

## Differentiation of Transmission and Incubation Temperatures for Wheat Spindle Streak Mosaic Virus

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### ABSTRACT

The optimum and maximum constant temp for the development of wheat spindle streak mosaic on wheat (*Triticum aestivum*) were 10 C and 17 C if the plants were inoculated manually, and 8 C and 15 C if the plants were grown in infectious soil. More plants developed symptoms from soil-borne infection at temp that fluctuated between 5 C and 17 C than at any constant temp.

Differences in temp requirements for infection from soil and for the development of symptoms were found by growing seedlings in infectious soil for periods up to 4 wk, then

removing, washing the roots free from soil, and replanting them in noninfectious soil before subjecting them to specific incubation temp. The optimum and maximum temp for infection (15 C and 20 C) were each 5 C higher than for incubation after infection. In some tests, all wheat plants developed spindle streak mosaic if they were grown in infectious soil at 15 C for 4 wk then transplanted to noninfectious soil and kept at 10 C for 8-10 wk.

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In years favorable for the development of wheat spindle streak mosaic (WSSM), the disease occurs with remarkable uniformity throughout many fields in southern Ontario. Usually all plants are affected in patches or throughout most fields with long histories of wheat production. The absence of escapes in many such fields, indicates a thorough distribution of the pathogen in the soil and efficient transmission.

Temperature was recognized as a major factor affecting the development of WSSM (3). Our earliest successes in demonstrating that the disease was caused by an infectious agent in the soil were achieved by seeding wheat in late September in boxes of soil from fields where the disease was found. The boxes of soil were left outside through the fall and into the winter when they were moved to a greenhouse in which the temp remained predominantly below 15 C. Under these variable conditions, all the test plants developed WSSM symptoms (2).

Although the disease also developed on wheat plants inoculated by rubbing with juice from diseased leaves then incubated at constant temp of 5 C to 15 C, and on plants grown in infectious soil at constant temp of 5 C to 13 C, the percentages of plants that developed WSSM symptoms were low, in contrast to the high incidence of the disease under natural conditions (3).

The following report includes the more significant results from many experiments done over several years to determine the major effects of temp on the transmission and incubation of WSSMV in wheat.

**MATERIALS AND METHODS.**—Winter wheat (*Triticum aestivum* L. 'Kent'), which is moderately resistant to powdery mildew, but susceptible to WSSMV, was used as the test host for most experiments, but 'Genesee' was used for some tests.

For artificial inoculations, wheat was grown to the two- to three-leaf stage in a greenhouse at about 20 C. The plants were usually placed in a growth room at 10 C the day before inoculating, but the results were equal if the plants were inoculated at 20 C then moved within 1.0 h to the specific incubation treatment temp. The inoculum was prepared by grinding leaves with distinct mosaic symptoms in 0.1 M  $\text{Na}_2\text{SO}_3$  (1 g leaves: 4 ml solution), filtering out leaf fragments and spraying the suspension on the test plant leaves with an artist's airbrush, using about 21 kg/cm<sup>2</sup> air pressure and holding the spray nozzle 2-3 cm from the leaf surface (4).

Infectious soil was collected from fields of wheat in which WSSM was prevalent in May. The soil was dried in the greenhouse, passed through a screen with 5-mm openings and stored dry in a greenhouse or in an unheated shed.

Many tests were done by sowing the seeds in a 3- to 4-cm layer of infectious soil on top of noninfectious steamed potting soil in 12.5-cm clay or fibre plant pots, then growing the plants at the specified conditions. Other experiments involved exposing wheat to infectious soil for specific periods. In these experiments, wheat seeds were sprouted on moist filter paper at about 20 C for 2 days. About 20 of these seedlings were transferred to a 7.5-cm diam pot of infectious soil (about 200 g) and maintained at specific temp for specific periods. The seedlings were then removed from the soil, and the roots

were washed thoroughly to remove soil particles. The seedlings were replanted in noninfectious (steamed) soil, in 12.5-cm diam clay or fibre pots and maintained at specific temp for development of symptoms.

Temperatures for infection and incubation were maintained in cabinets regulated for constant temp of 10 C, 12.5 C, 15 C, 17.5 C and 20 C with normal variations of about  $\pm 0.5$  C, and in rooms at 5 C and 8 C  $\pm 2$  C. For some experiments, cabinets were regulated to alternate from a lower temp during the dark period to a higher temp during the light period. The light intensity was about 10,000 lux, 12 h per day.

**EXPERIMENTS AND RESULTS.**—*Effects of temperature on the development of symptoms on manually inoculated wheat.*—In three experiments plants inoculated by the artist's airbrush spray technique were incubated at a series of temp ranging from 8 C to 20 C. In each experiment, some of the plants developed mosaic symptoms in 21 to 28 days at all temp from 8 C to 17.5 C, but additional plants developed symptoms up to about 42 days after inoculation. The highest percentages of plants with symptoms as well as the most severe mosaic symptoms developed at 8 C to 10 C (Fig. 1-A). The symptoms were very mild at 15 C and 17.5 C. None of the plants developed symptoms at 20 C in any of the tests.

*Effects of constant temperatures on the development of symptoms on wheat growing in infectious soils.*—Experiments were done in which wheat was sown

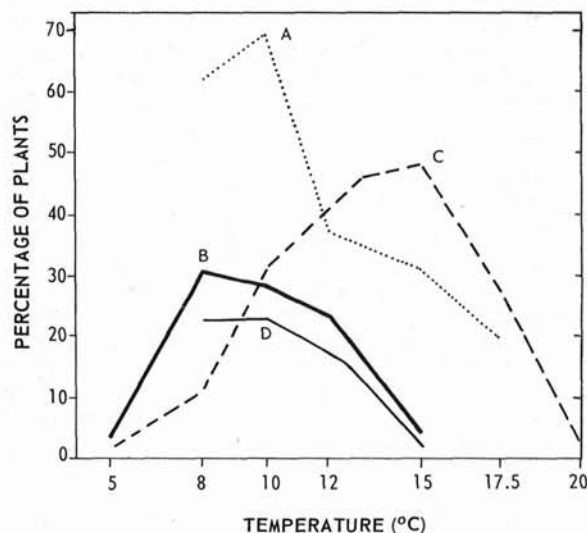


Fig. 1-(A to D). Percentages of wheat plants infected with wheat spindle streak mosaic virus and developing mosaic symptoms at different temp. A) Plants inoculated by the artist's airbrush spray technique. (Results from three experiments with 40-50 plants in each at each temp.) B) Plants grown in infectious soils at constant temp. (Results from four experiments including a total of five collections of soil as shown in Table I.) C) Infection of plants from soil. (Plants grown in infectious soil at the specific temp for 32 days, then removed from soil, roots washed, replanted in sterile soil and incubated at 6-12 C for symptoms to develop.) D) Incubation of symptoms after infection from soil. (Plants grown in infectious soil at 15 C for 1 wk, then removed, roots washed, replanted in sterile soil and incubated at specific temp.)

in a series of pots of each of two or three infectious soils and incubated at constant temp ranging from 5 C to 20 C. In each experiment, some plants began to show symptoms in about 40 days at temp of 8 C to 12 C, but some additional plants developed symptoms until the tests were terminated, about 120 days after seeding. Few plants developed symptoms at 5 C or 15 C. None developed symptoms at 17.5 C or 20 C (Fig. 1-B). Even at the most favorable temp (8 C), the percentage of plants developing symptoms was low (avg 31%). The percentages differed greatly between soils and between experiments with the same soils at the same temp, but even the highest percentage of plants developing symptoms (89% in one test) was lower than should be expected in these soils which came from fields in which all the plants were diseased when the soils were collected.

*Effects of fluctuating temperatures on the development of WSSM on wheat growing in infectious soil.*—Each year some experiments with infectious soil were done between October and March in small greenhouses in which the temp could be kept predominantly between 15 C and 5 C. Usually a higher percentage of the plants growing in infectious soil developed WSSM under these fluctuating conditions than at constant temp of 8 C or 10 C maintained in growth rooms.

Other experiments were done in which wheat was sown in pots of infectious soil then kept at different temp for different periods before moving to 8 C to incubate for symptom development. Generally the percentages of plants developing symptoms were decreased if the seedlings were grown at 18-24 C for 1-8 wk, or at 0-3 C for 4-8 wk. However, the percentages of plants developing symptoms were generally increased by exposing the seedlings outdoors for two wk in October when the daily mean temp ranged from 15 C to 5.5 C; in growth cabinets at 15-18 C; or at temp cycling daily between 5 C and 17 C or 8 C and 18 C. These results suggested that the optimum temp for infection from soil may differ from the optimum temp for the development of symptoms.

*Infection time for plants growing in infectious soil at different temperatures.*—In the first experiments to determine infection threshold time, wheat was sown in infectious soil and grown in a greenhouse in which the temp fluctuated from 5 C to 15 C, but was predominantly about 12 C. At different times after seeding, seeds or seedlings were removed, washed to remove all infectious soil, replanted in steamed noninfectious soil and returned

to the greenhouse. The results of three similar experiments showed that infection could occur soon after the seeds had sprouted, between 4 and 8 days after seeding at these temp. Maximum infection had occurred by the time the primary leaf had emerged through the soil, between 10 and 17 days after seeding.

To determine the effects of temp on infection, wheat seeds sprouted in moist chambers at 22 C for 2 days, were planted in infectious soil (20 seedlings/200 g soil) and kept in growth cabinets at a series of temp from 5 C to 20 C. After periods of 1 to 32 days, seedlings were removed and washed to remove all infectious soil, replanted in noninfectious soil, and grown in a growth cabinet at 6-12 C for about 90 days for symptoms to develop.

The results of three such experiments using two infectious soils in each showed that differences in numbers of plants developing symptoms were related to the soils, the times of exposure in the soils, the temp and to other unexplained factors. In one test, all of the plants became infected during 32 days in one of the soils at 10 C and 12 C, but in another test with the same soil, few plants were infected at any temp. The combined results from the two soils in the three experiments (Table 1) show that in 1 day no infection occurred at any temp, in 2 days a low percentage of infection developed at 10 C, and in 16 days infection occurred at all temp from 5 C to 20 C. In 32 days, infection ranged from 1.2% at 5 C and 3.1% at 20 C to 49.4% at 15 C (Fig. 1-C).

Four other experiments, in each of which wheat was grown in two infectious soils at a series of temp, confirmed that the highest percentage of infection occurred at 10-15 C, the latter being optimum.

*Temperature in relation to the development of spindle streak mosaic in wheat after an inoculation period in infectious soil.*—To determine the effects of temp on the development of spindle streak mosaic following infection, sprouted wheat seeds were planted in infectious soil and grown at 15 C for 1 wk for infection, then removed, washed free from infectious soil and planted in pots of noninfectious soil. Four pots with about 18 seedlings each were then placed at each of a series of constant temp ranging from 5 C to 20 C. This procedure was repeated three times with the same infectious soil. The combined results from the four replicates show that in 3 mo mosaic symptoms developed on plants incubated at all temp from 8 C to 15 C, but 8 C and 10 C were optimum (Fig. 1-D). Few plants developed symptoms at 15 C, and none at 17.5

TABLE 1. Development of spindle streak mosaic on wheat grown in infectious soil at different temp for different periods before transplanting to noninfectious soil and incubating at 6-12 C

Temp (C)	Seedling infection (%) after growth in infectious soil <sup>a</sup>					
	1 day	2 days	4 days	8 days	16 days	32 days
20	0.0	0.0	1.2	1.2	1.2	3.1
17.5	0.0	0.0	0.0	6.6	27.7	27.1
15	0.0	0.0	2.2	2.4	19.5	49.4
12.5	0.0	0.0	1.1	0.0	8.0	46.1
10	0.0	1.2	0.0	8.0	21.0	31.0
7.5	0.0	0.0	0.0	0.0	3.6	15.8
5	0.0	0.0	0.0	0.0	1.1	1.2

<sup>a</sup>Percentages based on 82 to 92 plants tested in three experiments, except at day 1 where 52-65 plants were tested.

C, even though 15 C is optimum and 17.5 C highly favorable for infection (Fig. 1-C). Additional tests showed that more of the plants exposed to infectious soil at 15 C for 14 days developed symptoms at both 10 C and at 15 C if they were kept for an intervening period of 7 to 21 days at 5 C.

*Daily fluctuations of temperature in relation to transmission of WSSMV from soil and its incubation in wheat.*—To determine if the percentages of wheat plants infected from soil and developing symptoms are increased by daily fluctuations in temp, wheat was sown in infectious soil then grown in four cabinets, each regulated at 15 C during a 12-h light period and at 15 C, 10 C, 7.5 C, or 5 C during a 12-h dark period. After 14 days the plants were removed from the soil, washed and replanted in noninfectious soil. Four pots of seedlings from each temp were placed back at the same temp, and four other pots were placed at 10 C to incubate for the development of symptoms. During 4 mo of incubation, significant differences in disease development occurred between treatments (Table 2). As indicated by the percentages of plants that developed mosaic symptoms after incubation at 10 C, infection during a 14-day period in infectious soil differed little among the four day-night temp regimes, i.e., 15 C-15 C, 15 C-10 C, 15 C-7.5 C and 15 C-5 C. However, large differences in disease development occurred if the plants were kept continuously at the same temp regimes. The lowest percentage of disease development occurred when the plants were kept at 15 C constantly, and the highest developed at the 15 C-5 C cycle. These results confirm the conclusion, that although the optimum temp for infection of wheat with WSSMV from infectious soil is about 15 C, exposure to lower temp periodically or continuously is necessary for optimum development of symptoms.

**DISCUSSION.**—The results of these experiments confirm earlier reports (3) that WSSMV has a very low temp requirement. The optimum temp for symptom development on wheat plants infected either by manual inoculation or by growing in infectious soil was 8-10 C. This appears to be the lowest optimum temp for any known plant virus. The maximum temp at which symptoms developed were 17.5 C for manually inoculated plants, and 15 C for plants infected from the soil. These optimum and maximum temp are several degrees lower for WSSMV than for the wheat soil-borne mosaic viruses from Illinois and Nebraska, which appear to develop optimally at about 16 C, and will also develop at 20 C (1).

The most unexpected results of the temp study were those showing that infection could occur in soil at temp higher than those at which symptoms will develop. The optimum temp for infection, 15 C, and the maximum, 20 C, are about 5 C higher than the optimum and maximum

TABLE 2. Percentage of wheat plants developing spindle streak mosaic after 14 days in infectious soil at different daily temp regimes, followed by incubation in noninfectious soil at the same temp regimes or at 10 C<sup>a</sup>

Infection temp (C)		Seedling infection (%) with incubation temp	
Day	Night	Same as infection	10 C
15	15	15	90
15	10	52	85
15	7.5	53	68
15	5	83	96

<sup>a</sup>Least significant difference ( $P = 0.05$ ) = 23.3; ( $P = 0.01$ ) = 31.7.

temp for symptom development on plants infected from soil. Notwithstanding the high percentage of plants that became infected from soil at 15 C, few of these plants developed symptoms if kept at 15 C unless they were exposed to lower temp (5 C to 10 C) daily, or for 1 wk or longer before returning to a constant temp of 15 C.

Although infection of roots may take place most rapidly at 15 C, the virus requires a lower temp to become established in the plant. Unreported results (J. T. Slykhuis, *unpublished*) show that manually transmissible virus is lost from plants kept at temp of 20 C or higher. Few plants develop symptoms when grown in infectious soil at any constant temp because 8-10 C, which is optimum for the development of symptoms, is not the optimum for infection. One of the more effective schedules for testing the infectivity of soil has been to sow wheat in the soil and grow it at 15 C for 4 wk, then remove the plants, wash their roots, transplant them to noninfectious soil and incubate at 10 C for 8-10 wk. Using this procedure, WSSM has developed on all plants in some tests with some soils.

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