Identification of Wheat Spindle Streak Mosaic Virus and Its Role in a New Disease of Winter Wheat in Kansas

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ABSTRACT

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Severe mosaic, yellowing, bronzing, and stunting symptoms in patterns typical of the *Polymyxa graminis*-vectored wheat soilborne mosaic virus (WSBMV) appeared in early March 1984 and again in the spring of 1985 in south central Kansas. Many of the hard red winter wheat cultivars showing symptoms were considered resistant to WSBMV. Electron microscopic examination revealed WSBMV particles and other long, flexuous rods. Examination of thin sections revealed pinwheel and amorphous inclusion bodies. From these observations and with Western blot analysis, it was determined that the plants were also infected with wheat spindle streak mosaic virus (WSSMV). WSBMV and WSSMV particles were observed in all symptomatic plants in a ratio of about 20:1, respectively. A comparison of wheat plants resistant to wheat soilborne mosaic (WSBM) in soils infested with WSBMV and WSBMV plus WSSMV indicated that WSBMV infected WSBM-resistant cultivars and increased to about the same levels as in WSBM-susceptible cultivars in the presence of a WSSMV infection. Environmental conditions during the springs 1984 and 1985 were optimal for wheat spindle streak mosaic (WSSM) symptom development. The sudden recognition of this disease is attributed to several new WSBM-resistant cultivars that show vivid symptoms when infected with WSBMV plus WSSMV and the conducive environmental conditions. WSSM has now been shown to be widely distributed throughout the eastern half of Kansas and, in many cases, WSSM entirely overlaps in range with WSBM.

Wheat soilborne mosaic virus (WSBMV) causes a major disease of hard red winter wheat in Kansas. The virus is vectored through the soil by the fungus *Polymyxa graminis* Led. (9). Once the soil is infested with the fungus and virus, it remains infested indefinitely (8). Wheat soilborne mosaic (WSBM) symptoms appear early in the spring with cool and wet weather conditions with an optimal temperature of 15 C (1). Symptoms generally disappear in the late spring as temperatures warm up. WSBM-resistant hard red winter wheat cultivars are available. The resistant cultivars do not show the typical WSBM symptoms and suffer little detectable yield loss (3,4). WSBMV and the vector fungus are endemic throughout the eastern two-thirds of Kansas, with a particularly intense infestation in the south central region. As a result, WSBM-resistant cultivars are widely sown in these regions.

Wheat spindle streak mosaic virus (WSSMV) is a long, flexuous rod (16 × 1,775 nm) (5) that produces distinctive pinwheel inclusion bodies in infected tissue (17). WSSMV, like WSBMV, is vectored by *P. graminis* (12). WSSMV requires temperatures below 17 C for symptom development with an optimal temperature of 10 C (11). Slykhuis (11) has reported symptoms of wheat spindle streak mosaic (WSSM) to include chlorotic spindle-shaped streaks, stunting, and reduced tillering. WSSMV has been reported to occur in North America from as far north as Ontario, Canada (13), as far south as Kentucky (18), and as far west as Nebraska (2). There has been no report of WSSMV yet in Kansas, although J. K. Uyemoto (*unpublished*) made a preliminary identification in 1981 (2).

In late February 1984, large acreages of WSBM-resistant wheats in south central Kansas developed disease symptoms that initially resembled WSBM. Yellowing turning to bronze, mosaics, stunting, and reduced tillering were observed in discrete areas within fields generally corresponding to areas of lower terrain. The initial assumption was that the symptomatic wheat was infected with WSBMV. Closer inspection of the symptoms revealed spindle-shaped streaks and a bronzing of the tissue, which is generally not observed with WSBMV infections.

This paper describes WSSMV in Kansas and its involvement with the ubiquitous WSBMV.

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MATERIALS AND METHODS

Electron microscopy. Leaf-dip preparations were made by adding one drop of leaf sap to one drop of 1% potassium phosphotungstate, pH 7.0, on a 300-mesh copper grid coated with Formvar. The drops were allowed to remain on the grid for 5–10 min before blotting off excess liquid. The preparations were then examined with a Philips EM-201 transmission electron microscope (EM).

Thin sections. Portions of field-collected wheat leaves with symptoms were excised and fixed in 4% glutaraldehyde, then treated in 2% OsO_4. The fixed tissue was dehydrated by successive washes in a series of ethanol baths. The tissue was cleared with acetone and embedded in plastic resin. Blocks were thin-sectioned with a microtome and poststained with uranyl acetate and 0.4% lead citrate. The sections were then observed with the EM.

Western blot analysis. Purified virus preparations were electrophoresed in 12% sodium dodecyl sulfate (SDS) polyacrylamide gels and electroblotted to nitrocellulose as described by Towbin et al (14). Proteins were fixed to the membranes by a 15-min incubation in 10% acetic acid and 25% ethanol. Membranes were incubated in virus-specific antiserum diluted with TBS:T100 (50 mM Tris-HCl, ph 7.4, 200 mM NaCl, and 0.1% Triton X-100). A WSSMV antiserum (5) was provided by K. Z. Haufler, Michigan State University, East Lansing (WSSMV-MS sera) and was used at a 1:200 dilution. An antiserum (WSSMV-KS sera) manufactured from a Kansas isolate of WSSMV electrophoretically purified 36-kDa (6) capsid protein (T. L. Kendall and S. A. Lommel, *unpublished*) was used at a 1:1,000 dilution. WSBMV antiserum was used at a 1:3,000 dilution. After antibody incubation, the membranes were rinsed in TBS:T100 and incubated for 2 hr in a 1:1,000 dilution of protein A-alkaline phosphatase conjugate (Sigma, St. Louis, MO). After rinsing in TBS-T100, the membranes were developed for 30 min in the dark with napthol AS-MX phosphate alkaline solution (Sigma) and fast violet B salt (Sigma).

Field studies. Cultivar plots were sown at two locations. A replicated cultivar test was planted at the Hesston, KS, experimental field on 17 September 1983 and harvested on 27 July 1984. This soil was naturally infested with WSBMV only. The second location, at Clearwater, KS, was naturally infested with both WSBMV and WSSMV, and contained cultivar demonstration strips. The Clearwater plot was not replicated; however, each strip was about one acre. The cultivar demonstration was planted on 29 September 1983 and harvested 3 July 1984.

Weather data. Temperature data were collected at 12-hr intervals from the airport at Wichita, KS, about 10 mi. northeast of the WSBMV plus WSSMV field plots.

RESULTS

Field observations. A pronounced yellowing, bronzing, and stunting was observed in mid-February 1984 throughout south central Kansas. Gross field symptoms included yellowing with an overall bronze appearance to the field, stunting, mosaic, and reduced tillering. The symptoms were always strongest in lower portions of the field (Fig. 1B). In late spring, after several warm days, the affected plants lost symptoms but remained stunted compared with uninfected plants.

Cultivar reactions. A hard red winter wheat demonstration trial was planted in soil infested with WSBMV plus WSSMV in September 1983 (Fig. 1A). By late February 1984, the cultivars Vona and Chisholm were so severely affected by the two viruses that many of the plants were dead (Table 1). Newton and Arkan, two widely planted WSBM-resistant cultivars, showed significant disease symptoms in the field infested with WSBMV plus WSSMV compared with the field infested with WSBMV only. In the WSBM-only field, the two cultivars had a disease rating of 1.00 with no observable symptoms, whereas in the presence of WSSMV, they had ratings of 3.25 and 4.00, respectively (Table 1). Hawk (Agripro) and Tam 108 had the lowest disease ratings of the cultivars tested at Clearwater (Table 1). Mustang (Agripro) had the highest disease rating (4.75) of the WSBM-resistant cultivars tested. In general, the WSBM-susceptible cultivars were most severely affected by the disease, whereas the WSBM-resistant cultivars varied from severe to light symptoms.

Etiology. Field samples were collected beginning in early March 1984. Leaf-dip preparations from symptomatic wheat plants were examined for virus with the EM. All symptomatic plants contained stiff, rodlike particles of two lengths (20× 280 and 140 nm) that were found to be WSBMV (Fig. 1C). In addition, all samples contained a few thin, flexuous, rodlike particles (Fig. 1C). These particles were about 13–16 nm in diameter and ranged from 100 to 1,500 nm long. In all samples examined, the number of long, flexuous rods was always less than the number of WSBMV particles. The shorter rodlike viruses were confirmed to be WSBMV by sandwich enzyme-linked immunosorbent assay (ELISA) (7) and by Western blots (Fig. 2).

Thin sections were prepared from the same field samples and observed with the EM. The infected tissue contained prominent pinwheel inclusion bodies and areas

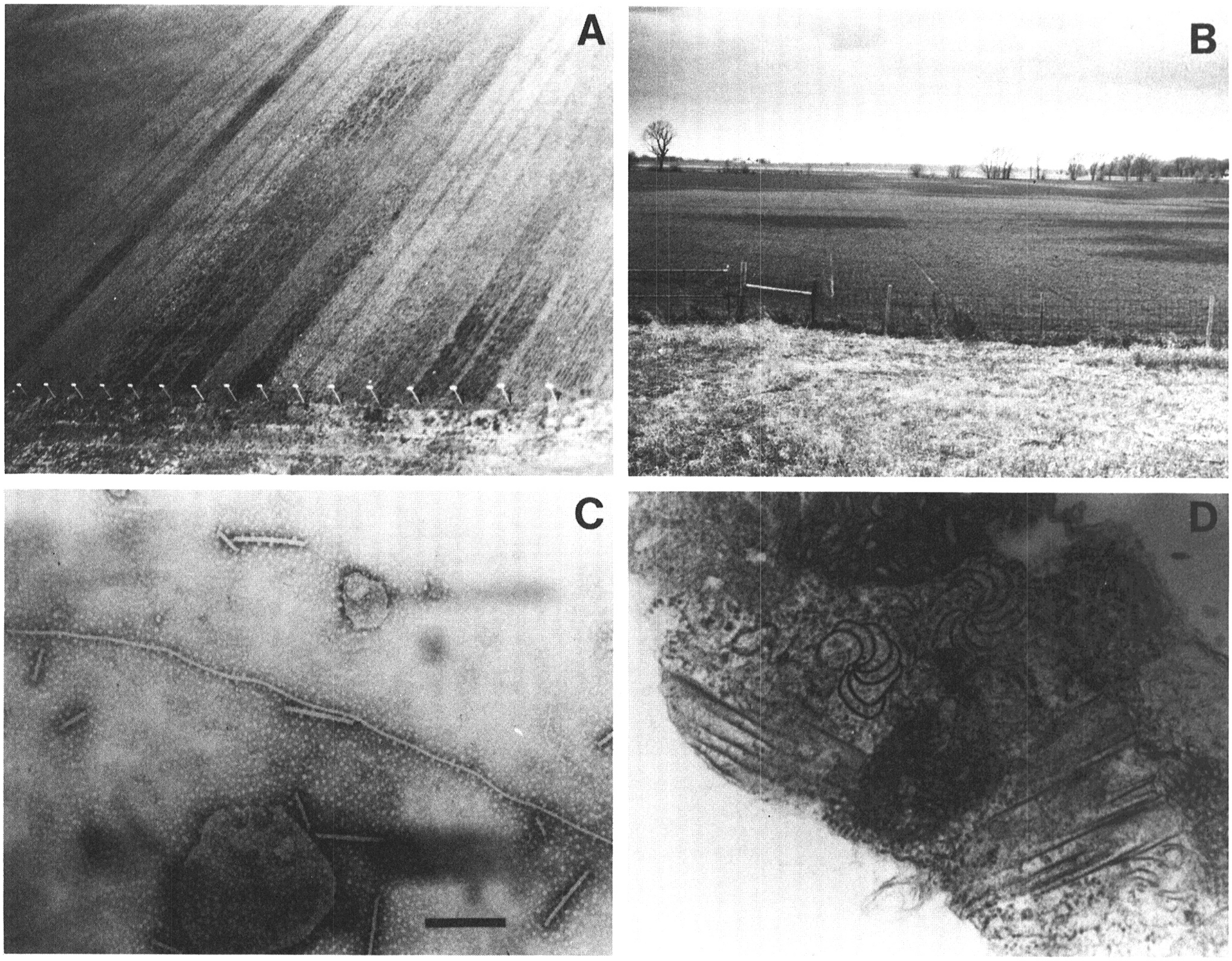

Fig. 1. (A) Clearwater cultivar demonstration strips on 20 March 1984. Soil was naturally infested with wheat soilborne mosaic virus (WSBMV) and wheat spindle streak mosaic virus (WSSMV). **(B)** Commercial field of Newton wheat (WSBM-resistant) on 27 February 1984 infested with WSBMV and WSSMV. Dark areas are locations of higher terrain and have symptomless plants. Light areas are plants showing mosaic and stunt symptoms. **(C)** Leaf-dip preparation of symptomatic Newton wheat collected from field shown in B. The shorter, wider rod particles are WSBMV, and the long, flexuous rod is WSSMV. **(D)** Thin section of symptomatic wheat leaf tissue from field shown in B. Membranous bodies and pinwheel inclusions are clearly present. Scale bars = 300 nm.

Table 1. Disease ratings, yields, and virus content of hard red winter wheat cultivars from field plots infested with wheat soilborne mosaic virus (WSBMV) and WSBMV plus wheat spindle streak mosaic virus (WSSMV) during the 1983–1984 growing season

Cultivar or hybrid	Field reaction to WSBMV[a]	WSBMV-infested field plot[b]				WSBMV- and WSSMV-infested field plot[c]			
		Disease rating[d]	Yield (bu/acre)	WSSMV[e]	WSBMV[e]	Disease rating	Yield (bu/acre)	WSSMV	WSBMV
Hawk	R	1.25	48	–	–	2.00	37	+	+
Bounty 201	MR	2.25	52	–	+	2.50	30	+	+
Newton	R	1.25	44	–	–	3.25	30	+	+
Arkan	R	1.00	47	–	–	4.00	25	+	+
Mustang	R	1.00	47	–	–	4.75	20	+	+
Tam 105	S	5.00	40	–	+	6.00	15	+	+
HW1010	MS	4.50	43	–	+	7.25	14	+	+
Vona	S	6.00	33	–	+	7.75	N.H.[f]	+	+
Chisholm	S	5.50	37	–	+	9.00	N.H.	+	+

[a] R = resistant, MR = moderately resistant, MS = moderately susceptible, and S = susceptible to WSBMV as determined by field reactions.
[b] Plots located at Hesston, KS. Soil naturally infested with WSBMV only.
[c] Plots located at Clearwater, KS. Soil naturally infested with WSBMV plus WSSMV.
[d] Disease ratings are for mosaic, yellowing, and stunting, where 1 = no symptoms and 9 = severest symptoms.
[e] Virus presence was determined by analysis of leaf-dip preparations with the electron microscope.
[f] N.H. = not harvested.

of amorphous membranous bodies (Fig. 1D). These cytological features and the unique particle morphology suggested WSSMV.

The possibility that the long, flexuous rods were not actually wheat streak mosaic virus (WSMV) was examined. WSMV is a common virus of wheat in Kansas. The virions are long, flexuous rods about 13 × 700 nm, and pinwheel inclusions are produced in vivo (10). Symptomatic wheat tissue collected from fields was mechanically inoculated to N28Ht inbred corn, an excellent indicator host for WSMV (15). No symptoms developed after 20 days, indicating the absence of WSMV. In addition, field-collected samples were assayed for WSMV by ELISA (7) with negative results.

The long, flexuous rods were definitively confirmed to be WSSMV by Western blot analysis using a known WSSMV antiserum (WSSMV-MS). Both the WSSMV-MS and WSSMV-KS antiserum detected the 36-kDa (6) WSSMV capsid protein (Fig. 2). There appeared to be cross-reactivity of the WSSMV-MS antiserum to WSBMV capsid protein and healthy plant protein components. The WSSMV-KS antiserum manufactured to isolated WSSMV capsid protein detected only the 36-kDa WSSMV polypeptide.

Distribution. In the spring of 1984, five counties in south central, one in north central, and one in northeastern Kansas were shown to be infested with WSSMV and WSBMV as assayed by the EM. By the spring of 1985, 43 counties, all in the eastern half of the state, were shown to have wheat fields infested with WSSMV and WSBMV (Fig. 3).

Environmental conditions. Optimal temperatures for symptom development are 15 C for WSBMV (8) and 10 C for WSSMV (11). At the Clearwater plots, the average daily mean temperature for February 1984 was much higher than average, above 5 C. The March temperatures were close to average (Fig. 4), and the temperatures for April and May were

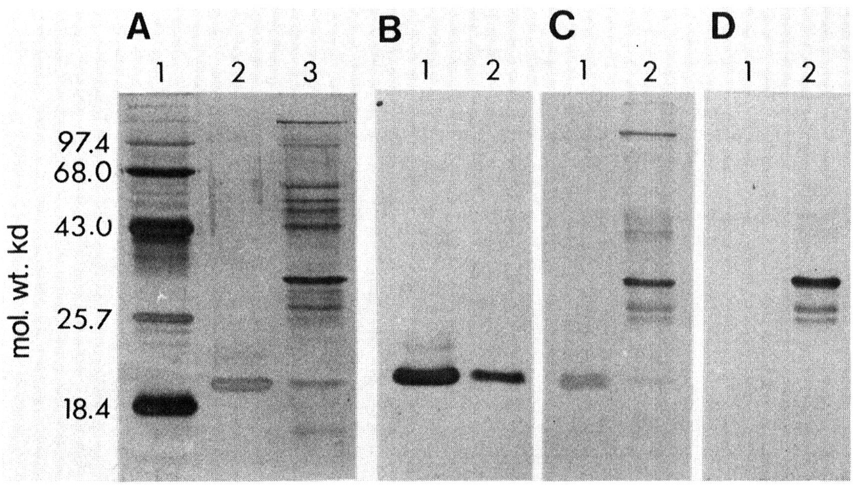

Fig. 2. Western blot analysis of wheat soilborne mosaic virus (WSBMV) and wheat spindle streak mosaic virus (WSSMV) with WSBMV and WSSMV antiserum. **(A)** Silver-stained 12% SDS-polyacrylamide gel. (1) Protein molecular weight standard: phosphorylase B, 97.4 kDa; bovine serum albumin, 68.0 kDa; ovalbumin, 43.0 kDa; α-chymotrypsinogen, 25.7 kDa; and β-lactoglobulin, 18.4 kDa. (2) Purified virus preparation of WSBMV. (3) Purified virus preparation of WSSMV. **(B)** Western blot of (1) WSBMV and (2) WSSMV detected with WSBMV antiserum (1:3,000 dilution). **(C)** Western blot of (1) WSBMV and (2) WSSMV detected with WSSMV-MS antiserum (1:200 dilution). **(D)** Western blot of (1) WSBMV and (2) WSSMV detected with WSSMV-KS antiserum (1:1000 dilution).

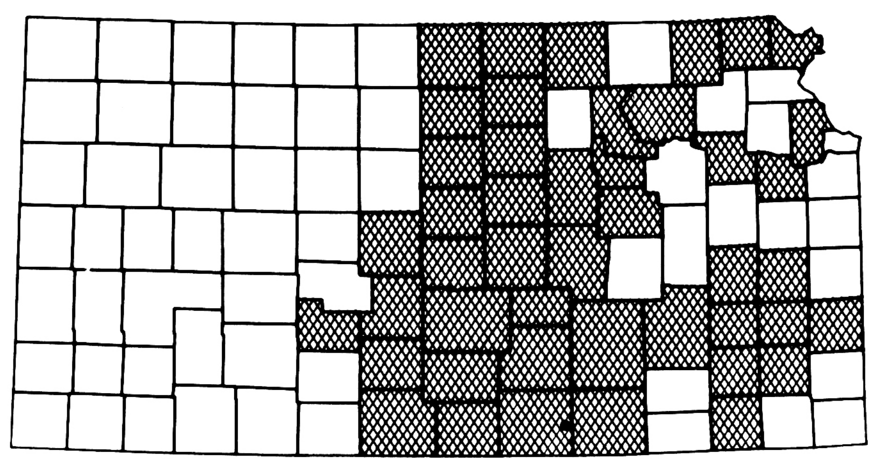

Fig. 3. Distribution of wheat spindle streak mosaic virus (WSSMV) in Kansas in spring 1985. Cross-hatched counties indicate that at least one field of infected wheat (WSSMV) within the county has been identified. All samples tested throughout the state were infected with wheat soilborne mosaic virus (WSBMV) and WSSMV except the location marked (•); this was the only field identified with only a WSSMV infestation.

cooler than normal. Therefore, for a 4-mo period, the average daily mean temperatures were within the optimal range of temperatures for both WSBMV and WSSMV symptom development. Weather conditions during the spring of 1985 were not as conducive for WSBMV and WSSMV symptom development, and consequently, the disease ratings for most of the cultivars were lower in 1985.

Cultivar reaction comparisons in fields infested with WSBM only and WSBM plus WSSMV. A study was performed using several popular cultivars resistant and susceptible to WSBM that were grown in soil infested with WSBMV only and with WSBMV plus WSSMV. The cultivars tested have similar yield potential under non-WSBM conditions (16). In the WSBM-only plots, all cultivar reactions and yields were consistent with historic reactions (Table 1). No WSBMV was observed in many EM grid sections in leaf-dip preparations from the WSBM-resistant cultivars, except in the moderately resistant Bounty 201 in which only a few particles were detected. In contrast, many WSBMV particles were visible in preparations from the WSBM-susceptible cultivars (Table 1). No WSSMV particles were detected in any of the plants from the WSBM-only plots.

In plots with both viruses, WSBMV and WSSMV particles were easily detected in both the cultivars susceptible and resistant to WSBM (Table 1). Numerous WSBMV particles were observed in the WSBM-resistant cultivars in about equal concentrations as the WSBM-susceptible cultivars. The disease ratings for the WSBM-resistant cultivars varied from minor (Agripro Hawk) to vivid and severe symptoms (Agripro Mustang) (Table 1). The WSBM-susceptible cultivars were all severely affected, with some killed when infected with WSBMV plus WSSMV. For all cultivars tested, the grain yields corresponded directly with the disease ratings (Table 1).

Comparisons of virus particle counts in cultivars susceptible and resistant to WSBM. A virus particle count study was performed on a WSBM-resistant cultivar (Newton) and a WSBM-susceptible cultivar (Vona) in both a field infested with WSBMV only and one field infested with WSBMV plus WSSMV. Leaf-dip preparations of Newton (WSBMV-resistant) from the WSBMV-only plot were devoid of virus particles, whereas Newton in the field infested with WSBMV plus WSSMV had an average of 20 WSBMV particles and one WSSMV particle per grid section (Table 2). Vona (WSBMV-susceptible) had 20 WSBMV virus particles per grid section in both field plots and one WSSMV particle in the doubly infested field. In essence, when Newton is coinfected by both viruses, the concentration of WSBMV particles is equal to that of a WSBM-susceptible cultivar.

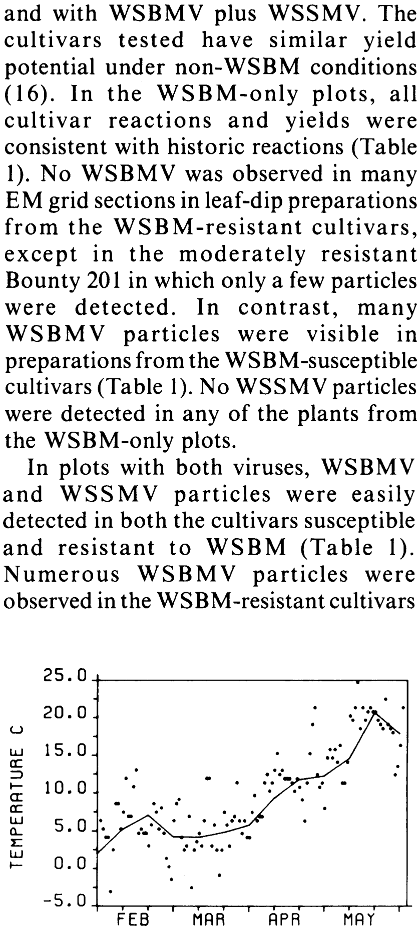

Fig. 4. Average mean temperatures for the spring 1984. Data collected at the Wichita, KS, airport. Each dot represents the average mean temperature for each day. Line represents average mean temperature averaged over a week.

DISCUSSION

WSSMV has now been identified in Kansas. The diagnosis was confirmed by unique particle morphology of the virus, the presence of pinwheel inclusions, and by a serological Western blot analysis. The possibility that the virus was misidentified and was actually WSMV was eliminated. The presence of WSSMV along with the endemic WSBMV has resulted in a new disease situation in hard red winter wheat. This preliminary evidence suggests that the two viruses interact synergistically, resulting in a severe yield-reducing disease of wheat resistant and susceptible to WSBM.

WSBMV and WSSMV are both widely distributed throughout North America. It is evident that in Kansas, the infestations of WSBMV and WSSMV overlap over scattered regions of the eastern half of the state. All samples assayed during the 2-yr study had either WSBMV alone or WSBMV plus WSSMV. There was only a single identification in a commercial wheat field sown to Newton wheat that was infected by only WSSMV (Fig. 3).

In the Western blots, the WSSMV-MS antiserum reacted at low levels to WSBMV capsid protein. These data may mean that there are common antigenic sites between the capsid proteins of the two viruses, or more likely, they suggest that wheat plants in Michigan may have been infected by both viruses and that the antiserum may have antibodies to WSBMV.

Given the fact that WSBMV and WSSMV are vectored by the same soil-inhabiting fungus, *P. graminis*, which is persistent, it appears that WSSMV is now endemic in many regions of Kansas and will persist in the soil. For this reason, it is not surprising that WSBMV and WSSMV are found together in Kansas. WSBMV is firmly established in the eastern two-thirds of the state, with a particularly intense and concentrated infestation in south central Kansas. WSSMV has the immediate potential to invade wherever WSBMV is already established.

In 1984, wheat in four counties of Kansas was found to be infected with WSSMV. In 1985, wheat in 43 counties, all in the eastern half of the state, was confirmed to be infected with WSSMV as well as WSBMV. This does not indicate that WSSMV is spreading rapidly but does reflect a more intensive effort to determine the distribution.

Most soilborne diseases spread slowly and predictably. Given that WSSMV is widely distributed throughout the state as well as in Nebraska (2) and the fact that it is vectored by *P. graminis*, it seems contradictory that only recently have detectable disease symptoms been observed. We believe an explanation for these confusing observations involves the spring weather for 1984 and 1985 and the widespread planting of several new WSBM-resistant wheat cultivars.

Newton, a popular WSBM-resistant cultivar released in 1977, periodically showed mild WSBM-like symptoms (W. G. Willis, *unpublished*). Newton scored a disease rating of 3.25 under optimal environmental conditions for disease in a plot known to be infested with WSBMV plus WSSMV (Table 1). Two other WSBM-resistant cultivars, Arkan and Mustang, which gained rapid popularity within the past 2 yr, had significantly higher disease ratings of 4.00 and 4.75, respectively (Table 1). Before 1983, Newton, which was widely grown in WSBMV-infested areas under nonideal weather conditions for disease, was probably infected with both WSBMV and WSSMV but did not show striking symptoms. In the fall of 1983, other WSBM-resistant cultivars

Table 2. Relative concentrations of virus particles from wheat cultivars resistant and susceptible to wheat soilborne mosaic virus (WSBMV) that were grown in soils infested with WSBMV only and WSBMV plus wheat spindle streak mosaic virus (WSSMV)

		Average no. of virus particles per grid section[b]			
		WSBMV-infested soil[c]		WSBMV- + WSSMV-infested soil[d]	
Cultivar	Field reasction to WSBMV[a]	WSBMV	WSSMV	WSBMV	WSSMV
Newton	R	0	0	20	1
Vona	S	20	0	20	1

[a] R = resistant and S = susceptible.
[b] Average number of morphologically similar virus particles from 50 grid sections, as examined with the electron microscope.
[c] Plots located at Hesston, KS. Soil naturally infested with WSBMV only.
[d] Plots located at Clearwater, KS. Soil naturally infested with WSBMV plus WSSMV.

were released commercially. The wide planting of these new cultivars, coupled with the conducive spring weather conditions, resulted in the sudden development of dramatic symptoms. These conclusions all assume that WSSMV has been present in Kansas soils for some time.

An observation of particular concern is that WSBM-resistant cultivars appear to be fully susceptible to WSBMV when coinfected with WSSMV. The data in Table 2 illustrate that the virus concentration in Newton (WSBM-resistant) is equivalent to that in Vona (WSBM-susceptible) when grown in soil infested with WSBMV plus WSSMV. Some WSBM-resistant cultivars appear to have some levels of natural resistance to WSSMV or the WSSMV-WSBMV complex. For example, both Hawk and Mustang are WSBM-resistant, but Hawk had a very low disease rating whereas Mustang produced quite vivid symptoms. It is difficult to speculate without further information whether Hawk is resistant or tolerant to the WSBMV-WSSMV complex, because both viruses can be detected in infected tissue.

In conclusion, WSSMV is widely distributed throughout Kansas and has the potential to infest every location where WSBMV already exists. Therefore, until new cultivars become available that are resistant to WSBMV and WSSMV together, a severe disease situation has the potential to occur when spring weather conditions are conducive for symptom development.

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