Identification and Occurrence of Wheat Streak Mosaic Virus in Winter Wheat in Colorado and Its Effects on Several Wheat Cultivars

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ABSTRACT

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Winter wheat plants showing symptoms presumed to be those of streak mosaic were collected in northeastern Colorado in 1979 and 1980 to determine if wheat streak mosaic virus (WSMV) was the causal agent. Inoculum was prepared from each plant sample and hand-rubbed onto Carborundum-dusted Parker wheat seedling leaves. Six of 73 samples produced streak mosaic symptoms on inoculated seedlings. These six samples were confirmed as WSMV isolates by using appropriate indicator plants, stability in plant sap, occurrence of characteristic inclusion bodies, electron microscopy, and leaf-dip serology. Differences in symptom expression and percent infection were found among the six Colorado isolates when compared on Parker seedlings. The presence of WSMV was subsequently confirmed in 11 severely diseased commercial fields. In all cases, either nearby fields had many volunteers because of severe late-season hail damage or corn or millet had been grown in an adjacent area during the previous growing season. Volunteer wheat plants, corn, or millet apparently served as primary inoculum sources for early-fall infection of newly emerged winter wheat seedlings. Eight winter and four spring wheat cultivars were evaluated for symptom expression and yield loss after inoculation with a single WSMV isolate in the greenhouse. Significant reductions occurred within all cultivars. Regression analysis revealed no significant correlation between reductions in yield and plant height or fertile tiller number. Seedling disease severity ratings could not be used to predict relative rankings in percent reductions.

Wheat streak mosaic virus (WSMV) is a serious threat to wheat production in the United States, Canada, and many other wheat-producing areas of the world (22). The estimated average annual loss to wheat streak mosaic (WSM) in Kansas from 1971 to 1976 was 13 million bushels, with 30 million bushels lost in 1974 alone (13). The annual loss to WSM in Colorado has been less than 5%, but individual growers can sustain severe losses. The wheat curl mite (*Aceria tulipae* Keifer) is the only known WSMV vector in the United States (17,19), and virus epidemiology seems closely correlated with mite population dynamics (6). Many wild grasses also may serve as alternate hosts for mites and the virus (16,19,20).

McKinney (9) reported in 1949 that WSMV was the causal agent of mosaic symptoms in common wheat (*Triticum aestivum* L.) collected near Akron, CO.

Some of the information presented is a portion of the first author's M.S. thesis.

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McKinney's identification was based only on host range, which included positive reactions on bromegrass (*Bromus tectorum* L.), corn (*Zea mays* L.), and sugarcane (*Saccharum officinarum* L.) (9). McKinney reported that WSMV damaged Colorado wheat in 1953 (10) but did not include Colorado in his 1967 review (11) of states sustaining WSM losses in wheat. Sill and Agusiobo (15) reported that sugarcane was not a host of WSMV. Thus, the host range discrepancy casts some doubt on the initial report of the virus in Colorado. However, the early observations of WSM in Nebraska in 1922 (4) and in Kansas in 1929 (8) indicate a continuing spread to Colorado.

Our study was initiated to obtain and positively identify WSMV isolates from Colorado-grown winter wheat, to observe major yield loss situations in the state, and to determine if cultivar seedling disease severity ratings could be correlated with reductions in plant height, number of fertile tillers, and yield in the greenhouse.

MATERIALS AND METHODS

Seventy-three winter wheat plants showing mosaic symptoms were collected from commercial fields in northeastern Colorado from the fall of 1979 through the fall of 1980. Tissue samples from these plants were separately prepared as inocula by macerating mosaic leaves in 0.02 M, pH 7, potassium phosphate buffer (1:5, w/v). Inoculum was hand-rubbed onto Carborundum-dusted leaves of Parker wheat seedlings at the three- to four-leaf stage. After inoculation, seedlings were placed in a Percival growth chamber (Percival Refrigeration & Manufacturing Co., Boone, IA 50036) programmed with 12 hr of fluorescent and incandescent light (about 5,000 lux) followed by 12 hr of darkness. This production of systemic WSM symptoms on Parker wheat seedlings held at 27 and 33 C was recorded. Separate inocula of individual isolates were prepared from seedlings that developed systemic mosaic symptoms. Fifteen seedlings each of *Agropyron intermedium* L., *Hordeum jubatum* L., and oat (*Avena sativa* L. 'Russell') were then inoculated with the isolates. These plant species have been used as indicator plants to aid in the identification of WSMV (18). Inoculated plants were placed in the growth chamber at 27 C and observed for development of systemic WSM symptoms.

Percent infection and symptom expression of the Colorado WSMV isolates and a Nebraska WSMV isolate (obtained from M. K. Brakke, University of Nebraska, Lincoln) were assessed. Each isolate was hand-rub-inoculated on 30 Parker seedlings and placed in the growth chamber at 27 C under 12 hr of light followed by 12 hr of darkness. After 2 wk, the number of plants expressing WSM symptoms was recorded for each isolate. Diseased plants were rated for severity on a scale of 1–5, where 1 = light green streaks, 2 = mostly light green and a few yellow streaks, 3 = mixed green and yellow streaks, 4 = yellow streaks, and 5 = severe yellow streaks.

Light microscopic observations for cytoplasmic inclusions were made using the calcomine orange-brilliant green technique for staining epidermal strips (3). Epidermal strips obtained from WSMV-inoculated and uninoculated Parker wheat seedlings were stained using the calcomine orange-brilliant green technique (2) and examined for inclusion bodies with a compound light microscope (×1,000). The leaf-dip serology method (1,5) was used for electron microscopic observations. WSMV antiserum was supplied by J. K. Uyemoto, Kansas State University, Manhattan. Samples were observed with an AE1-6B electron microscope (Picker Nuclear, formerly Associated Electrical Industries Limited, Harlow, Essex 26761, U.K.) at 60 kv.

From May through July of 1980 and 1981, diseased plants showing wheat streak mosaic symptoms were collected from 11 severely diseased fields. The presence of WSMV was confirmed by inoculating 10 Parker wheat seedlings with inocula prepared from these diseased plants. The cropping and environmental history of diseased fields and adjacent areas was correlated with WSM incidence.

Eriophyid mite (*A. tulipae*) transmission of the virus was attempted by placing a pot containing WSMV-infected and mite-infested 4-wk-old plants in the center of a flat containing uninoculated 14-day-old Parker seedlings. A small electric fan generated air movement to help disseminate the mites. The fan was periodically moved around the group of plants to facilitate mite movement in all directions. A similar arrangement with nonviruliferous mite-infested plants was used as a control.

Eight winter and four spring wheat cultivars were grown to maturity in the greenhouse and evaluated for symptom expression and yield reduction after inoculation with WSMV. One hundred seeds of each winter wheat cultivar were placed in petri dishes lined with filter paper and germinated under light at room temperature. After 3 days, they were transferred to a cold room (1 C) without light and vernalized for 72 days. Eight milliliters of distilled water, containing 0.27 g/L Terracoat (pentachloronitrobenzene) to reduce fungal growth, was first added to each petri dish. Five milliliters of solution was added to each dish every 10 days to maintain adequate moisture. Twenty-four plastic pots (15.5 × 15.5 cm) containing soil, peat, perlite, and vermiculite (2:1:1:1, v/v) were sown at the rate of two seedlings per pot for each of the eight vernalized winter wheat cultivars and two seeds per pot for each of the four spring wheat cultivars. One week later, the plants were thinned to one per pot.

Inoculum from Parker seedlings inoculated with WSMV 2 wk earlier was prepared by macerating infected leaves in a 1:20 (w/v) dilution of leaf tissue to 0.02 M potassium phosphate buffer (pH 7.0). The extract was filtered through cheesecloth, and Carborundum was added as 1.5% (w/v) of the filtrate. At the four-leaf stage, 12 plants of each cultivar were hand-rub-inoculated. The remaining 12 plants of each cultivar were treated similarly with buffer and Carborundum only. Two weeks later, inoculated seedlings were rated for disease severity. At maturity, plant height, number of fertile tillers, and total grain yield were recorded for each plant. Percent reduction was obtained from each of the 12 pairs of each cultivar and transformed using the arc sine conversion (21). A two-way analysis of variance and regression analysis were performed on the transformed data (21). Yield data means were ranked and separated using Duncan's multiple range test (21).

Table 1. Percent infection and average disease severity obtained from 30 Parker wheat seedlings hand-rub-inoculated with an isolate of wheat streak mosaic virus

Isolate	Percent infection	Avg. disease severity
NB 1[a]	100	5[b]
CO 1	100	5
CO 2	93	3
CO 3	90	3
CO 4	83	3
CO 5	73	1
CO 6	60	3

[a] Nebraska and Colorado isolates.
[b] 1 = Light green streaks, 3 = mixed green and yellow streaks, and 5 = severe yellow streaks.

RESULTS

Inocula prepared from six of the 73 samples produced typical wheat streak mosaic symptoms and leaf drooping within 1 wk. Subsequent indicator plant inoculations with these six isolates produced no symptoms on *A. intermedium* and *H. jubatum*, whereas all the oat cultivar Russell plants developed typical systemic streak mosaic symptoms within 19 days of inoculation. Only inocula prepared from plants showing WSM symptoms could transmit the virus to susceptible seedlings. Sap from plants infected with each isolate remained infective to Parker seedlings after storage at room temperature (about 20 C) for 4 days.

Leaf epidermal cells from Parker wheat plants inoculated with each of the six isolates contained cytoplasmic masses of cylindrical inclusions typical of those reported by Christie and Edwardson (3). No such inclusions were found in the epidermal cells of leaves obtained from uninoculated Parker seedlings. Electron micrographs of wheat leaf-dip preparations revealed nearly identical flexuous-rod-shaped particles from each isolate. The shape and size of the particles were within the range of described WSMV particles (2). Virus particles were not found in uninoculated control wheat leaves. The virus particles from all infected samples reacted positively with the WSMV antiserum during leaf-dip serology procedures.

Typical mosaic symptoms on many of the plants surrounding the mite-infested, WSMV-infected plants indicated that mites had transmitted the virus. No streak mosaic symptoms appeared in any of the plants surrounding the uninoculated mite-infested plants. Mites were observed on all plants in both treatments.

Nine of the 11 severely WSM-infected commercial winter wheat fields had been planted near winter wheat fields that had sustained severe hail damage late in the growing season and contained abundant volunteer wheat plants. One field had been planted adjacent to corn, and the other field had been planted adjacent to foxtail millet (*Setaria italica* (L.) Beauv.). Therefore, all the new wheat crops were adjacent to living alternate hosts of the vector and virus during the fall.

The effects of seven WSMV isolates hand-rub-inoculated on Parker wheat seedlings are presented in Table 1. Variation in percent infection and disease severity among the isolates is demonstrated. Percent reduction of height, fertile tillers, and yield of 12 cultivars after inoculation with a single WSMV isolate are presented in Table 2. The average seedling disease severity rating is also included. There were significant differences among cultivars for all traits but not within cultivars. Significant differences in yield reduction among cultivars are demonstrated in Table 3. Regression analysis revealed no significant relationships between reduced yield and reduced plant height, reduced fertile tillers, or seedling disease severity.

Table 2. Average percent reduction in height, fertile tillers, and yield and average seedling disease severity of 12 wheat cultivars hand-rub-inoculated with an isolate of wheat streak mosaic virus and grown to maturity in the greenhouse

Cultivar	Height (%)	Fertile tillers (%)	Yield (%)	Avg. seedling disease severity
Ariz	38.4	16.7	52.4	5[a]
L.S. 25	26.6	51.9	70.5	2
Lugimi	58.0	92.5	91.4	1
Super 'X'	15.8	12.6	50.2	5
Baca	59.9	54.2	81.7	2
Centurk	51.9	65.1	88.6	1
Duke	67.9	57.3	76.2	2
Sandy	46.9	46.6	78.5	2
Scout	40.5	43.9	70.5	3
Vona	40.0	31.3	62.6	4
Warrior	38.4	37.1	67.1	4
Wichita	54.7	63.6	89.0	1

[a] 1 = Light green streaks, 2 = mostly light green and a few yellow streaks, 3 = mixed green and yellow streaks, 4 = yellow streaks, and 5 = severe yellow streaks.

DISCUSSION

Occurrence of WMSV in Colorado has been assumed for many years and there is no reason to believe that it was not present, because the virus has caused considerable damage in Kansas and Nebraska for some time (4,7,11).

However, the host range discrepancy in the original report on WSMV occurrence does leave some doubt as to the time of appearance and subsequent losses attributable to this disease in Colorado (9). The low isolation frequency of WSMV from winter wheat showing streak mosaic symptoms in this study may help explain the host range discrepancy. WSMV was confirmed in only six of the 73 plants showing symptoms, and it is possible that a different virus, another type of pathogen, or certain environmental conditions caused the mosaic symptoms in the remaining plants. We have not been able to identify a pathogen associated with these symptoms. Many of the 73 mosaic plant samples were collected from commercial fields where WSM-like symptoms were not widespread. Subsequent samples from fields sustaining widespread infection, with a disease gradient from an inoculum source, consistently yielded WSMV.

Isolation and confirmation of WSMV from other than severely infected areas appears to be difficult. Our difficulty in using visual symptoms for field identification casts some doubt on past yield-loss estimates attributed to WSMV in Colorado. Perhaps, the original Akron mosaic-symptom plants were obtained from mildly to moderately infected fields, and thus, the chance of isolating WSMV was much less than from severely infected fields. Laboratory verification of WSMV seems necessary for confirmation of this disease in Colorado, at least in fields suspected of being mildly to moderately infected.

All of the severe WSM occurrences brought to our attention could be correlated with an adjacent field that, during the previous year, had either sustained severe late hail damage or was planted to corn or millet. The resultant proximity of living mite and virus alternate hosts seems to be the most important condition contributing to severe incidence of WSM in Colorado. In addition to the thrashing action on the grain heads, hail provides the moisture essential for mature seeds to germinate as volunteers. Many volunteer wheat plants occur in these fields and persist after harvest. Volunteer wheat plants and corn or millet then can act as a green tissue bridge between crops where the mites and virus can survive and multiply. If fall weather remains mild, permitting mite activity and sustained plant longevity and growth, the virus can be spread to the new wheat crop.

Table 3. Ranked means of percent yield reduction for 12 wheat cultivars inoculated with a single wheat streak mosaic virus isolate

Cultivar	Mean percent reduction[z]
Wichita	89.0 a
Lugimi	91.4 a
Centurk	88.6 a
Baca	81.7 abc
Sandy	78.5 abcd
Duke	76.2 abcd
L.S. 25	70.6 bcd
Scout	70.5 cd
Warrior	67.1 d
Vona	62.6 de
Ariz	52.4 e
Super 'X'	50.2 f

[z]Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

WSMV observed in naturally infected fields revealed that severity decreased rapidly as distance increased from the inoculum source. Generally, in Colorado, significant losses caused by WSMV do not occur in fields farther than 1 km from the inoculum source. Breaking the mite cycle has been recommended for disease control in other wheat-growing areas (6). Planting winter wheat at least 1 km from corn, millet, or volunteer wheat, or destroying volunteer wheat plants at least 3 wk before planting, seem to successfully control the disease in Colorado. The virus seems to be endemic at low levels throughout Colorado, either in wheat fields or wild hosts, as demonstrated by its ability to cause losses whenever favorable conditions occur.

All virus identification methods, including the eriophyid mite transmission in the greenhouse, contributed to the positive identification of the six Colorado isolates. The combined information identifies the causal agents as WSMV. However, inoculation of Parker wheat seedlings seems to be a convenient and accurate WSMV indicator when testing plants showing WSM symptoms.

The ranges of symptom expression and infection efficiency on a single wheat cultivar indicate pathogenic variability within the Colorado population of WSMV. A program to screen or breed for increased WSMV resistance or tolerance should include as many isolates as possible in the inoculum. The interaction of various factors, such as time of infection, temperature, soil moisture, soil fertility, and cultivars with symptom expression and yield (20), should also be recognized in such a program. Seedling disease severity ratings and plant height and fertile tiller reductions did not correlate significantly with yield. It might have been practical to select at the individual plant level within a large heterozygous (segregating) wheat population if a high correlation of yield and one or more of the factors existed. The 12 cultivars sustained an average yield loss of 50–91%, which indicates genetic variation in resistance or tolerance to WSMV. Similar variation in resistance or tolerance has been observed in the field (14). Lack of correlation between field symptoms and yield (12) may result from variation in percent and time of infection as well as interactions with environmental conditions. Yield tests seem to be the best available method for identifying cultivar differences in tolerance or resistance to WSMV. Further research investigating WSMV variability and cultivar interactions is in progress.

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