# Survival of Wheat Streak Mosaic Virus in Grass Hosts in Kansas from Wheat Harvest to Fall Wheat Emergence

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**ABSTRACT**

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Field samples of annual and perennial grasses were tested to determine which can be alternate hosts to wheat streak mosaic virus (WSMV) and can survive and sustain the virus from wheat harvest to fall wheat emergence in Kansas. Sites sampled included Conservation Reserve Program (CRP) hectares, government set-aside fields, wheat stubble fields, and waterways. Samples were collected in August of 1988 and at four approximately monthly intervals in 1989 and were tested for the virus by indirect ELISA. None of the perennial grasses sampled were infected, but five annual grass species—*Setaria viridis, S. faberi, Eriochloa contracta, Echinochloa crusgalli*, and *Panicum capillare*—plus volunteer wheat were infected throughout this period. This is the first report of *S. faberi* and *E. contracta* as hosts of WSMV. WSMV was detected in 24.1% of the samples of susceptible grass species in 1988 and 27.5% in 1989. Grasses susceptible to WSMV are abundant over much of Kansas, including in CRP fields, and infected plants could serve as an inoculum source for infection in fall-seeded wheat.

Wheat streak mosaic (WSM) is an important, endemic disease of wheat (*Triticum aestivum* L.) throughout the Great Plains region of the United States. Since 1976, WSM is estimated to have caused an average wheat yield reduction of 2.6% per year in Kansas (W. G. Willis, *unpublished*). In 1988, however, an epidemic of WSM resulted in an estimated statewide yield loss of 13%. This was the largest yearly loss since 1959 and the second highest reduction in the last 40 yr.

A high incidence of WSM is usually associated with the presence of volunteer wheat, which serves as a reservoir for wheat streak mosaic virus (WSMV) and its only known vector, the wheat curl mite (*Eriophyes tulipae* Keifer) (10). High incidence and severity of WSM are also associated with early planting dates, susceptible cultivars, extended warm fall weather, and warm, dry spring weather.

The impact of WSM is usually the greatest in the western third of Kansas because the wheat-fallow-wheat rotation system used in this region enhances the summer survival of volunteer wheat and thus the survival of WSMV and wheat curl mites. In 1988, WSM damage was severe in the north central region and light to moderate in the eastern part of the state. Cropping systems in these areas normally do not result in a high incidence of volunteer wheat; hence, the damage in these areas was surprising.

The high incidence of WSM in locations where volunteer wheat was not abundant suggested that other grass species might be serving as hosts for wheat curl mites and WSMV. Since 1986, Kansas farmers have committed almost 1.2 millon ha in the Conservation Reserve Program (CRP). These consigned hectares must be planted to approved perennial grasses or trees. Annual grasses grow naturally in these fields, especially in the first few years during stand establishment, and could create a potential virus reservoir.

Studies by different researchers have shown that several oversummering grasses may be hosts to WSMV. Included are the following common annual grasses: green foxtail (*Setaria viridis* (L.) P. Beauv.) (1,4,9,11-13), barnyard grass (*Echinochloa crusgalli* (L.) P. Beauv.) (1,4,8,9,11,12), sandbur (*Cenchrus pauciflorus* Benth.) (1,4,9,12), common witchgrass (*Panicum capillare* L.) (1,4,9,11-13), crabgrass (*Digitaria sanguinalis* (L.) Scop.) (1,4,9,11,12), stinkgrass (*Eragrostis cilianensis* (All.) Link ex Vign.) (1,4,9,11-13), and bristly foxtail (*Setaria verticillata* (L.) P. Beauv.) (1,9,11). Only a few perennials are cited as possible hosts, including: Virginia wildrye (*Elymus virginicus* L.) (1,4,9,12), Canada wildrye (*E. canadenis* L.) (1,9,12), and hairy grama (*Bouteloua hirsuta* Lag.) (1,9,12). However, most of these studies were conducted in the greenhouse where plants were inoculated mechanically or by introduced wheat curl mites to determine susceptibility to the virus. In some studies, grasses were collected in the field and examined for virus symptoms and the presence of curl mites. Most grasses were only sampled once, and reports did not indicate whether the plants remained alive and viruliferous until fall wheat emergence.

The objectives in this study were to: 1) determine which grass species were infected with WSMV in Kansas fields, including CRP and government set-aside land; 2) quantify the incidence of WSMV in different grass species; and 3) determine if commonly infected grass hosts are capable of maintaining WSMV through the summer and fall until the time of wheat emergence.

## MATERIALS AND METHODS

**1988 Survey.** The survey in 1988 involved collecting annual and perennial grasses that were found growing naturally or planted in CRP, government set-aside fields, waterways, and wheat stubble fields to test for the presence of WSMV. Samples were collected in August from several sites in 11 central Kansas counties where WSM incidence was high the previous spring and from Brown County in northeastern Kansas where WSM incidence was low. Locations sampled included 27 wheat stubble fields, 11 CRP fields, 5 waterways, and 9 set-aside fields. Initially, all grass species were identified and collected in randomly selected plots (1 m in diameter) on a transect across the field at each sampling site. However, this sampling procedure failed to include several grass species that occurred at low population densities or had clumped distributions in the field. Therefore, selected samples of some species were collected. Samples were not selected based on symptoms. In each sampling plot, five to 10 leaves from each plant were harvested, the presence of viruslike symptoms on harvested leaves was recorded, and the samples were stored on ice until returned to the laboratory, where they were stored at −20 C until processed for detection of WSMV.

**1989 Survey.** In 1989, the scope of the sampling was narrowed to a specific set of five grasses that the 1988 survey indicated were potential sources of the virus. These grasses were green foxtail, common witchgrass, giant foxtail (*Setaria faberi* Herrm.), prairie cupgrass (*Eriochloa contracta* Hitchc.), and barnyard grass. Grass samples were col-

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lected on several sampling dates from wheat harvest to fall wheat emergence, the oversummering period for the wheat curl mite and WSMV. Samples were collected at four sites: one in Dickinson County (site 1), one in Washington County (site 2), and two in Nemaha County (sites 3 and 4). Sites 1, 3, and 4 were CRP fields and site 2 was a waterway. Each site was within 0.5 km of or adjacent to wheat fields. Plant samples were collected from each site three times at approximately monthly intervals between late June and early October as described above. The incidence of WSM within and among sites was not analyzed statistically because of nonrandom distribution of grass species and because not all species were present at each site. Two other common grasses, smooth brome (*Bromus inermis* Leyss.) and yellow foxtail (*Setaria pumila* (Poir.) Roem. & Schult. [= *S. lutescens* (Weigel) Hubb.]), were sampled again in 1989.

**WSMV assay method.** The presence of WSMV in grass samples was detected by indirect enzyme-linked immunosorbent assay (ELISA) as described by Lommel et al (5). Approximately 0.2 g of tissue was placed in 1.5-ml microcentrifuge tubes. The middle third of the youngest leaves was selected when possible, but because of differences in size, maturity, and physical condition (e.g., freeze damage) of some specimens, leaf selection was altered to include acceptable green tissue. Tissue samples were ground with a wooden applicator stick in 250 μl of indirect ELISA buffer (IEB: 0.01 M phosphate buffered saline [PBS], pH 7.4, 0.05% Tween 20, 1% polyvinylpyrrolidone 40, and 0.2% egg ovalbumin) and centrifuged for 30 sec at 13,600 *g*. Then 50 μl of sap was added to 200 μl of coating buffer (0.05 M sodium carbonate, 0.01% sodium azide, pH 9.6) in wells on microtiter plates (Immulon 1 flat, Fisher Scientific, St. Louis, MO). Samples were duplicated on each plate. Incubation for all steps was for 1 hr at 37 C, followed by one rinse and two 2-min interval washes (1× PBS and 0.05% Tween 20). After the washings, WSMV antiserum (provided by S. A. Lommel) was added and the samples were incubated at 1:500 dilution in IEB. After incubation and a washing, alkaline phosphatase conjugate (goat antirabbit IgG whole molecule, Sigma Chemical Co., St. Louis, MO), at 1:1,000 dilution in IEB, was added to the wells. After the final washing, phosphatase substrate tablets (Sigma 104) were added to substrate buffer (10% diethanolamine and 0.01% sodium azide, pH 9.8) at 1 mg/ml. This mixture was added to the plate wells and incubated at room temperature for 15 min. Reactions were recorded in a Titertek Multiskan photometer (Flow Laboratories, McLean, VA) at $A_{405nm}$. All readings over 2× the negative controls were considered positive.

The wheat cultivar Arkan was seeded in sterilized soil and grown at 27 C in the greenhouse for the positive and negative controls. Plants for the positive control were inoculated with WSMV (provided by S. A. Lommel) by hand-inoculation technique (7) 14 days after emergence. Infected leaves were harvested 2–3 wk after inoculation and stored at −20 C. The negative control plants were harvested and stored at the same time.

## RESULTS

**1988 Survey.** Of the 303 samples representing 28 annual and perennial grass species tested for WSMV in 1988, 8.6% were positive (Table 1). None of the perennial grasses and only six species of annual grasses (barnyard grass, giant foxtail, green foxtail, prairie cupgrass, sandbur, and volunteer wheat) sampled positive for WSMV. Within these six susceptible annual grass species samples, 24.1% were infected with WSMV.

**1989 Survey.** Some of the 385 samples from each of the five susceptible grass species tested positive for WSMV, but not at all sampling sites or dates (Table 2). On the first sampling date in late June, only 4.7% of the total susceptible grass samples at site 1 were positive for WSMV. Consequently, sampling at the other sites was postponed for 3 wk. Sites 2, 3, and 4 were infected with WSMV in 54.2, 31.8, and 8.3% of all grasses sampled, respectively. By late August, the overall incidence of WSM at the four sites was slightly higher, but the disease

**Table 1.** Annual and perennial grasses tested for presence of wheat streak mosaic virus in 1988 and 1989

| | | 1988 | | 1989 | |
|---|---|---|---|---|---|
| Common name[a] | Scientific name | No. sampled | No. positive | No. sampled | No. positive |
| Barnyard grass (A) | *Echinochloa crusgalli* (L.) P. Beauv. | 10 | 1 | 50 | 28 |
| Big bluestem (P) | *Andropogon gerardii* Vitm. | 22 | 0 | ... | ... |
| Blue grama (P) | *Bouteloua gracilis* (H.B.K.) Lag. ex Steud. | 4 | 0 | ... | ... |
| Buffalograss (P) | *Buchloë dactyloides* (Nutt.) Engelm. | 4 | 0 | ... | ... |
| Common witchgrass (A) | *Panicum capillare* L. | 8 | 0 | 55 | 4 |
| Crabgrass (A) | *Digitaria sanguinalis* (L.) Scop. | 18 | 0 | ... | ... |
| Fall panicum (A) | *Panicum dichotomiflorum* Michx. | 10 | 0 | ... | ... |
| Giant foxtail (A) | *Setaria faberi* Herrm. | 11 | 1 | 61 | 19 |
| Green foxtail (A) | *Setaria viridis* (L.) P. Beauv. | 38 | 15 | 148 | 30 |
| Hairy grama (P) | *Bouteloua hirsuta* Lag. | 3 | 0 | ... | ... |
| Indian grass (P) | *Sorghastrum nutans* (L.) Nash | 18 | 0 | ... | ... |
| Little bluestem (P) | *Andropogon scoparius* Michx. | 15 | 0 | ... | ... |
| Prairie cupgrass (A) | *Eriochloa contracta* Hitchc. | 8 | 3 | 71 | 25 |
| Prairie threeawn (A) | *Aristida oligantha* Michx. | 3 | 0 | ... | ... |
| Sand dropseed (P) | *Sporobolus cryptandrus* (Torr.) A. Gray | 3 | 0 | ... | ... |
| Sandbur (A) | *Cenchrus pauciflorus* Benth. | 12 | 1 | ... | ... |
| Side-oats grama (P) | *Bouteloua curtipendula* (Michx.) A. Gray | 19 | 0 | ... | ... |
| Silver beardgrass (P) | *Andropogon saccharoides* Swartz | 8 | 0 | ... | ... |
| Smooth brome (P) | *Bromus inermis* Leyss. | 14 | 0 | 34 | 0 |
| Stinkgrass (A) | *Eragrostis cilianensis* (All.) Link ex Vign. | 9 | 0 | ... | ... |
| Switchgrass (P) | *Panicum virgatum* L. | 15 | 0 | ... | ... |
| Tall dropseed (P) | *Sporobolus asper* (Michx.) Kunth | 6 | 0 | ... | ... |
| Tumble lovegrass (P) | *Eragrostis sessilispica* Buckl. | 2 | 0 | ... | ... |
| Tumblegrass (A) | *Schedonnardus paniculatus* (Nutt.) Trel. | 3 | 0 | ... | ... |
| Volunteer wheat (A) | *Triticum aestivum* L. | 29 | 5 | ... | ... |
| Western wheatgrass (P) | *Agropyron smithii* Rydb. | 3 | 0 | ... | ... |
| Yellow foxtail (A) | *Setaria pumila* (Poir.) Roem. & Schult. (= *S. lutescens* (Weigel) Hubb.) | 6 | 0 | 54 | 0 |
| Yellow nutgrass (P) | *Cyperus esculentus* L. | 2 | 0 | ... | ... |

[a](A) = annual grass, (P) = perennial grass.

incidence at individual sites varied. Incidence was 44.7% at site 1, 17.6% at site 2, 20.7% at site 3, and 54.1% at site 4. The last sampling period was after a freeze on 24 September. Leaf margins and tips on many grass species were desiccated; some species, such as barnyard grass and common witchgrass, were severely desiccated. In October, 32% of the grasses sampled at site 1, 12.5% of those at site 2, 37.5% of those at site 3, and 18.8% of those at site 4 were infected with WSMV. Site 4 was mowed before the last sampling date, so collection was limited to the unmowed areas.

Population densities of the grass species differed greatly among sites. At site 1, barnyard grass, green foxtail, and prairie cupgrass were abundant. At site 2, green foxtail and prairie cupgrass were the predominant annuals in the smooth brome waterway. At site 3, green foxtail was most prevalent, with common witchgrass sporadically dispersed, and site 4 was predominantly giant foxtail.

Over all sites and sampling dates, 20.3% of green foxtail, 7.3% of common witchgrass, 35.2% of prairie cupgrass, 56.0% of barnyard grass, and 31.1% of giant foxtail, or 27.5% of all these grass species together, were infected with WSMV.

Only 28% of the samples collected that tested positive for WSMV had visible leaf symptoms. Six samples with viruslike leaf symptoms were negative for WSMV. Green foxtail, prairie cupgrass, and barnyard grass showed leaf mosaic or mottling symptoms, whereas giant foxtail showed unusual elongated light green blotches. Smooth brome and yellow foxtail were considered as nonsusceptible grass species but important to test because of their abundance.

Smooth brome was sampled from sites 2 and 4. Site 2 was a predominantly smooth brome waterway; at site 4, the smooth brome was along the edges of the field. All smooth brome samples were negative for WSMV (Table 1). Yellow foxtail was abundant at sites 1 and 2, but none of the samples collected was infected with WSMV (Table 1).

**Table 2.** Number of samples positive for wheat streak mosaic virus per number of samples collected for five selected grass hosts sampled on four dates at four sites in 1989

| Host | Date | Site[a] 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| Green foxtail | 29 June | 0/16[b] | ...[c] | ... | ... |
| | 20 July | ... | 5/15 | 6/18 | 0/2 |
| | 30,31 August | 1/12 | 2/16 | 2/13 | 3/12 |
| | 2,3 October | 3/16 | 0/15 | 8/12 | 0/1 |
| Common witchgrass | 29 June | 0/5 | ... | ... | ... |
| | 20 July | ... | 0/0[d] | 0/3 | 2/6 |
| | 30,31 August | 0/2 | 0/4 | 1/10 | 0/3 |
| | 2,3 October | 0/8 | 0/3 | 1/11 | 0/0 |
| Prairie cupgrass | 29 June | 0/9 | ... | ... | ... |
| | 20 July | ... | 8/9 | 0/0 | 0/0 |
| | 30,31 August | 6/12 | 4/14 | 0/0 | 0/0 |
| | 2,3 October | 3/14 | 4/13 | 0/0 | 0/0 |
| Barnyard grass | 29 June | 2/13 | ... | ... | ... |
| | 20 July | ... | 0/0 | 0/0 | 1/1 |
| | 30,31 August | 10/12 | 0/0 | 2/5 | 3/6 |
| | 2,3 October | 10/12 | 0/1 | 0/0 | 0/0 |
| Giant foxtail | 29 June | 0/0 | ... | ... | ... |
| | 20 July | ... | 0/0 | 1/1 | 0/27 |
| | 30,31 August | 0/0 | 0/0 | 1/1 | 14/16 |
| | 2,3 October | 0/0 | 0/0 | 0/1 | 3/15 |

[a]Site 1 = Dickinson County, Conservation Reserve Program; site 2 = Washington County, waterway; and sites 3 and 4 = Nemaha County, Conservation Reserve Program.
[b]Number positive/number sampled.
[c]Not tested on date shown.
[d]Species not found during sampling.

## DISCUSSION

This study is the first to report giant foxtail and prairie cupgrass as hosts for WSMV and confirms that green foxtail, common witchgrass, and barnyard grass can be alternate hosts to WSMV in their natural environment. It also shows that these grasses can maintain virus inoculum throughout the period from wheat harvest to wheat emergence. The low infection rate in late June could be associated with the sequence of wheat just maturing in nearby fields and the movement of the wheat curl mite. Thus, grasses recently infested with wheat curl mites may not yet be systemically infected. The slight reduction of WSM incidence in October could be attributed to the early freeze.

Because farmers have often implicated smooth brome as a source for WSMV, it was necessary to test this perennial grass. Likewise, sampling yellow foxtail was deemed necessary because it is in the same genus as green foxtail and giant foxtail and also is a common grass throughout the state. In both years of the study, neither of these grass species was found to be infected with WSMV, which supports the findings of earlier studies (1,12). Hence, smooth brome and yellow foxtail do not appear to be hosts to WSMV.

To function as an efficient oversummering bridge for WSMV and the wheat curl mite vector in quantities sufficient to cause an epidemic, host grasses (as a population if not as individual plants or species) must: 1) thrive in significant population densities in or adjacent to wheat fields, 2) emerge in the spring or early summer before wheat matures and survive until the fall-seeded wheat emerges, 3) be susceptible to WSMV infection and multiplication, and 4) maintain adequate wheat curl mite populations. Whether these grasses can contribute to an outbreak or epidemic of WSM depends on their habitat and distribution and on how well they fit the above criteria.

This study and other studies found that green foxtail, prairie cupgrass, barnyard grass, and giant foxtail are routinely intermixed and can be found abundantly in most areas of Kansas (2,3,6). Common witchgrass was found less often. Giant foxtail is mostly confined to the eastern half of the state, whereas prairie cupgrass grows throughout the state except the northwest corner. These species produce seeds from July to September, with plants maturing into October. Prairie cupgrass and barnyard grass develop slightly later than the other species. Most are prevalent in cultivated fields, in waste areas, and along roadsides, with barnyard grass and prairie cupgrass also adapted to moist areas. Therefore, these species are present at the right time and in sufficient numbers to potentially serve as oversummering reservoirs for WSMV.

We assume that all infected grasses in this study were naturally inoculated by wheat curl mites. However, we do not know whether these grass hosts can maintain sufficient populations of wheat curl mites to be a signficant epidemiological source of viruliferous mites. Somsen and Sill (12) reported that green foxtail was susceptible to wheat curl mites, but quantitative data are lacking. More research is required on the population dynamics of wheat curl mites on various alternate grass hosts.

Conservation Reserve Program hectares, government set-aside areas, wheat stubble fields, and weedy waterways provide an opportunity for large populations of annual grasses to develop. It has yet to be proved that these grass hosts can initiate serious epidemics of WSM in adjacent wheat fields. However, it would be prudent to: 1) manage CRP fields by mowing or burning to suppress annual grasses and 2) avoid planting susceptible wheat cultivars adjacent to areas with high populations of these annual grasses.

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