REACTION OF PARKER AND EAGLE WHEATS TO WHEAT STREAK MOSAIC VIRUS

by

JOHN ROBERT YOUNG

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Approved by:

Major Professor

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Wheat streak mosaic (WSM) caused by wheat streak mosaic virus (WSMV) is a serious threat to wheat production in the Great Plains. Yield reduction by WSM in Kansas alone was estimated at 30 million bushels in 1974 (10). There is no effective high level resistance to WSMV in present agronomically suitable wheats (Triticum aestivum L., em Thell). A low level of resistance is available in some cultivars. This includes 'Scout' and many of its derivatives including 'Eagle'. It is indeed fortunate these cultivars are being used in the Great Plains. Such widespread use undoubtedly reflects the resistance of these cultivars to WSM. In inoculated field tests as well as in natural epidemics these cultivars are clearly superior. Under moderate infection these cultivars yield up to 40% more than susceptible cultivars. Although these losses are considerable (averaging 17%), one must consider the potential losses if the recently released and highly susceptible cultivars 'Centurk', 'HiPlains', 'Homestead', 'Kirwin', and 'Trison' (average loss = 50%) become as widely grown as Scout and its derivatives. We should analyze and characterize this mechanism of resistance so that it may be readily identified in screening tests and incorporated into subsequent cultivars.

Differences in response of wheat cultivars to WSMV were first reported by McKinney (7) in greenhouse tests, but he did not associate this with field response. Brakke (3) noted virus concentration differences in cultivars, but did not relate this to seedling or field response. Results from inoculated field plots (11,14) and natural infection (15) support the contention that differences in response to WSMV do exist. Similar results in inoculated plots and under natural infection support the fact that reliable data may be obtained in the absence of the natural vector of WSMV, the wheat curl mite (Aceria tulipae, Keifer).

MATERIALS AND METHODS

The strain of WSMV used in these investigations was isolated near Russell, Ks. The virus was maintained on 'Ohio-28' corn (Zea mays L.), Parker or Eagle wheat. Test plants were inoculated seven days after seeding with an extract of infected tissue ground 1:10 W:V in 0.01M potassium phosphate buffer, pH 7.0 (KPO_4). Plants were dusted with carborundum and the leaves rubbed with the above extract as suggested by McKinney (6). Experiments were conducted in growth chambers at 22C, and 1400 ft-c of fluorescent lighting with a 14 hour photoperiod.

At 14 days postinoculation leaves of Parker and Eagle wheat were harvested and cut into 6 mm sections. Fifteen grams were ground in a Waring blendor for no longer than two minutes in 10 ml of KPO_4 and expressed through two layers of cheesecloth. The extract was clarified by heating 60 minutes in a 40C water bath and centrifuging for 10 minutes at 13,000g (3). The supernatant was collected and the virus concentrated by centrifugation for 1.25 hours at 192,000g. Pellets were immediately disrupted with a glass rod and allowed to resuspend overnight in 2.5 ml of KPO_4. The preparations were then centrifuged for 10 minutes at 13,000g and the supernatant subjected to density gradient centrifugation. Gradients were prepared by layering 5, 9, 9, and 9 ml of solutions containing 100, 200, 300, and 400 mg sucrose/ml dissolved in KPO_4. The gradients were allowed to equilibrate overnight. Virus preparations were layered on the gradients and centrifuged for two hours at 100,000g and 7C. Gradients were scanned at 254nm with the ISCO UA-2 UV analyzer and Model D density gradient fractionator attached to an external recorder. UV absorbing peaks were quantitated by planimetry and fractions were collected for infectivity assays.

Samples studied by electron microscopy were removed from the above preparations prior to heating and diluted to 1:10, 1:40 and 1:80 with 0.05 M Tris-HCL buffer, pH 7.2. Fomvar coated grids were floated for 30 minutes on drops of WSMV antiserum diluted 1:100 with Tris buffer. The grids were rinsed five times by floating them on drops of buffer and then placed on drops of the diluted WSMV preparations for one hour at 24C. Grids were rinsed again by floating on drops of deionized water and finally in a stream of deionized water from a wash bottle. The grids were air dried and negatively stained using the stain and method of Ball and Brakke (1). Grids were viewed in a Phillips 201 electron microscope at 60 KV and 10,000 magnification. The number of virus particles was counted in 3 randomly selected fields.

Samples of the two cultivars were tested for infectivity before heating and diluted to 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} with KPO_4. Three pots each of Eagle and Parker were inoculated with each dilution. Data were recorded as the percentage of plants showing visible symptoms and transformed to the arcsin as variances were unequal among treatments.

Field responses to WSMV were determined on Eagle and Parker wheats in 1974 and 1975. A split plot design was used with four replications. Each plot consisted of four rows 2.8 meters long and 30 cm between rows. The two center rows were harvested in 1974 and all four rows were harvested in 1975. Due to inclement weather inoculation was delayed until March 27, 1974 for the 1974 test. The 1975 test was inoculated on October 22, 1974. The inoculum was prepared as above except the dilution was 1:20 and four layers of cheesecloth were used. Carborundum was added (1.5% by weight) and the wheat plants were sprayed at a distance of 2.5 cm and at 95 psi. The extract was constantly agitated to keep the carborundum in solution. Analysis of variance was calculated and differences determined using the appropriate t test.

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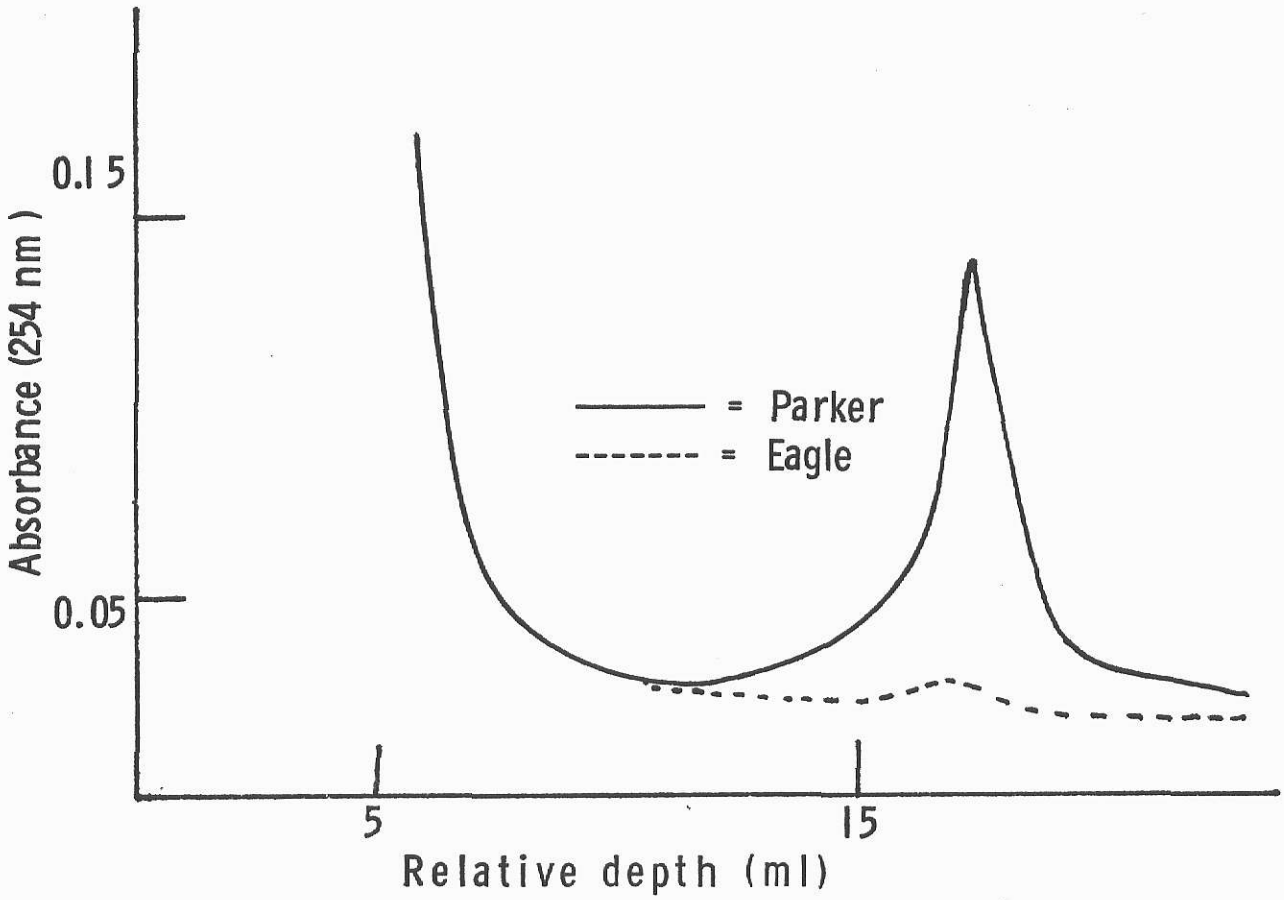
Fig. 1. Ultraviolet scanning patterns of density gradients containing wheat streak mosaic virus. Virus was concentrated from 15g of tissue from Eagle and Parker wheat.


0.15
0.05
Absorbance (254 nm)
= Parker
= Eagle
5
15
Relative depth (ml)

RESULTS

Virus concentration was measured by UV scanning of density gradients. Three experiments were conducted with two replications of each cultivar. The average concentration for Parker was A_{254}=0.136 and A_{254}=0.003 for Eagle (Fig. 1). Virus was detected in every experiment using Parker with values ranging from 0.170 to 0.050. Virus was detected in Eagle with difficulty and only one experiment yielded a significant amount of virus. Virus zones were collected and tested serologically, for infectivity and examined by electron microscopy to substantiate that WSMV was present. WSMV was found to be present by all three criteria.

Highly significant differences in number of virus particles per micrograph were seen by electron microscopy between Parker and Eagle (Table 1). Since a constant amount of antibody exist on the grids, lack of significant differences in the 1:10 dilution is not surprising. This probably reflects saturation of the antibody at the higher virus concentration.

Samples from WSMV-infected Parker were at least two times more infectious than those from Eagle (Table 2). This is in agreement with the particle counts by electron microscopy. Dilution assays for infectivity indicated significant differences between the two cultivars at dilutions of 10^{-2} and 10-3 (Table 2). Significant differences in infectibility were seen between Parker and Eagle. Eagle wheat was significantly more difficult to infect at dilutions of 10^{-2} and 10^{-3} than Parker. This infectibility difference was very evident when samples from WSMV-infected Parker were tested on Eagle wheat (Table 2). An arcsin or angular transformation was utilized. The data are binomial and this transformation, as suggested by Snedecor and Cochran (13) did homogenize the variances. Also, in these data zeros were replaced by $\frac{1}{4}N$=0.03 and 100's by $1-\frac{1}{4}N$=0.97. Actual comparisons were made with the transformed data (Table 2).

Effects of WSMV on the two cultivars are shown in Table 3. The 1974 results showed a greater reduction in grain yield. McKinney (8) suggested that plants with tillers offer more surface area for spray inoculation and therefore a greater infection efficiency. This and perhaps the period of warm weather following the spring inoculation in 1974 may have accounted for the severe response.

Parker and Eagle had similar yield potentials in Manhattan as shown by insignificant yield differences in control plots (Table 3). Yields of both cultivars were reduced by inoculation with WSMV, but Parker was reduced much more (average=38%) than Eagle (average=12%). Infection with WSMV also reduced plant height, tiller number, and kernel weight. Test weight was also reduced in Parker (about 20%) but was not analyzed as all four replications were pooled before the test weight was taken. Visual evaluations indicated that symptoms on Parker were much more severe than on Eagle in both field and growth chamber experiments.

Table 1. Counts of WSMV particles in extracts from Parker and Eagle wheats

Cultivar	Dilution		
	1:10	1:40	1:80
	Number of particles per micrograph		
Parker	35.8	19.7	15.8
Eagle	26.8	11.7**	6.3**

** denotes significance at the 1% level

Table 2. Infectivity dilution assay of WSMV infected Parker and Eagle wheat

Inoculum source	Cultivar inoculated	Dilution	%infected	Transformation
Parker	Parker	10^{-1}	95.6	77.89
		10^{-2}	92.2	73.78
		10^{-3}	37.3	37.64
		10^{-4}	9.3	17.78
Parker	Eagle	10^{-1}	89.7	71.28
		10^{-2}	36.5	37.17
		10^{-3}	12.1	20.36
		10^{-4}	0.0	9.98
Eagle	Parker	10^{-1}	90.6	72.15
		10^{-2}	41.9	40.34
		10^{-3}	10.6	19.00
		10^{-4}	0.78	5.07
Eagle	Eagle	10^{-1}	15.1	22.87
		10^{-2}	3.7	11.09
		10^{-3}	0.0	9.98
		10^{-4}	0.0	9.98

$LSD_{.05}$ = 12.20

$LSD_{.01}$ = 16.22

Table 3. Effect of WSMV on Eagle and Parker wheat

Cultivar	Yield (g/plot) C	Yield (g/plot) I	Height (cm) C	Height (cm) I	Tiller number C	Tiller number I	1000 kernel wt. (g) C	1000 kernel wt. (g) I
				1974				
Parker	299.2	120.2	38.1	28.1	113.8	82.5	26.2	17.0
	NS	**	NS	**	NS	NS	**	**
Eagle	350.5	300.8	40.0	38.8	111.5	102.0	29.0	28.7
				1975				
Parker	1166.2	780.0	38.4	31.1	118.5	98.3	27.2	27.2
	NS	**	NS	*	NS	*	*	**
Eagle	1126.2	987.5	39.3	37.6	120.5	114.0	30.8	31.4

C = control plot
I = inoculated plot
NS= non significant
* = significant.05
**= significant.01

DISCUSSION

The infectivity dilution results for Parker wheat were very similar to those reported by Brakke (2) and Haunold (4) with respect to dilution end point and slope of the dilution curve. Eagle had a similar slope but a much lower dilution end point. There was less virus in the density gradients of the Eagle preparations than indicated by electron microscopy or infectivity studies. Possibly there is some virus recovery problem when Eagle tissue is disrupted. For this reason the other tests are critical in measuring virus concentration.

Disease resistance depends on the ability to tolerate a pathogen or restrict its development. The problems of classifying plant-virus interactions was described by Schafer (12) in a review of tolerance. Tolerance is defined as in the glossary of a National Academy of Science publication:

> "the ability of a host plant to survive and give satisfactory yields at a level of infection that causes economic loss to other varieites of the same host species.

When virus titer or level of infection is unknown it is impossible to classify an interaction as tolerant or restrictive (resistant to pathogen colonization). All previous literature describes wheat cultivars as susceptible or tolerant to WSM. Tolerance may have existed but it was not measured as equivalence of virus concentration was not established with another cultivar. Also, tolerance as defined implies the ability to yield well at levels of infection detrimental to other cultivars. The inability of Eagle wheat to produce a satisfactory yield under severe infection seemingly puts some doubt on a tolerant reaction. We believe that the resistance in Eagle is not due to tolerance but to some virus restrictive mechanism.

Lower virus concentration in density gradients, fewer particles when examined under the electron microscope and a lower infection efficiency

in dilution assay experiments indicate a lower virus concentration and probably slower or reduced virus replication in Eagle. This combined with the apparent difficulty in infecting Eagle could certainly limit WSM epidemics.

Other cultivars are being studied for resistance to WSM. Their response is similar to that of Eagle in that a systemic infection develops but symptoms are abbreviated. Hopefully such resistances can be incorporated into existing resistant cultivars to increase the level and broaden the base of WSM resistance in the Great Plains. This type of resistance should be used in conjuction with the high level, hypersensitive sources of resistance from other genera which prevent systemic infection under field conditions (5).

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ABSTRACT

Two cultivars of wheat (Triticum aestivum L., em Thell), 'Parker' and 'Eagle' were compared in this study as they possess different responses to wheat streak mosaic virus (WSMV). Parker has a severe response and Eagle a moderate response. Under controlled conditions Eagle, while exhibiting systemic symptoms, was less damaged than Parker.

Eagle yielded 43% more than Parker in plots inoculated with WSMV, but no significant yield differences were found in uninoculated plots.

Virus concentrations were measured by ultraviolet scanning of density gradients. An average of 0.136 A_{254} units were obtained for Parker and 0.003 for Eagle. Significantly higher virus particle counts were observed in extracts from infected Parker than from infected Eagle by electron microscopy. Counts at a 1:80 dilution were 15.8 for Parker and 6.3 for Eagle. In infectivity studies, preparations from Eagle had significantly lower infectivity than preparations from Parker when both were assayed on Parker. The percentage of infection at dilutions of 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} were 90.6, 41.9, 10.6, and 0.78, respectively, with preparations from Eagle and 95.6, 92.2, 37.3, and 9.3, respectively for preparations from Parker.

The percentage of infection using inoculum from Parker and applying it to Eagle and Parker at the above dilutions was 91.9, 52.7, 21.6, and 1.6 when assayed on Eagle and 98.1, 91.1, 49.4, and 12.1 respectively when assayed on Parker. Significantly lower percentages were obtained with inoculum from Eagle, especially when assayed on Eagle. These results suggest that resistance in Eagle is not due to tolerance but to some virus restrictive mechanism.