

# Use of Symptomatology and Virus Concentration for Evaluating Resistance to Wheat Soilborne Mosaic Virus

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## ABSTRACT

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Visual assessment of symptoms, virus detection by electron microscopy, and virus concentration were compared for evaluating the resistance of 12 hard red winter wheat cultivars to wheat soilborne mosaic virus. Results showed that visual assessment used in conjunction with virus concentration are the best indicators of resistance. Symptomatic seedlings that developed after inoculation with root washings from infected source plants stored for 0, 2, 4, and 8 wk ranged from 45.3 to 96.4% compared with 6.2 to 28.8% when root washings were used from source plants stored for 24 wk.

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Wheat soilborne mosaic virus (WSBMV) was first reported in Oklahoma in 1952 (8). Currently, this disease is found primarily in the north and north central parts of the state. Although yield reductions from WSBMV in Oklahoma

have been reported (6,8), these reductions usually are not as severe, widespread, or numerous as in the Plains States north of Oklahoma. Symptoms observed on infected wheat plants in February and early March disappear with warming temperatures in late March and April. Thus, the most pronounced yield reductions from WSBMV in Oklahoma occur during years with a prolonged cool spring.

WSBMV can be transmitted artificially from infected to healthy plants (2-4). In addition, a procedure to identify resistance to WSBMV in wheat seedlings has been developed (1). Root washings from infected source plants are used to

drench flats of healthy wheat seedlings. Flats are kept in a growth chamber at 15 C and visually assessed for WSBMV symptoms after 6-8 wk. Unfavorable weather conditions are avoided in this procedure, and the test for WSBMV resistance is more reliable than selection of wheat planted in a field with a history of the disease (1). One difficulty with this procedure is that severe foliar chlorosis and necrosis of inoculated seedlings occurs frequently (R. M. Hunger, *unpublished*). This is especially evident if the infected source plants are taken from the field. When severe chlorosis and necrosis occur, the mosaic pattern characteristic of WSBMV is difficult to discern. Thus, identification of seedlings resistant to WSBMV would be more reliable if visual assessment of disease were coupled with another technique not confounded by the chlorosis and necrosis that result when seedlings are inoculated with root washings from infected plants.

Identification of resistance would be aided further if source plants used to obtain root washings could be collected in the spring and stored to be used as needed to provide inoculum during the summer and fall. This would eliminate

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the need for continuous maintenance of inoculum in wheat plants.

The purposes of this study were to examine the potential usefulness of coupling visual assessment with other methods for identifying resistance to WSBMV and to determine the method and length of time that plants from the field infected with WSBMV can be stored and still serve as a source of inoculum.

## MATERIALS AND METHODS

In October 1983, certified seed of the wheat cultivar Vona was sown in a field with a history of WSBMV. By late March 1984, the plants uniformly showed the mosaic pattern and stunting characteristic of WSBMV. These plants were used as the source of WSBMV inoculum (root washings) in our experiments.

Clumps of plants were removed from the field, taking care not to disturb the soil and roots for a depth of 15–20 cm. These source plants were stored in two ways. One set was placed in wooden flats (56 × 40 × 16 cm) and kept in a cold room at 4–10 C, 80 cm under a bank of eight Gro-Lux lights set for a 12-hr photoperiod. Flats were watered weekly with 2 L of tap water. Every third week, about 5 g of fertilizer (20-20-20) was added to each 2 L of water. The second set of Vona plants from the same field was stored in large plastic bags in ice chests in the cold room. Most of the soil was removed before placing these plants in the bags. The plastic bags and ice chests were left open in the cold room, and water was added as needed to keep the small amount of soil moist.

Twelve hard red winter wheat cultivars ranging in reactions to WSBMV were used in these tests. Certified seed was obtained from the Agronomy Department at Oklahoma State University. Fifty seeds of each cultivar were planted in rows in wooden flats (56 × 40 × 8 cm). A mixture of sandy loam soil, peat, and sand (1:1:1, v/v/v) was used in all experiments. Each flat had one row of each cultivar, and rows were arranged randomly. Three flats (replicates) were planted for each test. Flats were kept in a greenhouse and inoculated when the coleoptiles of most plants had emerged (4–5 days after planting).

Inoculum from source plants was prepared as described by Bockus and Niblett (1), except 130 and 500 g of root and crown tissue from source plants stored in flats and plastic bags were soaked in 950 and 1,500 ml of distilled H<sub>2</sub>O, respectively. Three 2,000-ml beakers with appropriate amounts of root and crown tissue and water were kept in the growth chamber. Each flat was flooded with 950 or 1,500 ml of root washings 1, 3, 5, and 8 hr after the roots and crowns of source plants were submerged in the water. Thus, each flat received a total of either 3,800 or 6,000 ml of root washings. During and after

inoculation, flats were kept in a growth chamber at 15 C with a 12-hr photoperiod. Each flat received 1,500–2,000 ml of tap water at 2-day intervals. Five grams of fertilizer (20-20-20) was added with every other watering.

One set of three flats was inoculated 1 day after collection of source plants. Subsequent tests used source plants kept in flats in the cold room for 2, 4, 8, and 24 wk. Source plants stored in plastic bags were used after 2 and 4 wk of storage in the cold room.

Seedlings were assessed for symptoms 6 wk after inoculation. Results were recorded as the percentage of seedlings with WSBMV symptoms. As seedlings were assessed, foliage of each cultivar was collected from each replicate. A single leaf showing a mosaic pattern was taken from each cultivar infected with inoculum from source plants stored for 2 wk and examined for virus particles with the transmission electron microscope (TEM). A small piece of freshly cut leaf blade was placed in a drop of uranyl acetate for 5 min on a Formvar-covered copper grid (200-mesh) stabilized with carbon. Excess uranyl acetate was removed with filter paper, then the grid was air-dried and examined for virus particles on the TEM at ×31,800.

The remainder of the foliage from each replicate of each cultivar was frozen at –20 C before analysis for WSBMV by gel electrophoresis. WSBMV was partially purified before electrophoresis by the procedure of Shirako and Brakke (7) to remove most host proteins. The frozen foliage was cut into 1-cm pieces and a 2-g sample was ground in 21 ml of 0.5 M borate buffer (pH 9.0) with 1 mM Na<sub>2</sub> ethylenediaminetetraacetate (EDTA) in a small Waring Blendor cup, filtered through cheesecloth, and centrifuged at 10,000 g for 10 min at 4 C. The supernatant was mixed with 7 ml of the grinding buffer containing 8% (v/v) Triton X-100. This was placed over a pad of 5 ml of 20% sucrose in grinding buffer and centrifuged at 29,500 rpm for 2 hr at 4 C in a Beckman type 30 rotor. The pellet was resuspended in 0.5 ml of the grinding buffer overnight, then centrifuged at 10,000 g for 10 min at room temperature. The supernatant was then used in electrophoresis. Uninfected material treated in a similar fashion before electrophoresis had no proteins detectable by Coomassie blue staining that comigrated with WSBMV capsid protein. The method of pelleting the virus and then examining the virus concentration by electrophoresis, although tedious, has the advantage over serological methods in that the amount of pelletable virus produced in the plant is assessed rather than the concentration of capsid protein alone.

Samples and WSBMV standards were heated at 95 C for 5 min in 62.5 mM Tris-HCl buffer (pH 6.8) with 10%

glycerol, 2% sodium dodecyl sulfate (SDS), 5% 2-mercaptoethanol, and 0.002% Bromophenol blue and electrophoresed in a 12% polyacrylamide running gel (pH 8.8) with a 4% stacking gel (pH 6.8). Under these conditions, the virions are degraded and the capsid protein subunits migrate as a single band. Electrophoresis buffer contained 25 mM Tris, 192 mM glycine, and 3.46 mM SDS (pH 8.3) (5). Electrophoresis was run in 1.5-mm slab gels at 20 mA until the tracking dye reached the running gel, then at 30 mA until the tracking dye reached the bottom of the slab. Gels were stained in 0.5% Coomassie blue in methanol-acetic acid-water (5:5:5), destained in methanol-acetic acid-water (50:7.5:52.5), and scanned at 590 nm with a Gilford Model 2600 spectrophotometer that determined peak areas. On each 10-lane gel, the three replicates of two cultivars were run with four known concentrations of WSBMV previously purified according to Shirako and Brakke (7). The concentration of WSBMV in samples was calculated on the basis of the peak areas of the capsid protein from the four WSBMV standards (12.5, 6.25, 3.13, and 1.56 μg) on each gel.

Results of visual assessment and determination of virus concentration were analyzed statistically by analysis of variance for a randomized block design. Duncan's multiple range test was used for mean separation.

## RESULTS AND DISCUSSION

**Visual assessment and virus detection by TEM.** Only the results obtained from inoculating wheat cultivars with root washings from Vona source plants stored in flats are presented. Use of inoculum obtained from source plants stored in plastic bags consistently resulted in extreme chlorosis and necrosis of seedlings, and visual assessment was not possible.

Percentages of seedlings with WSBMV symptoms are shown in Table 1. Cultivars Hawk and Newton, considered resistant to WSBMV, consistently showed a lower percentage of infected seedlings than the highly susceptible cultivars Vona and Wings. The remaining eight wheat cultivars had infection percentages between these two groups of resistant and highly susceptible cultivars (Table 1).

A high percentage of seedlings with WSBMV symptoms (45.3–96.4%) resulted when inoculum source plants were used after 0, 2, 4, and 8 wk of storage. The percentage of symptomatic seedlings was lower (6.2–28.8%) when inoculum source plants were used after 24 wk of storage. Using similar test procedures, Bockus and Niblett (1) also reported high percentages (74–98) of symptomatic seedlings, which they attributed to the severity of the screening procedure. We used a greater amount of root tissue than

**Table 1.** Percentage of seedlings of hard red winter wheat cultivars with symptoms of wheat soilborne mosaic virus (WSBMV) and virus concentration ( $\mu\text{g/g}$  fr wt) found in seedlings inoculated with root washings from source plants after 0, 2, 4, 8, and 24 wk of storage<sup>w,x</sup>

Cultivar and field reaction to WSBMV <sup>y</sup>	0		2 wk		4 wk		8 wk		24 wk	
	%	$\mu\text{g/g}$ fr wt	%	$\mu\text{g/g}$ fr wt	%	$\mu\text{g/g}$ fr wt	%	$\mu\text{g/g}$ fr wt	%	$\mu\text{g/g}$ fr wt
Hawk (R)	64.8 a <sup>z</sup>		68.7 a	1.9 a	46.6 a	13.6 a	50.6 a	0.5 a	6.2 a	0.0
Newton (R)	62.4 a		69.6 a	0.4 a	61.2 b	23.7 ab	45.3 a	0.0 a	7.5 a	0.0
Payne (MS)	65.5 a		67.6 a	55.4 de	63.3 b	77.0 cd	66.2 cd	38.0 bc	11.2 ab	0.0
Danne (MS)	86.3 b		75.0 a	49.4 bcd	80.7 cd	59.9 cd	50.2 bc	64.3 bc	26.6 c	0.0
TAM 105 (S)	86.9 b		87.9 b	78.3 ef	76.7 bc	53.6 bc	69.6 cd	30.3 b	21.4 bc	0.0
Sturdy (S)	96.1 b		64.8 a	52.7 cde	80.7 cd	53.3 bc	60.6 bc	44.5 bc	15.8 abc	0.0
Sage (S)	64.7 a		72.5 a	85.4 f	68.0 bc	112.6 d	74.7 d	53.8 bc	22.8 bc	0.0
Chisholm (S)	90.5 b		73.4 a	24.0 ab	75.8 bc	59.7 bc	69.9 cd	60.2 c	16.4 abc	0.0
Triumph 64 (S)	89.4 b		64.7 a	34.9 bcd	72.6 bc	64.6 bc	51.7 ab	39.3 bc	11.0 ab	0.0
TAM 101 (S)	92.3 b		63.6 a	27.1 abcd	74.5 bc	30.1 ab	73.1 d	37.7 bc	24.6 bc	0.0
Vona (S)	93.7 b		93.3 b	24.8 abc	83.2 cd	62.7 bc	88.8 e	36.6 bc	28.8 c	0.0
Wings (S)	89.3 b		96.4 b	48.7 bcd	94.0 d	63.4 bc	74.6 d	41.5 bc	27.9 c	0.0

<sup>w</sup>Source plants (cultivar Vona) showing WSBMV symptoms in the field were dug, placed in wooden flats with the soil around roots left intact, and stored at 4–10 C with a 12-hr photoperiod.

<sup>x</sup>Thirty seeds of each cultivar were planted in each of three flats, which were flooded with washings obtained from soaking 130 g of source plant root and crown tissue in 950 ml of distilled water. Each flat was flooded with 950-ml washings after root and crown tissue had soaked in the distilled water for 1, 3, 5, and 8 hr. Values are the mean of three replicates (15–30 plants per replicate).

<sup>y</sup>R = resistant, MS = moderately susceptible, and S = susceptible.

<sup>z</sup>Numbers followed by the same letter are not significantly different ( $P = 0.05$ ) according to Duncan's multiple range test.

Bockus and Niblett (1), which may explain the higher percentages of symptomatic seedlings we observed.

Detection of virus particles by TEM was not considered a suitable technique to indicate WSBMV resistance under these conditions, because few particles were found in leaves showing the mosaic pattern characteristic of the disease. Virus particles were found only in seedlings of cultivars Sage, Sturdy, Triumph 64, and Danne. No virus particles were found in leaf dips from seedlings of the resistant cultivars Hawk or Newton. This fact, coupled with the high percentage of symptomatic seedlings and the extremely low virus concentrations detected in Hawk and Newton (Table 1), indicate that something other than WSBMV may have caused some of the symptomatic seedlings. Wheat spindle streak mosaic virus has not been reported in Oklahoma but has been found in south central Kansas. This virus (also transmitted by *Polymyxa graminis* Led.), other organisms, or toxic compounds in the root washings may have contributed to the high percentage of symptomatic seedlings observed in our experiments.

**Virus concentration.** Significantly low virus concentrations were associated with the resistant cultivars Hawk and Newton compared with the high virus concentration associated with the cultivar Sage (Table 1). These results indicate that seedlings of Sage yield the greatest amount of virus and would be best to use to increase WSBMV. Amounts of virus found in the other nine cultivars ranged

between the amounts found in Hawk and Newton and the amount found in Sage. No pelletable virus was detected in foliar samples from seedlings inoculated with root washings from source plants stored for 24 wk.

Correlation between visual assessment and virus concentration was low. The  $r^2$  values for 2, 4, and 8 wk after inoculation were 0.047, 0.120, and 0.143, respectively. This may be explained by the possible effect of host genotype on virus replication. In some hosts, capsid protein may be synthesized but nucleic acid replication may be inhibited. In other hosts, nucleic acid may be replicated but no, or little, capsid protein is synthesized. Such differential effects on virus replication could explain the poor correlation between visual assessment and virus concentration observed in our study.

Our results indicate that source plants collected in spring and stored in flats at 4–10 C remained a good source of WSBMV inoculum for 8 wk. Inoculum prepared from source plants stored for 24 wk was still infective, but the percentage of symptomatic seedlings was reduced greatly.

In our study, visual assessment clearly differentiated between the resistant cultivars Hawk and Newton and the 10 susceptible cultivars only when coupled with virus concentration. Differentiation by visual assessment alone was not always defined clearly, because several cultivars (e.g., Payne) showed percentages of symptomatic seedlings similar to those of Hawk and Newton. Coupling visual assessment and virus detection by TEM

would not adequately indicate resistance because the leaf dip method for virus particle detection was inconsistent. Enzyme-linked immunosorbent assay (ELISA) may be more suited than pelletable virus concentration to indicate resistance to WSBMV because ELISA is less tedious and allows quicker screening of large numbers of plants. However, in cases where WSBMV antisera is not readily available, we suggest that visual assessment coupled with pelletable virus concentration is a useful procedure to identify WSBMV resistance.

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