlings of each cultivar in experiment 1 and five seedlings in experiment 2 were infested with three painted leafhopper adults (after a 14-day acquisition period on AWSMV-infected wheat) and covered with clear cylindrical cages as described above. The leafhoppers were allowed to feed on the test plants for 14 days and were then removed by fumigation as described above. Plants were then vernalized for 8 weeks at 4°C and an 8-h photoperiod of fluorescent light. Following vernalization, the plants were removed from the plant cells and planted into clay pots (12.5 cm deep and 15 cm diameter at top). A third set of plants was placed into the vernilizing chamber for 8 weeks before being infested with leafhoppers and planted into clay pots as described above. Once in clay pots, the plants were incubated in a green-

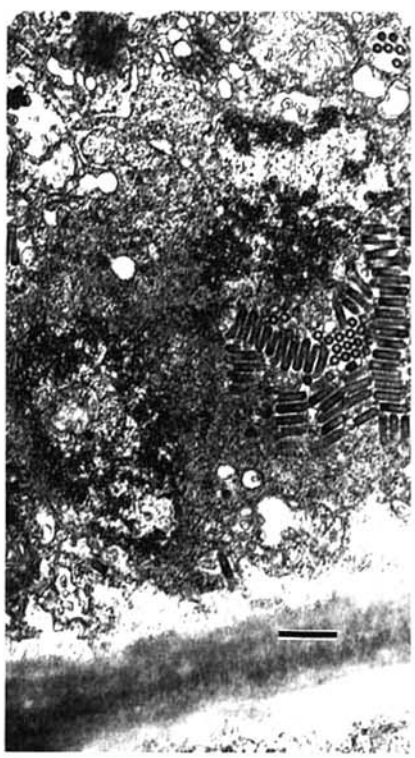

Fig. 1. Bacilliform particles in a thin-section of leaf tissue from Mesawheat expressing symptoms similar to those reported for American wheat striate mosaic virus. Bar represents 500 nm.

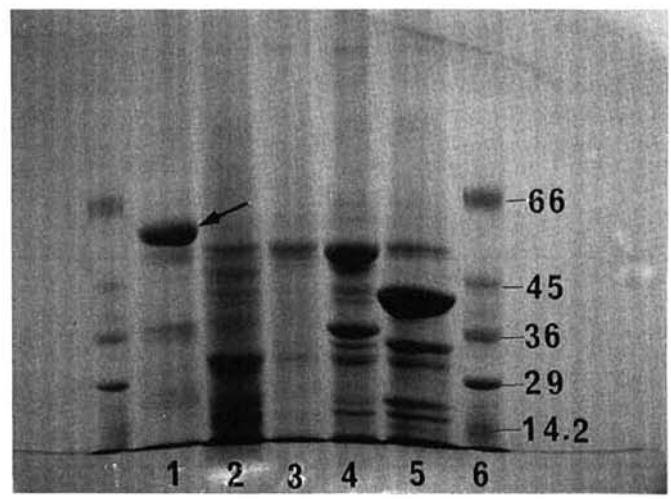

Fig. 2. American wheat striate mosaic virus (AWSMV) concentrated by minipurification; separated under sodium dodecyl sulfate–polyacrylamide gel electrophoresis; and stained with Coomassie blue (0.125% Coomassie blue R-250, 50% methanol, and 10% acetic acid). Lane 1, AWSMV-infected Mesa wheat, arrow indicates position of 59-kDa AWSMV protein; lane 2, healthy wheat; lane 3, ryegrass mosaic virus–infected *Lolium multiflorum;* lane 4, wheat streak mosaic virus–infected wheat; lane 5, Hordeum mosaic virus–infected wheat; and lane 6, molecular mass standards: bovine serum albumin (66 kDa), egg albumin (45 kDa), glyceraldehyde-3-P-dehydrogenase (36 kDa), bovine carbonic anhydrase (29 kDa), bovine pancreas trypsinogen (24 kDa), soybean trypsin inhibitor (20 kDa), and bovine milk a-lactalbumin (14.2 kDa). Numbers to right of lane 7 indicate the molecular masses (in kilodaltons) of protein markers. Electrophoresis was in a 10% gel 0.75 mm thick. Duration of electrophoresis was 3 h with starting and ending voltages of 90 and 250, respectively.

house (temperature range 19 to 30°C) under natural lighting conditions. Plants were rated for symptoms on the culms and glumes. Extracts of culms and glumes were also assayed for AWSMV by indirect ELISA. Pieces of culm and glume tissues were tested for bacterial and fungal pathogens following surface sterilization (30 s in 0.5% sodium hypochlorite followed by 30 s in 70% [vol/vol] ethanol), by plating them onto potato-dextrose agar, and incubating them for 3 weeks. The plates were sealed with Parafilm to prevent contamination. Controls (five plants of each cultivar) were infested with painted leafhoppers that had fed only on healthy wheat. Wheat cultivars used in this study were chosen because of symptoms expressed under field conditions: Mesa, Tomahawk, and Thunderbird had brown streaks on the culms and some glumes, and Ike, Karl, and TAM 107 did not.

RESULTS AND DISCUSSION

When the original Mesa wheat plants acquired from the field were placed in growth chambers at 25°C, their leaves developed fine chlorotic lines, which were more pronounced on the abaxial leaf surface, and necrosis in older parts. The symptoms were similar to those described for AWSMV-infected wheat (12) and extracts from such leaves tested positive for AWSMV in ELISA using AWSMV antiserum. WSMV, MDMV, SCMV-MDMV-B, johnsongrass mosaic virus (JGMV), and AMV were not found when leaf extracts from the same plants were tested in ELISA using the appropriate antisera. Tomahawk wheat, N28Ht corn, and G550E sorghum inoculated with extracts from symptomatic tissue from these plants failed to produce symptoms, whereas extracts from WSMV-infected wheat produced symptoms in 7 days in 10 of 10 wheat and corn plants but not in the sorghum assay plants.

Bacilliform-shaped particles (250 × 75 nm), characteristic of those described for AWSMV (11), were found in thin sections of Mesa leaf tissue (Fig. 1) but not in control tissue. Antiserum made against AWSMV (obtained from R. C. Sinha) reacted with extracts of infected wheat in indirect ELISA as well as with the ATCC isolate PV126, indicating that the two isolates were serologically related. Extracts from the Mesa leaf tissue used for thin-sectioning gave ELISA values two to three times those of the healthy wheat sample. Because virus particles were observed in the thin-sectioned tissue we maintained a value two times the healthy control as a positive threshold for ELISA in all studies. In SDS-PAGE of extracts from wheat infected with AWSMV or any of several other wheat-infecting viruses (WSMV, SCMV-MDMV-B, AMV) and virus-free wheat, a protein, unique to AWSMV-infected plants and of approximately 59 kDa, was consistently detected (Fig. 2). The same protein was detected in SDS-PAGE of extracts from symptomless TAM 107 wheat that tested negative in ELISA (Fig. 3A, lane 1), from symptomless TAM 107 wheat that tested positive to AWSMV in ELISA, and from Mesa wheat that showed typical symptoms of the disease (Fig. 3A, lanes 2 to 3 and 4 to 5, respectively). This protein reacted with AWSMV antiserum in Western blots (Fig. 3B, lanes 2 to 5). The positive label of the approximately 17-kDa protein in lanes 1 and 6 is a host protein. Although the protein was visible in Coomassie blue stain (Fig. 3A, lane 1) it was not visible in the corresponding lane in the Western blot, suggesting that its concentration might have been below the threshold for detection by ELISA and immunoblotting. All extracts that tested positive in immunoblots contained proteins of approximately 27 and 59 kDa, which are similar in mass to the N- and M- proteins of ASWMV (18). Two additional proteins of 92 and 145 kDa (surface G protein and L protein, respectively) were not detected, presumably because they were removed from the virus following use of Triton X-100 during purification.

Three of 30 plants infested with painted leafhoppers initially collected from the field at Hays, Kans., developed faint symptoms and tested positive in ELISA to AWSMV and negative to WSMV, indicat-

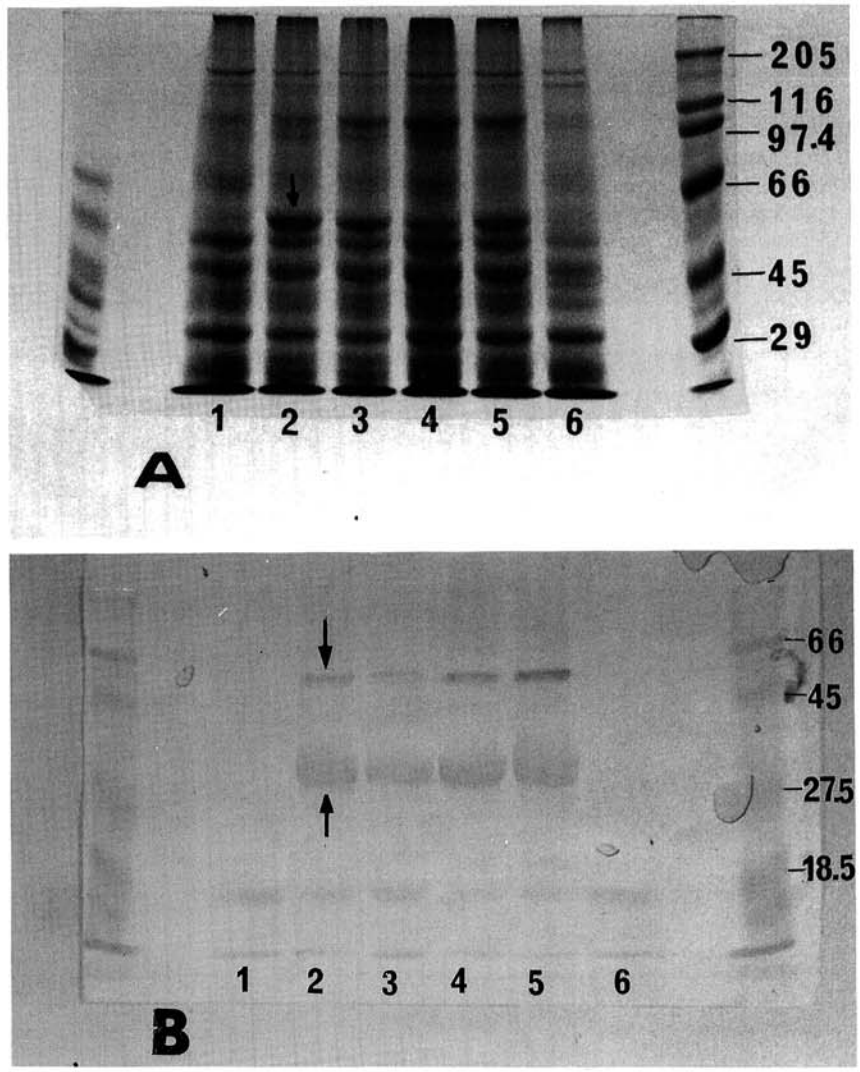

Fig. 3. Analysis of American wheat striate mosaic virus (AWSMV) proteins concentrated from wheat by minipurification and separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (A) and probed with antibodies to AWSMV (1:600 dilution) and anti-rabbit goat antibody:alkaline phosphatase conjugate (1:4,000 dilution) in a Western blot (B). **(A)** SDS-PAGE gel counterpart to that used for Western blot. Lane 1, TAM 107 negative in enzyme-linked immunosorbent assay (ELISA) and symptomless; lanes 2 to 3, TAM 107 positive in ELISA but symptomless; lanes 4 to 5, extracts from symptomatic Mesa wheat positive in ELISA; lane 6, extract from healthy TAM 107 wheat. Arrow marks a band of approximately 59 kDa found only in lanes 2 to 5. **(B)** Western blot of SDS-PAGE. Treatments in lanes are as described for (A). Arrows mark two bands (approximately 27 kDa and 59 kDa) in lanes 2 to 5 reacting to the AWSMV antiserum. A third band at the front of the gel is present in samples from healthy and AWSMV-infected wheat. Numbers (in kilodaltons) on right indicate position of molecular mass markers (prestained markers used for Western blot).

ing that some of the leafhoppers in Ellis County, Kans., had acquired AWSMV. The leafhoppers that fed on ELISA-negative wheat were used to rear leafhoppers to test transmission of AWSMV from the original plants (obtained in Comanche County, Kans.) to other wheat.

Thirty-seven percent of Mesa and 33% of TAM 107 plants, respectively, infested with the painted leafhopper (*E. inimica*) that had fed on AWSMV-infected source plants expressed typical symptoms of the disease. All plants expressing symptoms tested positive in ELISA for AWSMV and negative for WSMV. However, an additional 20% of the TAM 107 plants were symptomless, but a low level of ASWMV was detected by ELISA, perhaps indicating that a threshold level of virus is required for symptom expression. The time required for symptom expression in wheat at 30°C was 6 to 25 days, and longer at lower temperatures (15).

Wheat plants infested with *M. quadrilineatus* given access feeding on AWSMV-infected source plants remained symptomless and extracts of such plants did not react with antisera to either AWSMV or WSMV. Transmission of the Kansas isolate by *E. inimica* further suggests that the virus is AWSMV, previously reported only in Canada, Montana, Minnesota, North Dakota, and South Dakota (4,12,13,16).

During the experiments, three of five Mesa plants developed brown streaks on culms and on some glumes (Fig. 4) following their transfer from the field into a greenhouse. Affected culms and glumes were infected with AWSMV. The necrotic symptoms were typical of those of basal glume rot (19), black chaff (19), or Septoria glume blotch (19), but they did not exhibit shriveled seed, bacterial exudate, or pycnida, as would be expected for these diseases. Similar symptoms occurred on the culms of many ASWMV-infected Mesa plants in field plants in Comanche County but no such symptoms were observed on seven and four AWSMV-infected plants of TAM 107 and Ike, respectively, in Ellis County.

In two controlled experiments, plants of Ike, Karl, and TAM 107 were symptomlessly infected with AWSMV following infestation by viruliferous leafhoppers and virus could be readily detected in extracts of flag leaves by ELISA (Table 1). With but few exceptions, extracts of culms and glumes on infected plants tested positive in ELISA. Flag leaves of AWSMV-infected Mesa, Thunderbird, and Tomahawk exhibited symptoms and the culms and glumes of most of these plants exhibited brown necrotic streaks.

Upon plating of glume and culm tissues in experiments 1 and 2, only saprophytic fungi were recovered. There was no correlation between virus infection or absorbency of tissue extracts in ELISA and recovery of such fungi. Streak of culms and glumes was observed in the spring of 1994 in some ASWMV-infected cultivars and in some doubly infected with AWSMV and WSMV in a replicated yield test plot. The affected cultivars (Tomahawk, Rio Blanco, Laredo, Ponderosa, Ogallala, Vista, TAM 200, and Longhorn) represented cultivars from different breeding programs. As expected, plants of TAM 107, Karl, and Ike did not exhibit such symptoms. No attempts were made to isolate pathogenic bacteria or fungi from affected glumes and culms.

Because no other pathogens were isolated from affected glumes and culms, and control plants infested with nonviruliferous leafhoppers did not exhibit such symptoms, we conclude that culm and glume necrosis are due to infection by

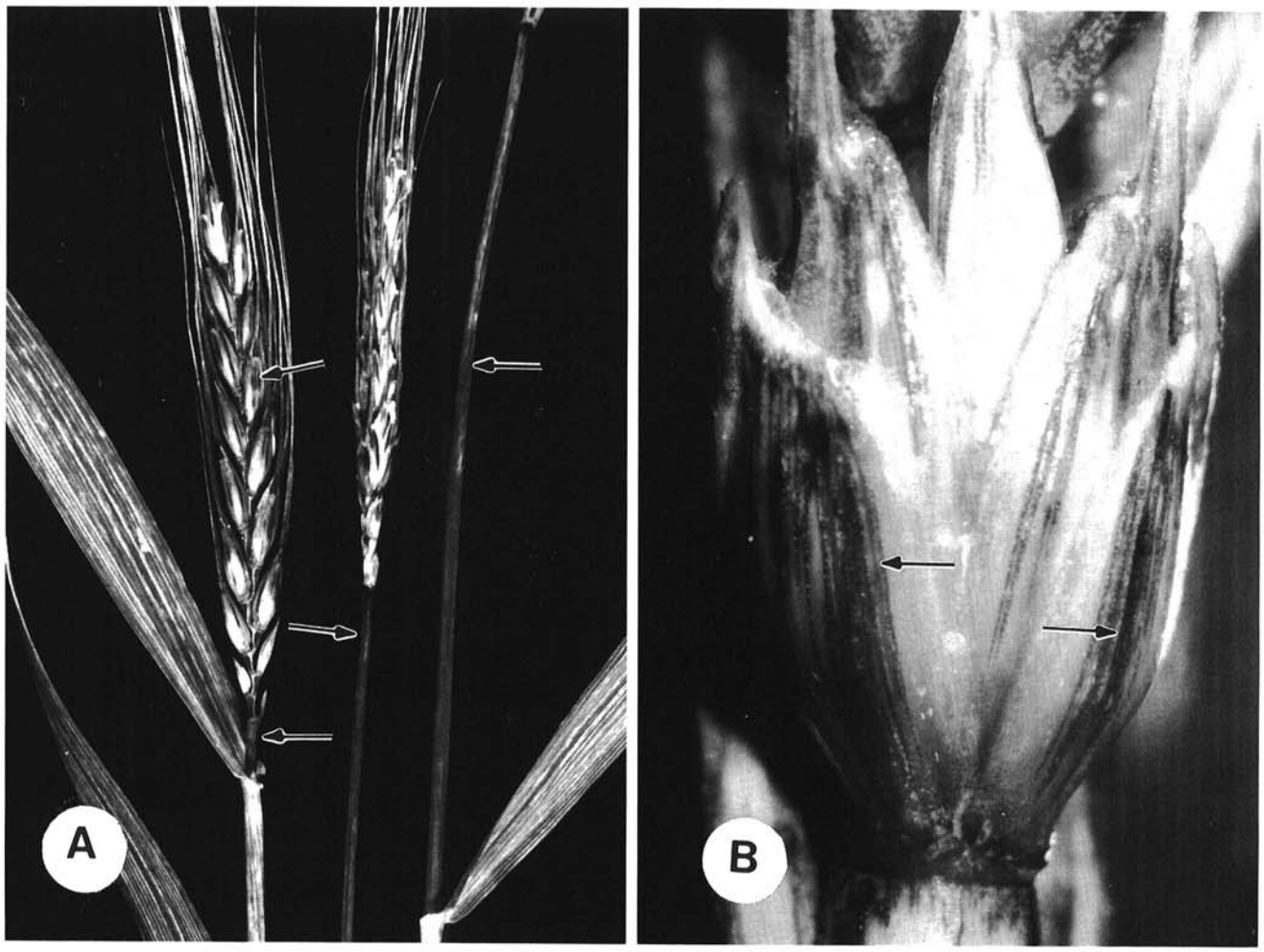

Fig. 4. Symptoms on Mesa wheat infected by American wheat striate mosaic virus: **(A)** arrows indicate black streaks and black discoloration of culm and glume tissue; **(B)** arrows indicate black streaks on glume tissue.

AWSMV. However, the reason for the sporadic incidence of necrosis is unknown. It is possible that necrosis is dependent on virus titer in affected tissues, but this was not tested because our ELISA tests were not quantitative. In any case, we found a strong effect of host genotype on symptoms expression, in contrast to the results of Timian (16), who observed no differences in symptom expression among spring wheat cultivars.

This is the first report of AWSMV in the Southern Great Plains. The Kansas isolate, which is serologically related to the PV126 isolate, is widely distributed in the state, occurring in planted and volunteer wheat plants in the following counties: Cheyenne, Comanche, Cowley, Edwards, Ellis, Finney, Ford, Hamilton, Harvey, Kearny, Kingman, Labette, Logan, Marion, Osborne, Riley, Saline, Scott, Stafford, Stevens, Thomas, and Wallace. In August 1994, AWSMV infection of dent corn, a known host of the virus (4) was confirmed by ELISA. Despite its wide distribution in Kansas, disease incidence across the state is usually less than 1%, and we have observed a maximum of 5% infected plants in production fields. The effect of AWSMV on wheat yield in Kansas remains to be assessed.

Table 1. Symptom expression of flag leaves (F), culms (C), and glumes (G) of greenhouse-grown wheat plants following infection by American wheat striate mosaic virus (AWSMV) and plant tissues positive (*) in enzyme-linked immunosorbent assay (ELISA)[x]

	Experiment					
	1			2		
	Symptoms			Symptoms		
Wheat entry and plant number	F	C	G	F	C	G
Ike						
1	–*	–*[y]	–*	–*	–*	–*
2	–*	–*	–*	–*	–*	–*
3	–*	–*	–*	–*	–*	–*
4	–*	–*	–*	–*	–*	–*
5	–*	–*	–*		No plant	
Karl						
1	–*	–*	–*	–*	–*	–*
2	–*	–*	–*	–*	–*	–*
3	–*	–*	–*	–*	–*	–*
4	–*	–*	–*	–*	–*	–*
5	–	–	–	–*	–*	–*
6	–	–	–		No plant	
7	–*	–*	–*		No plant	
8	–	–	–		No plant	
9	–*	–*	–*		No plant	
10	–	–	–		No plant	
Mesa						
1	+*[z]	–*	–*	+*	–	–
2	–	–	–	–*	–	–
3	+*	+*	+*	+*	–*	–*
4	+*	–*	–*	+*	–*	–*
5	+*	–*	–*	+*	+*	+*
6	–	–	–		No plant	
7	+*	–*	–*		No plant	
8	+*	–*	–*		No plant	
9	–	–	–		No plant	
10	+*	–*	–*		No plant	
TAM 107						
1	–	–	–	–*	–*	*
2	–	–	–	–	–	–
3	–	–	–	–	–*	–
4	–	–	–	–	–*	–*
5	–	–	–	–*	–*	–*
6	–*	–*	–*		No plant	
7		No plant			No plant	
8		No plant			No plant	
9	–*	–*	–*		No plant	
10	–*	–*	–*		No plant	
Thunderbird						
1	+*	+*	+*	–	–	–
2	+*	–*	–*	–	–	–
3	+*	–*	–*	+*	+*	+*
4	+*	–*	–*	+*	+*	+*
5	+*	+*	+*		No plant	
6	+*	–*	–*		No plant	
7	+*	–*	–*		No plant	
8	–	–	–		No plant	
9	–	–	–		No plant	
10	+*	–*	–*		No plant	
Tomahawk						
1	+*	+*	+*	+*	+*	+*
2	+*	+*	+*		No plant	
3	+*	No head	–	–	–	
4	–	–	–	+*	+*	+*
5	+*	+*	+*		No plant	
PV 126		0.579			0.871	
Healthy wheat	0.195	0.106	0.099	0.170	0.110	0.103

[x] ELISA conditions:AWSMV antiserum at 1:200 vol/vol dilution from a 1 mg/ml stock and antirabbit goat alkaline phosphatase-labeled antiserum was at a 1:1,000 vol/vol dilution. Plate reaction time was 30 min. An ELISA value was considered positive (*) if it was equal to or greater than twice that of the equivalent healthy control value.

[y] – = No symptoms.

[z] + = Leaf with striations or brown streaking and discoloration symptoms on culms and glumes.

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In the article "Conidial Germination and Appressorial Formation of *Colletotrichum capsici* and *C. gloeosporioides* Isolates from Pepper" by J. B. Manandhar, G. L. Hartman, and T. C. Wang on pages 361-366, reference 15 should read, Manandhar, J. B., Hartman, G. L., and Wang, T. C. 1995. Anthracnose development on pepper fruits inoculated with *Colletotrichum gloeosporioides.* Plant Dis. 79:380-383.