A cereal-infecting virus from orchardgrass

J.F. Peterson'

Extracts of orchardgrass *(Dactylis glomerata)* leaves showing mosaic symptoms in Frelighsburg, Quebec, were manually inoculated to wheat, oats, and barley, all of which developed similar symptoms. A few symptomatic orchardgrass leaves collected in Ste. Anne de Bellevue were infested with *Aceria tulipae,* an eriophyid mite vector of some viruses. Flexuous rod-shaped particles typical of other known grass viruses were associated with symptoms in experimentally infected barley and wheat, but the host range of the virus from orchardgrass, designated provisionally as "Orchardgrass mosaic virus" ('OGMV'), differentiated it from most of the other well-known grass viruses. Oat cultivars recommended for production developed fairly severe symptoms after inoculation, while symptoms were more moderate on spring and winter wheat cultivars.

Can. Plant Dis. Surv. 69:1, 13-16, 1989.

Un jus extrait de feuilles de dactyle *(Dactylisglomerata)* recueillies à Frelighsburg (Québec) et présentant des symptbmes de mosa'ique a été inoculé méchaniquement à du blé, de l'avoine et de l'orge, lesquels ont tous développé des symptbmes. Quelques plants de dactyle, recueillis à Ste. Anne de Bellevue, étaient de plus infestés par *Aceria tulipae* une mite de la famille des "Eryophyidae" qui est un vecteur de quelques virus. Des particules flexueuses en batonnets, caractéristiques d'autres virus connus des graminés ont été associées aux symptbmes observes sur l'orge et le blé inoculés. Toutefois, la gamme d'hôtes de ce virus, provisoirement nommé "virus de la mosa'ique du dactyle" ('DaMV'), le distingue de la plupart des autres virus connus s'attaquant aux graminees. Les symptômes manifestés par les variétés d'avoine recommandées au Quebec après inoculation ont été sévères, tandis que ceux du blé d'hiver et de printemps ont été plus légers.

Introduction

The growing of small grains fits in well with both dairying and some cash crop rotations in Quebec. Despite the importance of small grains to Quebec's agriculture, the only virus disease affecting them which has received much attention is the aphid-borne barley yellow dwarf virus. Comeau and co-workers have studied this extensively, and historically it has been the most important virus disease of cereals in Quebec (3, 4, 5).

In 1978, the writer observed virus-like symptoms on orchardgrass *(Dactylis glomerata* L.) occurring in Ste. Anne de Bellevue. During the late summer of 1984, orchardgrass plants showing typical virus-like symptoms were observed in both Frelighsburg and Ste. Anne de Bellevue. The virus was transmitted by manual inoculation to some cereals and orchardgrass. Because orchardgrass is common in the area and could represent a significant reservoir host, a greenhouse culture was established, and has been maintained for preliminary characterization by means of host range tests, determination of particle morphology, and investigations of reactions of currently recommended cereal cultivars. The virus from orchardgrass is designated provisionally as "Orchardgrass mosaic virus" ('OGMV').

1 *Department of Plant Science, Macdonald College of McGill University, 21,111 Lakeshore Road, Ste. Anne de Bellevue, Quebec H9X 1C0.*

Accepted for publication September 15, 1986.

Materials and methods

Establishment of primary culture. Orchardgrass leaves collected from naturally infected plants at the Agriculture Canada research station in Frelighsburg were homogenized in 0.1M potassium phosphate buffer, pH 7, containing 0.1% 2-mercaptoethanol, and a small amount of Celite (diatomaceous earth, Johns-Manville) was added to serve as an abrasive. The extract was rubbed onto leaves of Black Hulless barley (*Hordeum vulgare* L.) in the two-leaf stage with a cheesecloth pad, and the inoculated plants were then rinsed with tap water. All test plants were grown in the greenhouse at an average temperature of 19-22°C during the winter and 25-30°C during the summer.

Additional leaves of orchardgrass were stored 4-5°C for 11 to 21 days before extracts were similarly prepared and used to inoculate Selkirk (spring) and Genesee (winter) wheat (*Triticum aestivum* L.), Roxton and Clintland oats (*Avena sativa* L.) at the two-leaf stage.

Electron microscopy. A leaf segment of experimentally inoculated Black Hulless barley was minced in a drop of water on a glass slide, and a droplet of the resulting extract was placed on an electron microscope grid, blotted, stained with neutralized 2% phosphotungstic acid, and examined in a Zeiss EM-9A electron microscope. Additional examinations were made of extracts clarified and concentrated from Genesee wheat.

Host range tests and cereal cultivar inoculations. Various graminaceous species, and some cultivars of wheat, oats, and barley recommended for Quebec (9) were inoculated as described, with extracts prepared from stock cultures maintained in either orchardgrass or Genesee wheat, which were cut back periodically to promote new growth and symptom expression.

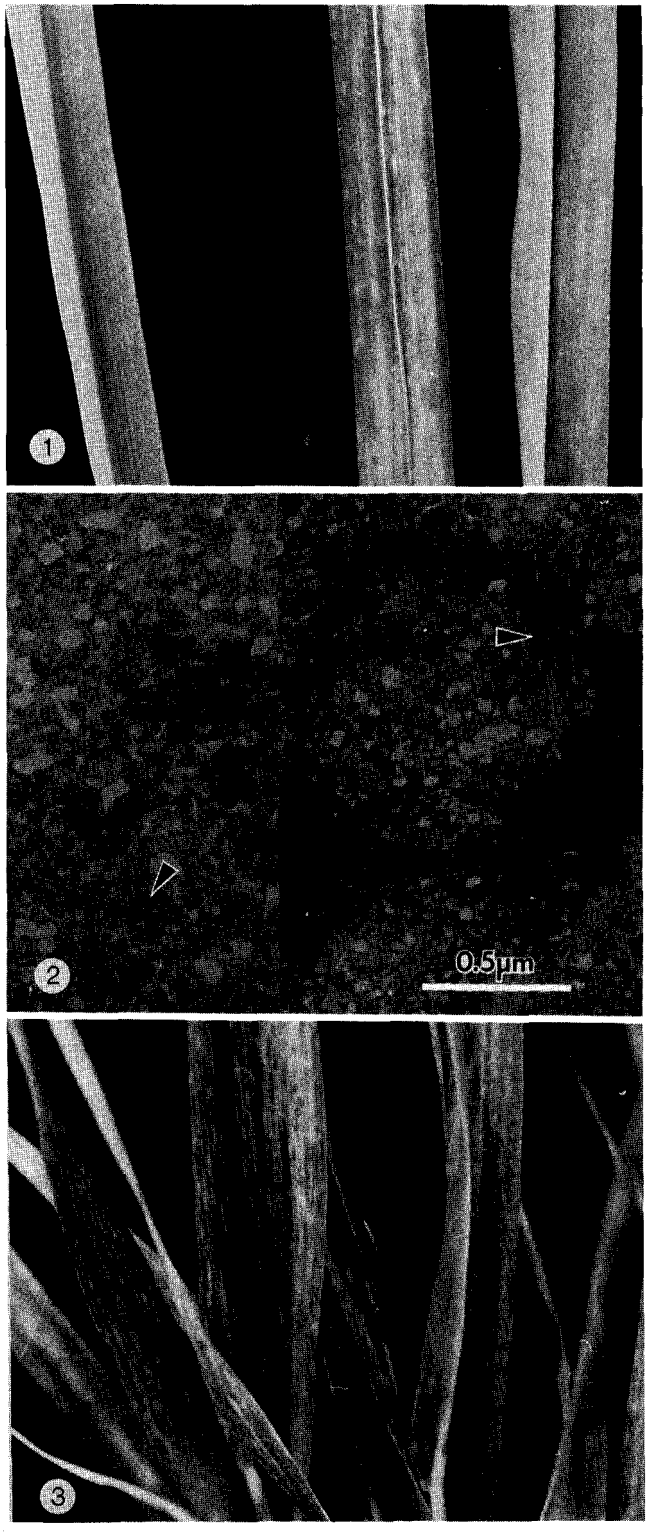

Figure 1. Leaves of experimentally inoculated orchardgrass showing typical symptoms. Healthy leaf on left.

Figure 2. Flexuous rod-shaped particles (arrowheads) in crude preparations from barley.

Figure 3. Symptoms shown by Lamar oats, 48 days after inoculation with 'OGMV'.

Forty to sixty test plants of each cereal cultivar, grown in four 10-cm pots, were inoculated with the extracts. Inoculation of plants in a fifth pot with only buffer and Celite served as a control. Counts of plants showing symptoms were made four and six weeks after inoculations. Various additional grasses and cereals were similarly inoculated.

Results

Symptoms on orchardgrass. Naturally or experimentally infected orchardgrass (Fig. 1) showed leaf markings typical of mosaics in grasses, generally consisting of light chlorotic streaking. Some naturally infected field plants, however, showed more prominent white streaking. Necrotic or brown areas usually were not present.

Primary culture establishment. Black Hulless barley, Genesee wheat, Selkirk wheat, and Roxton and Clintland oats all developed mosaic symptoms 17-21 days after inoculation with extracts from naturally infected orchardgrass leaves. Back inoculations from barley, Genesee wheat, and Roxton oats to orchardgrass resulted in symptom development after 3-4 weeks.

Electron microscopy. Examination of leaf mince preparations from Black Hulless barley revealed a low number of flexuous rod-shaped particles approximately 580-1000 nm long (Fig. 2). Similar particles were also present in partially clarified and concentrated extracts prepared from Genesee wheat leaves.

Host range and cereal cultivar reactions. The results of a series of inoculations of cereal cultivars are summarized in Table 1. Control (buffer/Celite inoculated) plants showed no symptoms. Symptoms on oats (Fig. 3) were generally more severe than those on wheat, and higher proportions of oats were infected, whether inoculum was prepared from orchardgrass or from Genesee wheat. Both wheat and oats showed typical mosaic symptoms, considerable stunting, and reduced or delayed heading (Fig. 4-6). Inoculations of barley cultivars yielded few meaningful results because of interference from a fungal pathogen, which did not appear later in the winter when Leger barley was inoculated.

Grasses which did not show symptoms after manual inoculation with extracts prepared from either orchardgrass or Genesee wheat included the following: ***Agropyron repens; Bromus inermis,*** cv. Saratoga; ***Lolium multiflorum,*** cvs. Merwester. Promenade; ***Lolium perenne; Phleum pratensis,*** cv. Drummond; ***Secale cereale,*** cv. Musketeer; ***Setaria italica;*** and ***Triticale*** × Triticosecale, cvs. Wintri, Decade.

Discussion

There are several flexuous rod-shaped viruses which infect grasses and cereals, and which are known to occur in other regions of Canada, e.g., wheat streak mosaic virus [WSMV (1)], ***Agropyron*** mosaic virus [AMV (11)], ryegrass mosaic virus [RMV (14)], ***Hordeum*** mosaic virus [HMV (13)], and oat necrotic mottle virus [ONMV (6)]. However, there are some host range differences between these viruses and 'OGMV', as

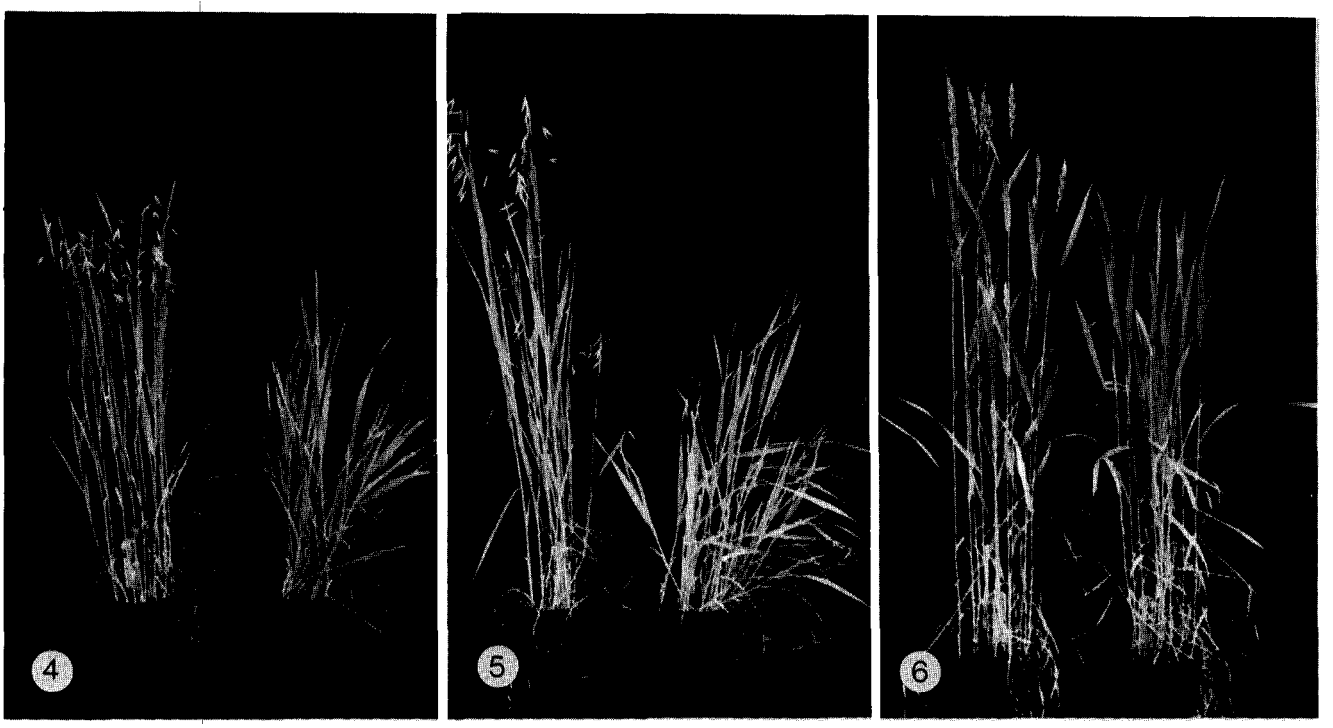

Figure 4-6. Stunting shown by oats and spring wheat, 48 days after inoculation. Left: control; Right: inoculated. 4. Lamar oats. 5. Laurent oats. 6. Laval-19 spring wheat.

Table 1. Reaction of cereal cultivars (no. plants with symptoms / no. inoculated).

Oats			Winter wheat		
Kamouraska	12/49*	49/49†	Frederick	36/40	29/61
Lamar	4/54	35/54	Yorkstar	40/41	32/51
Dorval	10/50	40/80	Valor	27/35	
Laurent		42/56	Lennox	39/50	
Spring wheat			Barley		
Columbus	22/46	20/52	Leger		14/52
Casavant	18/44	19/46			
Concorde	12/50	20/47			
Ankra	13/59	13/46			
Laval-19		33/54			
Dundas		24/58			

*Inoculum prepared from orchardgrass.

†Inoculum prepared from Genesee wheat.

shown in Table 2. AMV, HMV, and WSMV do not infect orchardgrass, and AMV does not infect oats (13). Oat necrotic mottle was originally described as not infecting wheat, barley, or orchardgrass (6), but it has been found to infect some cultivars of barley (Paliwal, *pers. comm.*). The literature is vague concerning infection of wheat by RMV; its description (14) refers to failures to infect wheat, but the description of AMV includes a statement suggesting that wheat is susceptible to RMV (11). At any rate, RMV infects *Lolium* spp., which have not shown symptoms when inoculated with 'OGMV'.

Some years ago, McKinney (8) described a virus from orchardgrass which caused symptoms resembling those seen in Quebec orchardgrass. However, the virus did not infect wheat or barley. Smith (15) implies that McKinney's virus was cocksfoot streak, but the usual British form of cocksfoot streak does not infect oats or wheat (2). Other British viruses infecting *Dactylis,* cocksfoot mottle virus and cocksfoot mild mosaic, are small spherical particles. McKinney also described a soil-borne oat mosaic virus, which does have flexuous rod-shaped particles, but infects only *Avena* spp. (7).

Whether 'OGMV' represents a potential threat to cereals in Quebec is difficult to determine at this point. Some symptomatic orchardgrass leaves found locally were infested by eriophyid mites, which were identified as Aceria *tulipae* by the Agriculture Canada Biosystematics Research Centre, but attempts to maintain a culture were unsuccessful. The association of Aceria *tulipae* with some symptomatic orchardgrass suggests a potentially hazardous virus-vector association, as this mite is known to transmit WSMV, and has been suspected as a vector of ONMV (6). However, there is as yet no evidence that it does in fact transmit 'OGMV' to other crops. Roots of naturally infected orchardgrass were not examined for presence of resting spores of Polymyxagraminis, which transmits some soil-borne cereal viruses (12).

Table 2. Usual host/symptom reaction of cereal viruses of similar morphology.

Host	Viruses					
	Agropyron mosaic (mi)	*Hordeum* mosaic	Wheat streak mosaic	Ryegrass mosaic	Oat necrotic mottle	'OGMV'
Agropyron repens L.	+	-	-	-	-	-
Avena sativa L.	-	+	+	+	+	+
Bromus inermis Leyss.	-	-	-		-	-
Dactylis glomerata L.	-	-	-	+	-	+
Hordeum vulgare L.	+	+	+	-	-	+
Lolium multiflorum Lam.	+	+	+	+	+	-
Lolium perenne L.	-	-	-	+	-	-
Secale cereale L.	+	+	+	-	-	-
Triticum aestivum L.	+	+	+	-	-	+
Reference:	**13**	**13**	**13**	**14**	**6**	present study

Virus infections of orchardgrass have not been reported previously in Quebec. Orchardgrass is recommended as a forage crop (10), and also occurs commonly in uncultivated situations. The symptoms shown by experimentally infected cereals indicate that 'OGMV' could represent a threat to their production if an efficient vector population were present. Determining whether 'OGMV' actually represents a previously undescribed virus or is simply a variant of one of the known grass viruses will require further work involving establishment of modal particle length, vector studies, and serological tests. The widespread occurrence of orchardgrass suggests that such studies could be useful.

Acknowledgements

Mr. Serge Lussier, Dr. B.E. Coulman, and Dr. A.K. Watson kindly provided seeds of currently recommended cereal cultivars and certain grasses. Danielle Mathieu, Thierry LeGros, and Josée Boisclair carried out inoculations of some cereals as portions of undergraduate projects. Drs. W.E. Sackston and R.D. Reeleder reviewed the manuscript and provided valuable suggestions for its improvement. Mrs. Roslyn James processed the words.

Literature cited

1. Brakke, M.K. 1971. Wheat streak mosaic virus. Descr. of Plant Viruses, No. 48. Commonw. Mycol. Inst., Surrey, England. 4 pp.
2. Catherall, P.L. 1971. Cocksfoot streak virus. Descr. of Plant Viruses, No. 59. Commonw. Mycol. Inst., Surrey, England. 4 pp.
3. Comeau, A., and J.P. Dubuc. 1977. ,Observation on the barley yellow dwarf virus epidemic in eastern Canada. Can. Plant Dis. Surv. 57:42-44.
4. Comeau, A., C.A. St-Pierre, and L. Couture. 1983. Effect of barley yellow dwarf virus and pink snow mold on winter survival and yield of winter cereals. Can. J. Plant Pathol. 5:203(Abstr.).
5. Comeau, A., and C.A. St-Pierre. 1983. Evaluation of cereals for barley yellow dwarf resistance: an international approach. Can. J. Plant Pathol. 5:203(Abstr.).
6. Gill, C.C. 1976. Oat necrotic mottle virus. Descr. of plant viruses, No. 169. Commonw. Mycol. Inst., Surrey, England. 4 pp.
7. Hebert, T.T.. and C.H. Pavizo. 1975. Oat Mosaic Virus. Descr. of plant viruses, No. 145. Commonw. Mycol. Inst.. Surrey, England. 4 pp.
8. McKinney, H.H. 1956. A virus from orchardgrass that infects oats. Plant Dis. Reptr. 40:524-526.
9. Ministere de l'Agriculture, des Plcheries, et de l'Alimentation. 1986. Céréales de Printemps — cultivars recommandbs en 1986. Conseil des Productions Végétales du Québec. 18 pp.
10. Ministere de l'Agriculture, des Pêcheries, et de l'Alimentation. 1987. Plantes Fourragères — cultivars recommandés en 1988. Conseil des Productions Végétales du Québec. 19 pp.
11. Slykhuis, J.T. 1973. ***Agropyron*** mosaic virus. Descr. of plant viruses, No. 118. Commonw. Mycol. Inst., Surrey, England. 4 pp.
12. Slykhuis, J.T. 1976. Virus and virus-like diseases of cereal crops. Ann. Rev. of Phytopathalogy 14:189-210.
13. Slykhuis, J.T. and W. Bell. 1966. Differentiation of ***Agropyron*** mosaic, wheat streak mosaic, and a hitherto unrecognized *Hordeum* mosaic virus in Canada. Can. J. Bot. 44:1191-1208.
14. Slykhuis, J.T. and Y.C. Paliwal. 1972. Ryegrass mosaic virus. Descr. of plant viruses, No. 86. Commonw. Mycol. Inst.. Surrey, England. 4 pp.
15. Smith, K.M. 1972. A textbook of plant virus diseases. Longman Group Ltd., London. 684 pp. p. 217-218.