VERIFICATION OF WHEAT SPINDLE STREAK MOSAIC VIRUS AS A CAUSE OF MOSAIC OF WHEAT IN ONTAR O'

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Abstract

Wheat spindle streak mosaic was observed in 62 of 107 fields of winter wheat surveyed in Ontario in May and June 1969. About 37% of the wheat surveyed was infected, which is moderate in comparison with none to 65% for other years since 1960.

The presence of the virus in field collections of plants and soil was verified by manual, root soak, and soil transmission to wheat. In manual transmission tests, wheat spindle streak mosaic virus was differentiated from wheat streak mosaic virus and agropyron mosaic virus by producing spindle streak mosaic symptoms on wheat kept in a growth room at 8–12C but not in a greenhouse at 18–25C. Manual transmission was not as successful from plants collected and tested in mid–June as in May.

Filamentous particles were observed with difficulty by the leaf dip electron microscope technique in young plants with spindle streak mosaic symptoms.

Introduction

A mosaic disease caused by a soil–borne virus has been recognized on winter wheat in Ontario since 1957 (4). Although the disease was at first assumed to be caused by a strain of wheat (soil–borne) mosaic virus (1,2,3), the cause is now recognized to be a different virus with filamentous rather than tubular particles and is designated wheat spindle streak mosaic virus (WSSMV) (5).

Surveys were made in Ontario in 1969 to compare with those of previous years. Special emphasis was placed on using transmission tests to verify the identification of the causes of symptoms observed in the field.

Materials and methods

The first general survey was made between May 12 and 16 when most wheat in most districts was in the late tillering stage. Another survey was made during June 16–19 when most wheat was headed and in or approaching flowering. In early May the presence of WSSM was often evident from the road by a yellowish–brown discoloration in patches or throughout the field. Similar discolorations are sometimes caused by other factors, but on close examination the presence of WSSM was indicated by a light green to yellow mosaic including spindle-shaped dashes parallel to the leaf axis. These markings could occur anywhere on the leaf from near the tip to the base. Sometimes dashes or blotches of tissue were grayish white and necrotic. During June the disease was identifiable principally by short, spindle–shaped streaks or light green to yellow mosaic on the youngest two or three leaves. The affected plants were usually only slightly stunted but had fewer tillers than mosaic-free plants.

Verification of the presence of WSSMV in field collections with mosaic symptoms was done by transmission from plant and soil samples and sometimes by electron microscopic examination of leaf dip preparations.

Manual transmission tests were done by grinding 1 g of leaf tissue with 3 ml of water and about 2 mg of celite, then rubbing the mixture on the leaves of 'Kent' winter wheat in the 2–3 leaf stage. Some plants were kept in a growth room at 8–12C with about 1200 ft–c of light for 12–16 hr/day. Similarly inoculated wheat plants and 'Clintland 60' oat plants were placed in a greenhouse at about 18–25C. The development of spindle streak mosaic symptoms on wheat kept up to 3 months at 8-12C, but not on plants at 18-25C, indicated the presence of WSSMV. The development of mosaic on wheat, but not on oats, at 18-25C indicated the presence of agropyron mosaic virus (AMV),

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while symptoms on oats indicated wheat streak mosaic virus (WSMV).

A root-soak transmission test was done by lightly washing the roots of each diseased plant to free them from soil, then placing them in water in petri dish with 15-20 sprouted wheat seeds. After one day at 20C and another at 8-12C, the seedlings were transplanted into a pot of noninfective soil and grown at 8-12C for 3 months.

Soil infectivity tests were done by placing a 4-cm layer of test soil on top of a noninfective potting soil mixture in a 5-inch clay pot, then seeding 'Kent' wheat (15-20 seeds) in the test soil and maintaining at 8-12C for 3 months. When necessary a satisfactory test could be done by mixing a few grams of the test soil with the potting soil at seed level.

Results

Field surveys, 1969

Wheat spindle streak mosaic was observed in 62 of the 107 (58%) fields of winter wheat observed in May and June. The estimated percentages of diseased plants varied from trace to 20% in 14 fields, 21 to 80% in 11 fields and 81 to 100% in 37 fields. The mean infection for all fields was 37.6%.

The incidence of WSSM in 1969 was moderate in comparison with the incidence in other years in the decade (Table 1). There were 6 years in which diseased plants were found in a higher percentage of fields and 5 in which the mean percentages of infected plants were higher.

Transmission tests for WSSMV in field samples

Although the number of test plants: that developed symptoms was variable, the combined results of the three types of transmission tests confirmed the presence of WSSMV in all Ontario fields from which plants with WSSM symptoms were collected (Table 2). The manual transmission tests indicated the presence of WSSMV in all plant samples with WSSM symptoms collected in May but failed for three of the four samples collected in June after the wheat was headed and had experienced very warm weather. The lack of mosaic development in oats kept at 18-25C after inoculation indicated that WSMV was not present in any of the Ontario samples tested. The development of symptoms in inoculated wheat kept either at 8-12C or 18-25C, but not in oats, confirmed the presence of AMV in one sample of wheat, No. 64, which had symptoms resembling agropyron mosaic. The root.-soak method confirmed the presence of WSSMV in 8 of the 11 samples tested, and the soil infectivity test confirmed the presence of WSSMV in 13 of the 16 samples tested, including the four samples collected in June.

Observations of particles with the electron microscope

Although particles in leaf dip preparations from plants with symptoms of

Table 1. Incidence of wheat spindle streak mosaic in winter wheat in Ontario, 1960-69

Year	Total fields	No. fields according to % diseased plants				Fields with disease		Mean % infection
		0%	tr-20%	21-80%	81-100%	No.	%	
1960	43	25	5	4	9	18	72.0	24. 7
1961	106	25	6	7	68	81	76.4	61. 6
1962	50	29	9	5	7	21	42.0	19.4
1963	39	5	13	6	15	34	87. 1	45.6
1964	33	25	3	1	4	8	24.2	13. 3
1965	24	24	0	0	0	0	0.0	0.0
1966	41	12	4	6	19	29	70.7	50.0
1967	140	19	8	26	87	121	86.4	65.1
1968	96	33	11	6	46	63	65.6	47.4
1969	107	45	14	11	37	62	57.9	37.6

Table 2. Verification of WSSMV in plant and soil samples from Ontario fields in which mosaic-disease plants were found in 1969

Field No.	Location	Mosaic-diseased plants Symptoms	%	Date samples collected	Manual transmission to Oats 18-25C	'Kent' wheat 18-25C	'Kent' wheat 8-12C	Infection from Root of dis. plants	Infection from Field soil	Virus identified WSSMV	Virus identified Other
1(a)	Ottawa	WSSM		May 27			8/29	1/17	1/21	t	
1(b)	Ottawa	WSSM		June 20		0/11	2/20	2/24	7/27	t	
4	Pt. Colborne	WSSM	100	May 13	0/20	0/22	5/34	0/20	7/71	t	
5	Pt. Colborne	WSSM or AM	1 plt	May 13	0/18	0/15	1/28			t	
7	Pt. Colborne	WSSM	<1	May 13	0/26	0/14	1/27		0/41	t	
24	Talbotville	WSSM	75	May 13	0/21	0/15	5/26	2/16	2/54	t	
27	Blenheirn	WSSM	75	May 13	0/29	0/17	6/32	1/17	12/18	t	
28	New Glasgow	WSSM	75	May 13	0/26	0/14	20/33		5/40	t	
30	Cedar Springs	WSSM	100	May 13	0/22	0/13	13/27	1/15	2/10	t	
47	Clandeboye	WSSM	25	May 13	0/26	0/14	7/41	1/15	4/47	t	
49	Clandeboye	WSSM or WSM?	<1	May 13	0/15	0/15	5/28	0/13	0/27	t	
51	Exeter	WSSM	90	May 13	0/15	0/10	9/27	0/15	0/16	t	
58	Tottenham	WSSM	50	May 13	0/20	0/12	9/21	2/16	5/65	+	
59	Bondhead	WSSM	100	May 13	0/29	0/13	1/21	2/14	2/13	t	
64	Renfrew	AM	1 plt	May 13	0/15	11/12	15/15			-	AMV
100	Clinton	WSSM	100	June 19		0/10	0/34		12/15	t	
101	Clinton	WSSM	75	June 19		0/9	0/22		1/12	t	
106	Newmarket	WSSM	100	June 19		0/11	0/37		5/16	t	
M4	WSMV control	WSM		May 14	25/25	15/16	11/29		0/27	-	WSMV
Ck	Potting soil mixture				0/25	0/16	0/15		0/27		

WSSM were more readily observed after shadowing with palladium than after staining negatively with phosphotungstic acid, they were generally much more difficult to find than particles of WSMV or AMV in plants infected with these viruses. Particles were rarely found in preparations from wheat collected in June. It was sometimes necessary to examine several grids prepared from a plant infected with WSSMV to find a few particles, which were usually partly obscured by non-specific, fine, granular material, but sometimes clumps of particles were found. The particles were thin (12-13 mμ) and flexuous and varied in length from 200 to >2000 mμ. Particles of WSMV and AMV were more readily found and were more distinctly visible, more uniform in size (19 x 650 - 750 mμ), but less flexuous than particles associated with WSSM (5,6). Although electron microscopy provided an additional means for indicating the presence of WSMV or AMV in mosaic diseased plants, the unreliability of this method for finding particles identifiable with WSSMV limited the usefulness of the leaf dip electron microscopic technique for routine determination of the presence of WSSMV.

Serology as an aid in distinguishing viruses causing mosaic symptoms in wheat

Microprecipitin tests with specific antisera (6) were useful for identifying WSMV and AMV in clarified juice from mosaic-diseased wheat plants. Sometimes the titre of AMV in field-collected plants was too low to detect the virus, but if young wheat plants were inoculated and the juice extracted after 12-14 days at 18-25C, the virus was usually detectable in precipitin tests.

Although attempts were made to develop an antiserum by injecting rabbits with concentrates of particles from plants infected with WSSMV, subsequent use of serum from the rabbits in precipitin tests failed to demonstrate the presence of WSSMV in clarified juice from diseased plants.

Discussion

Although the manual transmission, root-soak transmission, and soil infectivity tests each yielded postive results for the presence of WSSMV in most samples, and the combined results confirmed its presence in all samples from fields in which WSSM was observed, the precentages of test plants infected by each method were variable. Manual transmission was more successful for samples collected in May than in June when the wheat was more mature. The small number of test plants infected in any of the tests by the root-soak method probably indicates that temperature conditions during the infection period were not optimum for the vector or the virus. The variable results from soil transmission tests may reflect differences in infectivity of the soils as well as failure to provide optimum environmental conditions for infection.

Symptoms produced by WSMV and AMV on some plants may sometimes be difficult to distinguish from WSSM in the field. However,

the presence of these viruses can be determined easily by the differences in host reactions, the development of symptoms in one to two weeks at temperatures too high for WSSM development, lack of soil transmissibility,. differences in particle morphology, and reactions to specific antisera.

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