Factors Critical to Mechanical Transmissibility of Wheat Spindle Streak Mosaic Virus

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ABSTRACT

The infectivity of wheat spindle streak mosaic virus in extracts prepared by grinding 1 g leaves of diseased wheat in 3 ml of water was lost in 1 hour at 20 C and in 8-24 hours at 10 C. Extracts similarly prepared in 0.5 M sodium borate (pH 9.0), 0.1 M potassium phosphate (pH 7.0), or 0.1 M Na_2SO_3 were still infectious after 1 hour at 35-40 C, or 3-4 days at 10 C.

The virus was more readily transmitted from leaves than from roots of diseased wheat plants, and from the older, severely chlorotic leaves with islands of necrotic tissue than from the younger leaves with light green mosaic. Transmissibility from diseased plants was greatly reduced or lost in one week at temperatures of 20 C or higher, but diseased plants kept 6 weeks at 20 C or 25 C regained transmissible virus during a further 4 weeks at 10 C.

Susceptibility to mechanically-transmitted virus was very low in wheat plants grown outdoors at Ottawa in September or October, and was reduced in greenhouse-grown plants by transplanting, rubbing with Carborundum, growing under very high or very low light intensity, or by allowing them to wilt from insufficient moisture before inoculating.
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Early attempts to transmit wheat spindle streak mosaic virus (WSSMV) failed until the narrow temperature limitations of the virus were realized and the optimum found to be about 10 C (1, 2). Mechanical transmission was generally poor by the finger-rub method. It was better when the artist's airbrush or leaf tissue rub methods were used, but results were still unreliable (4). The variability of results of different tests appeared to be related to factors affecting the availability of the virus in the inoculum sources, the stability of the virus in the inoculum, and the susceptibility of the test plants.

Tests were done to determine factors that appeared to have major effects on the mechanical transmissibility of WSSMV.

MATERIALS AND METHODS.—WSSMV for transmission tests was obtained from winter wheat (*Triticum aestivum* 'Talbot', 'Genesee', or 'Kent') grown in infectious soil from a field at Ottawa. The diseased plants were grown in a room at 10 C with about 10,000 lux of daylight-type fluorescent light 12 hours per day. Leaves with distinct mosaic symptoms were selected for inoculum. Kent wheat grown for 10-14 days in a greenhouse at about 20 C was used for test plants.

For tests in which only one pot of plants was to be inoculated with samples from each of a number of plants, the leaf tissue rub method (4) was used, but most inoculations were done with an artist's airbrush. The inoculum for this method was prepared by grinding the source leaves in a diluting medium (1 g leaves: 3 ml diluent). Initially, several diluting media were compared, but 0.1 M Na_2SO_3 was used for most routine inoculations. After the leaves were ground, the juice was expressed from the pulp through a cloth, fine Carborundum powder was added (about 1 mg/ml), then the inoculum was sprayed on the entire leaf surface of test plants with a Paasche Model H airbrush operated at an air pressure of 2.1 kg/cm^2, while holding the nozzle about 3 cm from the surface of the leaves being sprayed. The plants were either inoculated at 10 C or were moved to 10 C within 1 hour after inoculation. At this temperature with 10,000-15,000 lux of daylight-type fluorescent light 12-16 hours per day, symptoms developed in 4-6 weeks after inoculation.

RESULTS.—*Effects of diluting medium on inoculum stability.*—No significant differences occurred in the dilution end points of extracts prepared by grinding diseased wheat leaves in distilled water, 0.5 M sodium borate (pH 9.0), 0.1 M potassium phosphate at pH 7.0 and at pH 9.0, or in 0.1 M Na_2SO_3 if the inoculations were done immediately after preparation at 10 C (Table 1). The differences were greater between replicates of the tests done at different dates than between preparations with the different diluting media. However, in tests for the longevity of infectivity of inoculum prepared with the different diluents and kept at 10 C, the inoculum prepared with water was infectious after 8 hours, but not after 24 hours. Inoculum prepared with 0.1 M phosphate buffer (pH 7.0) or 0.5 M borate buffer (pH 9.0) was infectious after 3 days, and with 0.1 M Na_2SO_3 after 4 days. In other tests, the virus was inactivated in 10 minutes at 30 C in the water preparation, and at 45 C in the preparations with the other media.

To determine if infectious juice could be clarified by heating, samples prepared with the different diluents were heated in water baths for 1 hour at temperatures ranging from 20 C to 45 C at 5-C intervals, then cooled to 10 C, before inoculating 30-40 test plants. The extracts prepared with water were not infectious after 1 hour at 20 C. Those prepared with 0.1 M phosphate (pH 7.0) and 0.5 M borate buffer (pH 9.0) were infectious after 1 hour at 35 C, while the Na_2SO_3 preparation was infectious after 1 hour at 40 C. However, none of the extracts that was heated to 35 C or 40 C was infectious after centrifuging at 3000 *g* for 15 minutes to remove the green solids.

Tissues used for inoculum.—In an experiment in which test plants were inoculated with extracts prepared from roots and from leaves of diseased plants infected either from soil or by mechanical transmission, the total numbers of plants that developed symptoms were 4/166 (2.4%) for root extracts, and 126/176 (71.6%) for leaf extracts. These and other results showed that inoculum

prepared from roots of diseased plants was too low in infectivity to be useful for routine tests for the presence of WSSMV in diseased plants.

About 60 test plants were inoculated with extracts prepared from young leaves with light green mosaic and from older leaves with distinct yellow mosaic and necrotic areas from diseased plants of different ages grown at 10 C. The percentages of plants infected with inoculum from the younger leaves with light green mosaic vs. the older leaves with distinct yellow mosaic and necrotic areas were, respectively, 29% and 86% for plants 78 days old (stooling stage), 22% and 48% for plants 165 days old (with elongated stems), and 51% and 58% for plants 250 days old (heading). It appears that the most infectious inoculum was from the older leaves with the most pronounced yellow mosaic and necrotic areas on the youngest plants with well-developed symptoms.

Transmissibility of WSSMV from leaves of diseased plants collected from the field at different times.—In some years WSSMV was transmitted mechanically from wheat plants with well-developed spindle streak mosaic symptoms collected from different areas in southern Ontario in May, and sometimes from plants collected in early June, but transmission from such field collections was too erratic and unreliable for diagnostic purposes (3). Tests for mechanical transmissibility of the virus from living leaves collected from diseased plants in a field at Ottawa at weekly intervals from late April through June, 1974, showed the following numbers of test plants infected per the numbers inoculated: 30 April - 0/56, 7 May - 1/70, 14 May - 31/59, 21 May - 59/75, 28 May - 28/67, 4 June - 0/49, 11 June - 10/60, 18 June - 4/58, 25 June - 0/57, and 4 July - 0/62. These results confirmed results in 1970 and 1971, showing that the virus became mechanically transmissible after growth of the wheat plants resumed and mosaic symptoms began to develop in early May. Each year, transmissibility increased to a maximum in mid- to late May, then decreased to zero by mid- to late June. The increases in transmissibility in May followed increases in daily mean temperature to 5-15 C. The decreases in June followed further increases in temperature above 15 C.

When diseased plants collected from the field in mid- to late June were replanted in pots of soil, the top growth removed, and the plants kept in a growth room at about 10 C, WSSMV usually was transmissible in about 4 weeks from new leaves that developed symptoms of WSSM.

Loss of transmissibility of WSSMV from diseased plants after moving to higher temperature, and recovery after returning to 10 C.—The effects of temperature on transmissibility from leaves of diseased wheat plants infected by mechanical inoculation, and plants infected by growing in infectious soil at 10 C, were tested by the tissue-rub method in two experiments with similar results. Inoculations from the plants before they were moved from 10 C infected 60-90% of the test plants. Inoculations done at weekly intervals for 6 weeks while the plants were at different temperatures, showed a depressing effect of the higher temperatures. While transmissibility from the control plants kept at 10 C changed little during the additional 6 weeks, it declined to about 10% after 2 weeks at 15 C or 20 C, and after 1 week at 25 C or 30 C. Transmissibility ceased in 3-4 weeks at 20 C and in 2 weeks at 25 C. However, plants from which the virus could not be transmitted after 6 weeks at 20 C or 25 C regained transmissible virus during a further 4 weeks at 10 C. The plants moved to 30 C died during the 4th week at 30 C, hence could not be tested for recovery of the virus at 10 C.

Factors affecting the susceptibility of test plants.—Inoculation of 35-40 plants at each of six stages of development ranging from one-leaf stage to tillering (ages 1-6 weeks) resulted in infection of 91-97% of the plants inoculated in the two-, three-, four-, and early five-leaf stages. Fewer plants developed symptoms from inoculations in the one-leaf stage (36%), and in the late tillering stage, 6 weeks after seeding (22%). These results showed a differentiation in age susceptibility not detected in earlier tests with less efficient transmission (4).

In attempts to infect wheat with WSSMV in the field in early October, using different methods of mechanical inoculation, few plants developed symptoms even though the plants were in the two-to-three-leaf stage and the temperature was suitable for infection (8-15 C).

TABLE 1. Dilution end-point, longevity and temperature tolerance of wheat spindle streak mosaic virus in leaf extracts prepared with different diluting media

Property tested	Test no.	Diluting medium: Distilled water	0.1 M Na_2SO_3 pH 9.0	0.1 M K phosphate pH 7.0	0.1 M K phosphate pH 9.0	0.5 M borate pH 9.0
Dilution end point (2 × series starting at 1/4)	1	512[a]	512	256	512	256
	2	64	32	32	32	32
	3	16	16	16	16	16
Longevity at 10 C		8 hours	4 days	3 days		3 days
Highest temperature tolerated for 1 hour (5 C intervals)		<20 C	40 C	35 C		35 C
Highest temperature tolerated when followed by centrifugation at 3000 *g* for 15 minutes		<20 C	30 C	30 C		30 C

[a]Each sample was tested by inoculating 15-25 test plants.

In an experiment to indicate the effects of outdoor vs. greenhouse growth on the susceptibility of Talbot wheat inoculated by the airbrush method then grown at 10 C, the numbers of plants developing symptoms per the numbers inoculated were as follows: plants grown outdoors from 22 September until inoculated 27 October - 0/26; grown outdoors, but placed in the greenhouse 8 days before inoculating - 3/28; grown in the greenhouse, but placed outdoors 8 days before inoculating - 8/43; grown in the greenhouse until inoculated - 27/29. These results indicate that plants grown outdoors became less susceptible to infection by mechanical inoculation than plants grown in the greenhouse. The difference in susceptibility appeared to be correlated with the tougher physical state of leaves of the plants grown outdoors.

Wheat plants grown in a greenhouse at about 20 C with 10,000-15,000 lux of light 16 hours per day, with moderate watering for 10 days, were then subjected to different conditions or treatments, before inoculating. In two experiments, symptoms developed on 43% and 80% of the control plants kept in the greenhouse until inoculated. No reduction in numbers developing symptoms resulted if the plants received excess moisture, or were grown at 5 C or 30 C with similar lighting for 7 days before inoculating. However, susceptibility was decreased significantly ($P = 0.01$) in plants that appeared wilted from deficient moisture when inoculated, and in plants grown with inadequate light (2,000 lux 12 hours per day), or with excess light (40,000 lux continuously) for 7 days before inoculating. Also, plants that were removed from the soil and replanted up to 2 days before inoculating, and plants that were rubbed with Carborundum powder in water to simulate the finger-rub method of inoculation before inoculating by the artist's airbrush method, were significantly less susceptible to infection.

DISCUSSION.—These and earlier results (4) demonstrate some of the factors which have caused erratic results and failures in tests involving mechanical transmission of WSSMV.

Temperatures predominantly between 5 C and 15 C are necessary not just for the incubation of the virus in inoculated plants, but also for the maintenance of transmissible virus in diseased plants used as sources of inoculum. Usually the virus is transmitted readily from plants with well-developed symptoms in early to mid-May, but it becomes less transmissible later in May and in June after increasing periods of higher temperature.

Because of variable and relatively low titres of the virus in plant extracts it has appeared desirable to use low dilutions (1 g leaves: 3-4 ml diluent) for general transmission tests. Although inoculum prepared by grinding leaves of diseased plants in water is initially as infectious, it is much less stable than inoculum prepared with a phosphate or borate buffer, or 0.1 M Na_2SO_3. The use of the latter for inoculum preparation has greatly increased the reliability of inoculations done at temperatures above 15 C.

Several conditions can critically affect the susceptibility of wheat plants. For maximum susceptibility, the plants should be grown with adequate (but not excessive) light, and with ample watering to avoid wilting or other shock before inoculating. The plants should not be transplanted for several days before being inoculated. The reduction in susceptibility caused by rubbing the leaves before inoculating indicates a reason why fewer plants become infected by the finger-rub method than by the airbrush method with the same preparations of inoculum.

Although other experiments showed that wheat plants growing in the field at Ottawa are susceptible to infection via soil in October, plants growing in noninfectious soil in the field in October are not susceptible to infection by mechanical inoculation procedures suitable for inoculating greenhouse-grown plants. This has hampered the use of mechanical inoculation to test the effects of the virus on wheat in the field.

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