Relationship Between Development of Hard Red Winter Wheat and Expression of Resistance to Wheat Soilborne Mosaic Virus

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ABSTRACT

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Expression of resistance to wheat soilborne mosaic in a field trial of susceptible winter wheat (*Triticum aestivum*) cultivars Sage and Vona and resistant cultivars Newton and Hawk was evaluated using symptomatology, the enzyme-linked immunosorbent assay (ELISA), and polyacrylamide gel electrophoresis. All three evaluations showed that resistant cultivars became infected by wheat soilborne mosaic virus, and symptoms were most pronounced during early jointing in February and March. In resistant cultivars, high ELISA values and high virus concentrations occurred later in the growing season than those in susceptible cultivars. Results from a study conducted in a growth chamber indicated that the late-season rise in ELISA values observed in resistant cultivars is related to tiller maturity rather than to sampling date.

Winter wheat (*Triticum aestivum* L.) is thought to be inoculated with wheat soilborne mosaic virus (WSBMV) following infection of seedling roots by the fungal vector *Polymyxa graminis* Ledingham during cool, wet periods primarily in the autumn (2,9). A mottling of the leaves is visible on susceptible cultivars as early as November, but symptoms usually are not apparent in Oklahoma until February or March. Symptom expression appears to be favored by early spring temperatures below 20 C (13), and symptoms fade with warming temperatures in April and May (5).

Visual assessment of wheat is the most commonly used method to evaluate resistance to wheat soilborne mosaic (WSBM), but other factors may mimic macroscopic symptoms of WSBM. Fertility and other viruses can induce chlorosis similar to the mosaic associated with WSBM (3,5), and *P. graminis* may cause stunting (11). This mimicry of WSBM symptoms induced by agents other than WSBMV presents a need to confirm visual assessments in programs involving breeding for resistance to WSBM.

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Enzyme-linked immunosorbent assay (ELISA) has been used in conjunction with visual assessment to verify the presence of WSBMV. During late spring, however, ELISA values in resistant cultivars often increase to levels nearly comparable to ELISA values in susceptible cultivars (6). The objective of our study was to use ELISA, polyacrylamide gel electrophoresis (PAGE), and symptomatology to examine the relationships between capsid production, virion concentration, and the expression of resistance by hard red winter wheat to WSBM.

MATERIALS AND METHODS

Field trial. A field trial located near Stillwater in north central Oklahoma consisted of four replications of two hard red winter wheat cultivars resistant to WSBM (Hawk and Newton) and two susceptible cultivars (Vona and Sage). The trial was planted in a locale with a history of severe WSBM. Five 3-m rows of each cultivar were seeded with 100–150 kernels per row. Cultivars were assessed six times during 1988 (14 February, 1 and 24 March, 8 and 21 April, and 6 May) for WSBM symptoms, which were rated on a scale of 0–3, where 0 = no mosaic or stunting, 1 = mild mosaic and little or no stunting, 2 = moderate mosaic and stunting, and 3 = severe mosaic and stunting.

Leaves were collected at random from the second and fourth rows of each plot 12 times, commencing in November 1987 and ending in May 1988. From each sample, 5-g subsamples of 5- to 8-cm leaf segments were taken. These leaf segments were passed through a leaf squeezer (Piedmont Machine & Tool Inc., Six Mile, SC), and expressed sap was rinsed into 50 ml of grinding buffer (0.5 M sodium borate, pH 9, with 0.001 M EDTA, 1:10, w/v, dilution) to produce a stock sap solution.

Virus extractions. Aliquots of 21 ml were removed from each stock sap solution for virus extraction, which was performed as previously described (5). These viral extracts were stored at −20 C until run against standards in PAGE to determine the relative absorbance (A_{595nm}) and estimate virus concentration as previously described (5). Six samples and four standards (20, 10, 5, and 2.5 μg of virus per well) were applied in 100-μl aliquots to each gel. The 12 samples of a cultivar/replicated plot combination were run concurrently, each sample being run on two gels. Samples were randomly distributed among the wells of a gel. Mean absorbances were plotted against virus concentrations for the standards. This plot was used to estimate virus concentration for the samples.

ELISA procedures. The remaining stock sap solutions were stored at −20 C until all sampling had been completed. All sap samples were analyzed concurrently by ELISA. Aliquots of stock sap solution were diluted (1:10, v/v) with sample buffer (phosphate-buffered saline with 2% polyvinylpyrrolidone and 0.05% Tween 20) applied in 200-μl aliquots to five wells on each of two ELISA plates.

Rabbit polyclonal antiserum was prepared to WSBMV, and ELISA was performed using standard flat bottom plates by the direct double-antibody sandwich procedure described previously (1). Alkaline phosphatase-labeled IgG conjugate was used at a 1:1,600 dilution. The reaction was stopped by the addition of 50-μl aliquots of 5 M NaOH 30 min after the addition of substrate. Absorbance values were measured at 405 nm with an EIA Reader (Model EL-307, Bio-Tek Instruments, Inc., Burlington, VT). Sixteen wells on each ELISA plate contained aliquots of sap extracted from virus-free leaves of cv. Blue Jacket, and the mean absorbance from these wells was used as a negative check to correct for background absorbance for each plate.

Growth chamber study. Seeds of cvs. Newton and Vona were soaked in a 0.26% sodium hypochlorite solution for 15 min on a reciprocal shaker at room temperature. Seeds were rinsed three

times with double distilled water, placed on filter paper in polyethylene petri dishes, saturated with double distilled water, and allowed to germinate on a bench top under laboratory conditions. Germinated seeds were then planted into a clay loam soil collected in September from the area with a history of severe WSBM used in the field trial. Seeds were separated by standard Monarch plant bands (6 × 4 × 4 cm) in wooden flats (51 × 38 × 7 cm). Four flats of each cultivar were planted and maintained in a Conviron Plant Growth Chamber-PGW36 under fluorescent and incandescent lights (180 $\mu E \cdot m^{-2} \cdot s^{-1}$ at plant level) at 15/10 C (11/13 hr day/night). Photoperiod remained constant throughout the experiment. Seedlings were trimmed to 5 cm when at growth stage 4 on the Feekes scale (7) to enhance foliar infection (10). When the seedlings reached growth stage 5, temperatures were reduced to 5 C for vernalization. After 6 wk, seedlings were transplanted into a 1:1:1 (v/v) peat-sand-soil mix in glazed 3.8-L clay pots, three plants to a pot, 44 pots per cultivar. Potted plants were returned to the growth chamber and kept at 5 C for 3 days after transplanting, then raised to 7/5 C (day/night) for 3 days, 10/7 C for 8 days, 15/10 C for 6 days, and 20/15 C for the duration of the experiment. Negative checks were treated similarly except for being planted in a steamed mixture of soil-peat-sand (1:1:1, v/v) and maintained in separate drainage pans throughout the experiment to avoid infection by WSBMV.

Leaves were collected at random from seedlings of each flat before vernalization and again during the day of transplanting. Samples were collected six additional times commencing 1 wk after growth chambers were set at 20/15 C and ending at senescence. Four pots of each cultivar were sampled from each growth chamber, three with infested soil and one check. The youngest two or three leaves were collected from tillers of similar maturity. On the last three sampling dates, a wide range of maturities existed among tillers within individual pots, so tillers were sampled according to maturity. Samples from plants maintained in growth chambers were processed, stored, and analyzed on the same weight-to-volume basis as samples collected from the field.

RESULTS AND DISCUSSION

Field trial. Frequently, symptoms were not uniform among plants within replicated plots. Differences in visual assessments, ELISA values, and virus concentrations were sometimes significant ($P = 0.05$) among replicated plots of the same cultivar (*data not shown*). These inconsistencies suggest that natural infection was not uniform among replicated plots within a field or among plants within individual plots. This lack of uniformity of infection may have been due to nonuniform irrigation, drainage, or inoculum densities across the field, although efforts were made to prevent these from being factors in the experiment. Infection of foliage within plants was also inconsistent. For example, within samples collected concurrently, the highest ELISA values were sometimes obtained from the youngest, second youngest, or third youngest leaves of different plants (*data not included*). Because of this nonuniformity, which was observed over several growing seasons, foliage samples of 6–10 g per 6-m row consisting of random leaves were collected for evaluation.

All three evaluations showed that resistant cvs. Newton and Hawk became infected by WSBMV; however, virus concentration and disease development differed among susceptible and resistant cultivars (Table 1). Virus concentrations, ELISA values, and visual assessments were often significantly different ($P = 0.05$) between Hawk and Newton and between Sage and Vona (Table 1). Symptoms were most pronounced during jointing in March. High ELISA values were obtained from susceptible cvs. Sage and Vona during February, and these values remained high into May, by which time the mosaic had partially faded. The highest ELISA values obtained from resistant cvs. Newton and Hawk were in April and May, respec-

Table 1. Comparisons of virus concentration means, enzyme-linked immunosorbent assay (ELISA) absorbance means, and visual assessment means for foliar samples of winter wheat cultivars Hawk and Newton (resistant to wheat soilborne mosaic [WSBM]) and cultivars Sage and Vona (susceptible to WSBM) collected during the 1987–1988 growing season from a field trial in a north central Oklahoma locale with a history of severe WSBM

	Virus concentration means (μg virus/g fresh leaf tissue)[v]						ELISA value means[w]						Visual assessment means[x]					
	Resistant cultivars			Susceptible cultivars			Resistant cultivars			Susceptible cultivars			Resistant cultivars			Susceptible cultivars		
Date	Hawk		Newton	Sage		Vona	Hawk		Newton	Sage		Vona	Hawk		Newton	Sage		Vona
1987																		
13 Nov.	0.383	*[y]	0.327	0.591		0.415	0.018		0.023	0.027	*	0.019	...[z]		...	...		...
10 Dec.	3.392	*	4.862	8.970	*	2.086	0.043		0.032	** 1.796	*	0.874	...		...	...		...
30 Dec.	4.234	*	7.199	3.028	*	4.648	−0.004		−0.001	** 1.535	*	1.051	...		...	...		...
1988																		
17 Jan.	2.525	*	9.020	** 8.267	*	10.746	0.005		−0.004	** 1.837	*	1.437	...		...	...		...
30 Jan.	2.111	*	8.392	** 32.430	*	15.996	0.017	*	0.129	** 2.093	*	1.791	...		...	...		...
13 Feb.	4.950		6.319	** 25.347	*	10.296	0.068		0.040	** 1.775	*	1.402	0.16	*	0.42	** 1.58	*	1.16
27 Feb.	2.474	*	7.249	** 12.812	*	19.429	0.105	*	0.341	** 1.970	*	1.829	...		...	...		...
10 Mar.	4.171	*	10.465	** 19.475	*	36.437	0.156	*	0.424	** 2.107		2.135	0.75	*	1.00	** 2.08		2.08
24 Mar.	3.920	*	10.050	** 19.118	*	24.554	0.272	*	0.412	** 2.079	*	1.998	0.58	*	0.92	** 2.25		2.25
8 Apr.	5.302	*	8.505	** 46.922	*	21.634	0.293		0.301	** 1.962	*	1.884	0.50	*	0.58	** 1.66		1.67
21 Apr.	5.113	*	20.299	** 38.161	*	43.986	0.348	*	0.614	** 2.115		2.079	0.24		0.33	** 1.33		1.24
6 May	7.299	*	11.212	** 30.581	*	38.130	0.895	*	0.566	** 1.975		2.026	0.33	*	0.66	** 1.66	*	1.24

[v] Leaves were collected at random from the second and fourth rows in each of four five-row plots for each cultivar. Sap from 5-g foliage subsamples was expressed into 50 ml of 0.5 M sodium borate with 0.001 M EDTA to produce 1:10 (w/v) dilution stock sap solutions. Aliquots of 21 ml of stock sap solution were used for virus extractions. Virus extracts were stored at −20 C until assayed by polyacrylamide gel electrophoresis. Four standards (20, 10, 5, and 2.5 μg of virus per well) were run on each gel, and mean absorbances were plotted against virus concentration for the standards. This plot was used to determine the mean virus concentration for each sample.

[w] Stock sap solutions were stored at −20 C until all sampling was complete, then were thawed and assayed by ELISA. Means of ELISA absorbances are from five wells on each of two ELISA plates for each of four replicated field plots.

[x] Averages from four replicated field plots, rated on a scale of 0 = no mosaic or stunting, 1 = mild mosaic and little or no stunting, 2 = moderate mosaic and stunting, and 3 = severe mosaic and stunting.

[y] * = Means of the two cultivars are significantly different and ** = means of the two resistant cultivars are significantly different from the means of the two susceptible cultivars by a least square means test ($P = 0.05$) used with the virus concentration means and by Fisher's LSD ($P = 0.05$) used with the ELISA value means and the visual assessment means.

[z] Visual assessment was not made.

tively. The highest virus concentrations were found in April for Newton, Sage, and Vona and in May for Hawk (Table 1). Multivariant analyses of variance were performed for ELISA and PAGE values by sampling dates. Although the late-season rise in viral antigen measured by ELISA in resistant cultivars might have been due, in part, to the rise in pelletable virions, the partial correlation coefficients for these data were low, −0.043319 (0.19%) for Hawk and −0.061453 (0.38%) for Newton. The partial correlation coefficients for the susceptible cultivars in field plots also were low, 0.079768 (0.69%) for Sage and −0.056826 (0.32%) for Vona. Production of capsid protein apparently is favored during more of the growing season than is viral assemblage. Although ELISA detects the presence of WSBMV, it is not necessarily a good indicator of virion concentration.

Larsen et al (8) suggested that resistance to *Polymyxa* zoospores carrying WSBMV and reduced movement of WSBMV within roots were possible mechanisms of resistance to WSBM. They felt their work eliminated tolerance to the virus, resistance to the virus at the cellular level, and resistance to the vector fungus alone (without virus) as possible mechanisms (8). The results presented in this paper (Table 1) and in a previous paper (4) demonstrate that symptoms, capsid production, and virion assemblage all occur in resistant cultivars, but at lower levels than in susceptible cultivars. This suggests that mechanisms that inhibit virus replication, virion assemblage, and/or virus movement are operating in resistant cultivars. Our results (Table 1) also indicate that capsid production and virus assemblage occur earlier in the season in susceptible cultivars than in resistant cultivars. This appears to support the proposal by Larsen et al (8) that the mechanisms of resistance to WSBM are resistance to *Polymyxa* zoospores carrying WSBMV or reduced movement of WSBMV within the roots of resistant cultivars. It seems doubtful, however, that these mechanisms alone are responsible for the expression of resistance to WSBM observed in cvs. Hawk and Newton.

Growth chamber study. No late-season rise in ELISA values was found in resistant cv. Newton when data were averaged by sampling date (Table 2). When ELISA values were averaged by maturity of the tillers in each sample, however, Newton showed a late-season rise (Table 3). Changes in photoperiod and the high temperatures that occur in the field late in the growing season were not present in the growth chamber and, therefore, do not account for the late-season rise in ELISA values. The late-season rise in ELISA values and virus concentration in resistant cultivars in field plots (Table 1) may be related to a reduced rate of viral activity and/or assemblage (4), but this explanation does not fit the pattern of ELISA values and virion concentrations found in the growth chamber (Tables 2 and 3). Virus concentrations were moderately high in Newton before and immediately after cold treatment, dropped during jointing, and rose substantially during and/or after anthesis. Thus, the late-season rise in ELISA values observed in resistant cultivars may depend on changes in host physiology associated with maturation and senescence.

Brakke et al (3) reported that seedlings maintained in a controlled environment developed pronounced leaf symptoms at 15 C, less pronounced leaf symptoms at 20 and 25 C, and faint, transient leaf symptoms in a few plants at 30 C. In our study, mosaic faded almost completely in Vona and completely in Newton during jointing in the growth chamber at 20 C with a constant photoperiod. This suggests that the disappearance of foliar symptoms may not result entirely from the change in photoperiod or high temperatures that occur in late spring and summer. Chlorophyll content in wheat increases as plants approach "sexual maturity," then falls rapidly after heading (12). Perhaps this natural increase in chlorophyll content partially or completely masks the mosaic of WSBM. Further work is required to more fully explain the interaction among

Table 2. Virus concentration and enzyme-linked immunosorbent assay (ELISA) value means for foliar samples of winter wheat cultivars Newton (resistant to wheat soilborne mosaic [WSBM]) and Vona (susceptible to WSBM)

	Newton		Vona	
Sampling date	Virus[v]	ELISA[w]	Virus	ELISA
10 January[x]	3.530	0.355	7.689	1.216
14 March[y]	4.849	0.139	31.004	1.548
10 April[z]	0.352	−0.003	13.961	0.869
24 April	0.504	−0.008	3.631	1.055
8 May	0.654	0.120	4.724	0.761
15 May	3.669	0.322	37.913	1.181
24 May	1.860	0.125	52.203	1.020
9 June	0.716	0.134	6.332	1.079

[v]Samples of leaves of similar age were collected from tillers of similar maturity, and sap was expressed into 0.5 M sodium borate with 0.001 M EDTA to produce a 1:10 (w/v) dilution stock sap solution. Aliquots of 21 ml of stock sap solution were used for virus extractions. Virus extracts were stored at −20 C until assayed by polyacrylamide gel electrophoresis. Four standards (20, 10, 5, and 2.5 μg of virus per well) were run on each gel, and mean absorbances were plotted against virus concentration for the standards. This plot was used to determine the mean virus concentration for each sample.

[w]Stock sap solutions were stored at −20 C until all sampling was completed, then were thawed and assayed by ELISA. Means of ELISA absorbances are from five wells on each of two ELISA plates.

[x]Germinated seeds were planted in soil from a locale with a history of severe WSBM, separated by standard plant bands in wooden flats, and maintained in a growth chamber. Sampling on this date was just before 6 wk at 4 C.

[y]Sampling on this date followed 6 wk at 4 C and corresponded to the transplanting of seedlings to 3.8-L pots, three seedlings per pot.

[z]Potted plants were kept at 5 C for 3 days after transplanting, then raised to 7/5 C (day/night) for 3 days, 10/7 C for 8 days, 15/10 C for 6 days, and 20/15 C for the duration of the experiment. Data means are averages for three pots per cultivar. Sampling on this date followed 1 wk at 20/15 C day/night, when plants were jointing.

Table 3. Means of enzyme-linked immunosorbent assay (ELISA) values averaged by maturity of tillers in foliar samples of winter wheat cultivars Newton (resistant to wheat soilborne mosaic [WSBM]) and Vona (susceptible to WSBM)

Tiller maturity of sample[w]	Newton	Vona
5 (before vernalization)[x]	0.355	1.216
5 (after vernalization)[y]	0.139	1.548
6–7[z]	−0.003	0.869
7–9	−0.008	1.055
9–10.5	0.102	0.958
10–11.1	0.228	1.033

[w]Samples of leaves of similar age were collected from tillers of similar maturity according to the Feekes scale (7), and sap was expressed into 0.5 M sodium borate with 0.001 M EDTA to produce a 1:10 (w/v) dilution stock sap solution. The solutions were stored at −20 C until all sampling was completed, then were thawed and assayed by ELISA. Means of ELISA absorbances are from five wells on each of two ELISA plates.

[x]Germinated seeds were planted in soil collected from a locale with a history of severe WSBM, separated by standard plant bands in wooden flats, and maintained in a growth chamber. This sampling was just before 6 wk at 4 C.

[y]This sampling followed 6 wk at 4 C and corresponded to the transplanting of seedlings to 3.8-L pots, three seedlings per pot.

[z]Growth chambers were kept at 5 C for 3 days after transplanting, then raised to 7/5 C (day/night) for 3 days, 10/7 C for 8 days, 15/10 C for 6 days, and 20/15 C for the duration of the experiment. Data means are averages for three pots per cultivar. This sampling followed 1 wk at 20/15 C day/night, when plants were jointing.

changes in host physiology associated with vernalization, maturation, and senescence and symptom expression, virus replication, and virus assembly.

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LITERATURE CITED

1. Bahrani, A., Sherwood, J. L., Sanborn, M. R., and Keyser, G. C. 1988. The use of monoclonal antibodies to detect wheat soil-borne mosaic virus. J. Gen. Virol. 69:1317-1322.
2. Brakke, M., and Estes, A. 1967. Some factors affecting vector transmission of soil-borne wheat mosaic virus from root washings and soil debris. Phytopathology 57:905-910.
3. Brakke, M., Estes, A. P., and Schuster, M. L. 1965. Transmission of soil-borne wheat mosaic virus. Phytopathology 55:79-86.
4. Hunger, R. M., Armitage, C. R., and Sherwood, J. L. 1989. Effects of wheat soilborne mosaic virus on hard red winter wheat. Plant Dis. 73:949-952.
5. Hunger, R. M., and Sherwood, J. L. 1985. Use of symptomatology and virus concentration for evaluating resistance to wheat soilborne mosaic virus. Plant Dis. 69:848-850.
6. Hunger, R. M., and Sherwood, J. L. 1985. Use of visual assessment and ELISA to evaluate the reaction of wheat cultivars to wheat soilborne mosaic virus. (Abstr.) Phytopathology 75:965.
7. Large, E. C. 1954. Growth stages in cereals. Illustration of the Feekes scale. Plant Pathol. 3:128-129.
8. Larsen, H. J., Brakke, M. K., and Langenberg, W. G. 1985. Relationships between wheat streak mosaic virus and soilborne wheat mosaic virus infection, disease resistance, and early growth of winter wheat. Plant Dis. 69:857-862.
9. Rao, A., and Brakke, M. 1969. Relation of soil-borne wheat mosaic virus and its fungal vector, *Polymyxa graminis*. Phytopathology 59:581-587.
10. Rao, A., and Brakke, M. 1970. Dark treatment of wheat inoculated with soil-borne wheat mosaic and barley stripe mosaic viruses. Phytopathology 60:714-716.
11. Teakle, D. S. 1969. Fungi as vectors and hosts of viruses. Pages 23-54 in: Viruses, Vectors, and Vegetation. K. Maramorosch, ed. Interscience Publishers, New York.
12. Whyte, R. O. 1948. History of research in vernalization. Pages 1-38 in: Vernalization and Photoperiodism: A Symposium. A. E. Murneed and R. O. Whyte, eds. Chronica Botanica Company, Waltham, MA.
13. Williams, E., Jr., and Young, H. C., Jr. 1981. Wheat soil-borne mosaic virus disease. Okla. State Univ. Ext. Facts 7629.