Separation of Montana Isolates of Wheat Streak Mosaic Virus on Michigan Amber Wheat

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ABSTRACT

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Eight isolates of wheat streak mosaic virus from winter wheat in central and eastern Montana were separated into two classes of variants or strains. Seven isolates were similar to the mild strain and one resembled the type strain of the virus. Separation was made by comparing the Montana isolates with the mild and type strains of the virus on the basis of symptoms caused in mechanically inoculated Michigan Amber wheat in the greenhouse.

For some time, the diagnosis of wheat streak mosaic (WSM) in Montana has been puzzling when based solely on symptom expression. Leaves from diseased plants of winter and spring wheat collected in commercial fields have had symptoms varying from a light green mottle to a severe chlorosis. The chlorosis symptom has often been indistinguishable from leaf yellowing caused by barley yellow dwarf virus or from nitrogen deficiency. Only immunosorbent electron microscopy and mechanical transmission tests have allowed us to determine that the responsible agent was wheat streak mosaic virus (WSMV) and not barley yellow dwarf virus. A similar chlorosis of entire wheat leaves due to WSMV was reported many years ago (7). In 1976, a greenhouse study was undertaken to determine whether symptom variations associated with WSM were caused, in part, by variants or strains of WSMV.

Variants or strains of WSMV have been recognized for some time, but few studies have dealt with their biological or physicochemical properties. The virus is presently believed to exist as filamentous particles about 700 nm long and 15 nm in diameter. Each virus particle is thought to be composed of a single molecule of ribonucleic acid, and treatment with ribonuclease or formaldehyde suggests that it is single stranded (2). However, virus characterization has been difficult because the virus is unstable (10) and because it has been associated at times with the wheat spot mosaic agent (8,10). Like WSMV, this agent is transmitted by *Aceria tulipae* Keifer, but unlike WSMV it is not mechanically transmissible. To date, the nature of the wheat spot agent remains a mystery (10). Possible associations may also occur between WSMV and other viruses or agents. For example, L. C. Lane has discovered an inexplicable protein (mol wt about 29×10^3) in addition to the coat polypeptide (mol wt $40-43 \times 10^3$) in protein preparations of WSMV (M. K. Brakke, *personal communication*).

In this article, we report the separation of Montana isolates of WSMV into two classes of variants or strains. The separation was made by comparing the Montana isolates with the mild and type strains of WSMV on the basis of symptoms caused in mechanically inoculated Michigan Amber wheat in the greenhouse. For the purpose of this study, it was assumed that each isolate or strain studied was a variant of what is presently believed to be WSMV. A preliminary report of this work has been published (11).

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MATERIALS AND METHODS

Isolates of WSMV in diseased leaves were collected from wheat plants in central and eastern Montana and named for the towns closest to collection sites. The Kansas 1, Nebraska 2, and Nebraska 5 isolates of the virus were obtained in diseased wheat leaves from C. L. Niblett, formerly of Kansas State University, Manhattan. An isolate of WSMV from corn (I isolate) was received in corn leaf material from J. H. Hill, Iowa State University, Ames. The type culture PV57 (formerly AC 29, ATCC) and the mild strain PV91 (formerly AC 85, ATCC) of WSMV were supplied by M. K. Brakke, University of Nebraska, Lincoln, in diseased wheat leaves. Diseased corn and wheat leaves were chopped into small strips, dried in vacuum desiccators over Drierite, transferred to large test tubes containing fresh Drierite, and placed in a freezer until needed.

All isolates and strains of the virus were transferred by mechanical inoculation to about 12 seedlings of Winalta (CI 13670) winter wheat in the three- to five-leaf stage (four seedlings per 20-cm pot). Inoculum prepared by grinding diseased leaf tissue in distilled water (1 g/5 ml) using a mortar and pestle was rubbed onto Carborundum-dusted seedlings with cotton swabs. Infected Winalta plants served as reservoir hosts for the isolates and strains of WSMV.

Wheat cultivars used as differential hosts were the hard red winter wheat (*Triticum aestivum* L.) cultivars Centurk (CI 15075), Cheyenne (CI 8885), Winalta, Eagle (CI 15068), and Parker (CI 13285); the soft red winter wheat cultivar Michigan Amber (CI 11379); the hard red spring wheat cultivars Fortuna (CI 13596) and Olaf (CI 15930); and Ward (CI 15892) durum spring wheat (*T. durum* Desf.).

Corn (*Zea mays* L.) lines tested as differential hosts were Oh28, Oh28Rf, N28Ht, B37Ht, H84, H93, and Seneca Chief sweet corn. Using the procedures described above, sap preparations from infected Winalta wheat plants were applied to wheat and corn seedlings growing in 20-cm (8-in.) pots, one to four plants per pot. Plants were checked for symptoms at 2, 4, 6, and 8 wk after inoculation. Isolates or strains of WSMV were separated into two categories based on symptom severity in Michigan Amber wheat.

All plants were grown in steam-sterilized potting soil and irrigated about three times weekly with liquid greenhouse fertilizer (20-20-20 NPK). Wheat hosts were grown from late autumn to early summer in the greenhouse at Bozeman, MT. Temperatures ranged from 13 to 33 C. The plants received supplementary light daily (1600–2200 hr) from metal halide lamps (about 40,000 lux at plant height). Corn plants were grown during the summer or autumn when temperatures ranged from 16 to 38 C, and the light regime was the same as that used for wheat plants.

To check the WSMV isolates and strains for possible contamination with brome mosaic virus (BMV), agropyron mosaic virus, and hordeum mosaic virus, selected indicator test plants (contamina-

Table 1. Symptom severity[a] in greenhouse-grown wheat plants of different cultivars mechanically inoculated with Montana isolates and selected strains (variants) of wheat streak mosaic virus

Isolate/Strain	Centurk	Cheyenne	Winalta	Parker	Eagle	Fortuna	Olaf	Ward	Michigan Amber
Bozeman, MT[b]	MI	MO	MO	MI	MO	MO	S	MO	MI
Choteau, MT	S	MI	MO	MO	MI	MO	MO	MO	MI
Conrad, MT	S	S	S	S	S	MO	MO	S	S
Culbertson, MT	MI	MI	MI	MI	MI	MI	S	MI	MI
Froid, MT	S	MI	MI	MI	S	MO	S	MO	MI
Geraldine, MT	S	MI	MO	S	MO	MO	MO	MO	MI
Lewistown, MT	MI	MI	MO	MI	MI	MO	MO	MI	MI
Sidney, MT	MI	MI	MI	MO	MI	MO	S	MO	MI
Kansas 1	MO	MO	MO	MO	MO	MO	MO	MO	MI
Nebraska 2	S	MO	S	MO	S	MO	MO	MO	S
Nebraska 5	MO	MO	MO	MO	MO	MO	MO	MO	MI
Iowa	MO	MO	MO	MO	MO	MO	MO	MI	MI
Mild	MI	MO	MO	MI	MI	MO	MO	MO	MI
Type	S	S	MO	S	MO	MO	S	MO	S
Uninoculated (control)	N	N	N	N	N	N	N	N	N

[a] Symptom severity on leaves: MI = mild (mosaic or light green mottle, spots or dashes); MO = moderate (yellow green mottle, dashes and streaks); S = severe (yellow mottle, dashes and streaks); N = no symptoms.
[b] Field isolates collected in Montana.

tion check hosts) were inoculated mechanically with leaf sap preparations from the Winalta reservoir hosts. Four to eight plants were inoculated per isolate or strain as described earlier. Golden Bantam Cross or Golden Giant sweet corn was used to detect BMV (1), quackgrass (*Agropyron repens*) was used to screen for agropyron mosaic virus, and foxtail barley (*Hordeum jubatum*) was used to detect hordeum mosaic virus (9). In addition, immunodiffusion tests (4) and immunosorbent electron microscopy (3,5) were used to check WSMV strains, isolates, and cultures for possible contamination with barley stripe mosaic virus and BMV. Immunosorbent electron microscopy was also used to monitor WSMV in reservoir and differential hosts.

RESULTS AND DISCUSSION

No contaminating viruses or agents were detected in the isolates of WSMV collected in Montana wheat or in donated strains of the virus. Only filamentous particles about 700 nm long and 15 nm wide were observed for each variant of WSMV by immunoelectron microscopy.

After the 14 isolates and strains of WSMV were inoculated onto 2,211 differential host plants representing nine cultivars of wheat, 96.3% of the plants expressed symptoms. Sixty-one uninoculated control plants remained symptomless throughout the work. Some inoculated but asymptomatic plants and many uninoculated control plants were assayed by immunosorbent electron microscopy, and all were found to be free of typical WSMV particles.

The eight Montana isolates of WSMV were separated into two groups based on the symptom severity on Michigan Amber wheat, using the mild and type strains of the virus as reference standards. Seven isolates were similar to the mild strain and one resembled the type strain (Table 1). Mild symptoms consisted of a mosaic or light green mottle, spots or dashes, whereas the severe symptoms included a yellow mottle, dashes and streaks. The symptom severity was relatively uniform throughout all combinations of the different Montana virus isolates in Michigan Amber wheat (Table 1). None of these isolates caused moderate symptoms (yellow green mottle, dashes and streaks). From 18 to 28 plants were observed per virus isolate in Michigan Amber wheat.

Although both Centurk and Parker wheat gave good separation of the Montana isolates of WSMV into variant groups, Michigan Amber wheat, when used with the mild and type strain reference standards, was our choice as a diagnostic species because it consistently provided the clearest separation of mild and severe variants in relation to that of Centurk, Parker, and the other wheats tested. Moreover, Michigan Amber wheat was our choice as a diagnostic species because it also has been used as a propagation species (2).

Winter wheat cultivars currently grown in Montana varied widely in their reaction to the Montana isolates and the mild and type strains of WSMV. Whereas Centurk reacted with severe symptoms to 5 of 10 isolates and strains, Cheyenne and Winalta only reacted with severe symptoms to 2 of 10 and 1 of 10 isolates or strains of the virus, respectively.

On the basis of the symptom severity of all wheat differential cultivars, the Conrad isolate was most severe, followed by the type and N-2 strains (Table 1). The K-1, N-5, and I strains produced mainly moderate symptoms, whereas the Culbertson, Lewistown, and Sidney isolates caused the mildest symptoms. The mild strain did not elicit any severe symptoms and the type strain did not induce any mild symptoms in the wheat differentials (Table 1).

Variants of WSMV reportedly can be differentiated into four strains on corn (6). However, our attempts with recommended corn cultivars and WSMV isolates were unsuccessful. We were able to infect the N28Ht and B37Ht corn cultivars with the N-2 and N-5 isolates of WSMV, making it impossible to distinguish the N-2 from the K-1 isolate. Consequently, we abandoned the use of the recommended corn cultivars to differentiate strains of WSMV.

Our results with the separation of Montana isolates suggest that other variants of WSMV may be categorized into strains using Michigan Amber wheat with the mild and type strains of the virus used as reference standards. Mild, moderate, and severe strains, or just mild (including moderate) and severe strains may be distinguished. Knowledge of the variants of WSMV in the virulence pool of a region would be useful in breeding programs there. For example, once any variant was separated into a strain and its ability to cause crop loss determined, it could be selected for the virus inoculum used in replicated field trials of various breeding lines. Additionally, knowledge of strain virulence on currently planted wheat cultivars would increase the accuracy of estimating crop losses of commercial fields.

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LITERATURE CITED

1. Bancroft, J. B. 1970. Brome mosaic virus. Descriptions of Plant Viruses. No. 3. Assoc. Appl. Biol./Commonw. Mycol. Inst., Kew, Surrey, England. 4 pp.
2. Brakke, M. K. 1971. Wheat streak mosaic virus. Descriptions of Plant Viruses. No. 48. Assoc. Appl. Biol./Commonw. Mycol. Inst., Kew, Surrey, England. 4 pp.
3. Brlansky, R. H., and Derrick, K. S. 1979. Detection of seedborne plant viruses using

serologically specific electron microscopy. Phytopathology 69:96-100.
4. Carroll, T. W., Gossel, P. L., and Batchelor, D. L. 1979. Use of sodium dodecyl sulfate in sero-diagnosis of barley stripe mosaic virus in embryos and leaves. Phytopathology 69:12-14.
5. Derrick, K. S. 1973. Quantitative assay for plant viruses using serologically specific electron microscopy. Virology 56:652-653.
6. Niblett, C. L., Newman, M. A., Livers, R. W., and Young, J. R. 1976. Strains of wheat streak mosaic virus. Annu. Wheat Newsl. 22:85-86.
7. Slykhuis, J. T. 1953. Wheat streak mosaic in Alberta and factors related to its spread. Can. J. Agric. Sci. 33:195-197.
8. Slykhuis, J. T. 1955. *Aceria tulipae* Keifer (Acarina: Eriophyidae) in relation to the spread of wheat streak mosaic. Phytopathology 45:116-128.
9. Slykhuis, J. T., and Bell, W. 1966. Differentiation of agropyron mosaic, wheat streak mosaic, and a hitherto unrecognized hordeum mosaic virus in Canada. Can. J. Bot. 44:1191-1208.
10. Wiese, M. V. 1977. Compendium of Wheat Diseases. Am. Phytopathol. Soc., St. Paul, MN. 106 pp.
11. Zaske, S. K., Carroll, T. W., and Brlansky, R. H. 1981. Utilization of Michigan Amber wheat to characterize Montana isolates of wheat streak mosaic virus. (Abstr.) Phytopathology 71:1008.