

PHYTOPATHOLOGICAL NOTES

Soil Transmission and Electron Microscopy of Wheat Spindle Streak Mosaic

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Michigan Agricultural Experiment Station Journal Series Article No. 5088. The authors are grateful to Roland Myers for assistance in embedding and sectioning.

ABSTRACT

Transmission of the wheat spindle streak mosaic disease to wheat seedlings (*Triticum aestivum* L. 'Genesee') grown in infested soil was markedly promoted by cool and freezing temperatures. Vernalization environments increased both symptom severity and the percentage of test plants affected. Affected tissues examined in an electron microscope contained viruslike rods and abundant pinwheel inclusions. *Phytopathology* 61:331-332.

Within the last decade, a new variegation disease developed in wheat grown in the Great Lakes area. Originally found in isolated fields, the disease currently affects vast acreages in Michigan and Ontario (3, 5). Until the work of Slykhuis (3), progress in characterizing the disease was minimal since its study largely relied on seasonal disease occurrence in the field. Slykhuis observed viruslike rods in sap from affected plants, and named the disease "wheat spindle streak mosaic" (WSSM). He reproduced the disease using infested soil and cool temp, but achieved low percentages of infected plants after several months' incubation.

Reported herein are results of soil transmission studies in which highly efficient and more rapid transmission of WSSM was realized through the use of vernalization environments. Further evidence for the viral nature of the disease was also obtained by direct observation of affected tissues.

Seedlings of Genesee wheat (*Triticum aestivum* L.) were started from seed in a greenhouse maintained at 20 ± 3 C. The soil in which the seedlings were grown was collected from wheat fields in Michigan in which the WSSM disease was prevalent. Prior to planting, the soil was air-dried and mixed with a small portion of sterile sand to improve aeration and seedling emergence.

Seven days after planting, the seedlings were transferred either to an outdoor cold frame or to a growth chamber maintained at 1 C. Plants in the cold frame were subjected to air temp of -2 ± 15 C and soil temp of 3 ± 5 C. After varying periods of cold treatment, the seedlings were transferred to a 10-C chamber or returned to the greenhouse and examined for WSSM development. The 1-C and 10-C chambers supplied 400 and 1,400 ft-c of a mixture of fluorescent and incandescent light, respectively, in alternate 12-hr intervals.

TABLE 1. Wheat spindle streak mosaic development in plants grown in infested soil at 10 C after preliminary cold treatment

Cold treatment	Days of cold treatment	Avg days for symptom development at 10 C	% Plants developing symptoms ^a
	0	110	46
1-C Chamber	15	64	48
	30	29	50
	60	25	72
Outdoor cold frame ^b	15	28	48
	30	12	100
	60	12	100
	90	12	100

^a Calculated from a min of 22 plants.

^b Air temp -2 ± 15 C. Soil temp -3 ± 5 C.

Soil transmission of WSSM was obtained if final incubation was at 10 C, but not at 20 C. These results agreed with those of Slykhuis (3). All plants incubated finally at 20 C failed to develop symptoms regardless of preliminary cold treatment. Seedlings transferred directly to 10 C without cold treatment developed symptoms after 95-120 days, whereas cold-treated seedlings developed symptoms more rapidly (Table 1). Cold treatment outdoors was more effective than cold treatment at 1 C. All seedlings remaining in the cold frame for 30 or more days developed severe WSSM symptoms when transferred to 10 C, and many plants died within 12 weeks after transfer. Within 4 weeks, such plants were visibly stunted and chlorotic as compared to similar plants grown in steamed soil (Fig. 1).

Seedlings grown in soil that was steamed (1 hr at 100 C) or treated with Fumazone 70E (0.06 ml 1,2-dibromo-3-chloropropane/kg), Dow Chemical Co., generally failed to become infected. Occasionally, how-

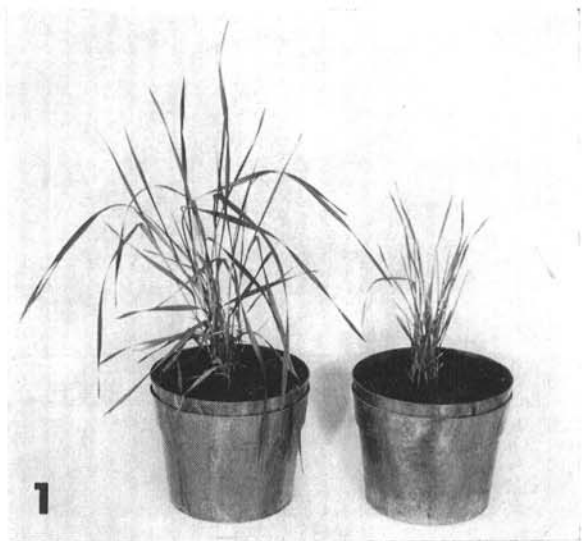


Fig. 1. Development of wheat plants grown in steamed (left) and untreated (right) wheat spindle streak mosaic-infested soil. Plants are shown after 4 weeks at 10 C following 30 days' cold treatment outdoors.

ever, mild WSSM symptoms occurred in individual plants after 120 days at 10 C. Transmission in soil treated with benomyl [methyl 1-(butylcarbamoyl)-2-benzamidazolecarbamate], 1 mg active/kg, Dupont Co.,

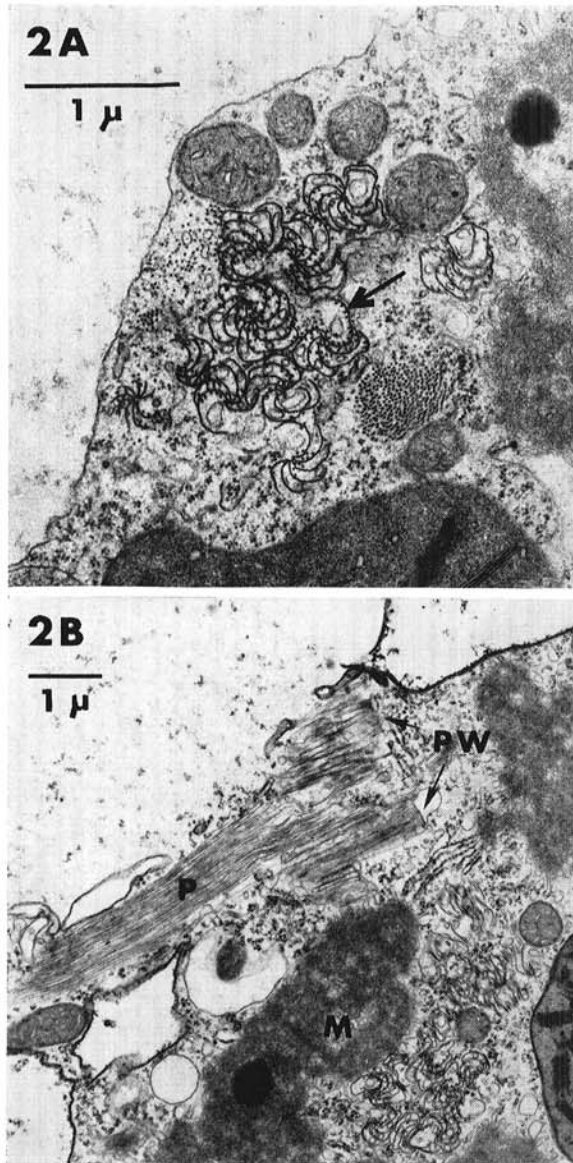


Fig. 2. Cytoplasmic detail of wheat spindle streak mosaic-affected wheat mesophyll. **A)** Cross section of pinwheels and an aggregate of rodlike particles. Note spaced particles on arm of pinwheel (arrow). **B)** Longitudinal section of pinwheels (pw) and an aggregate of rodlike particles (p). Note dense membranous inclusion (m).

was not appreciably different from that in untreated soil.

As WSSM developed, leaf segments from affected and symptomless plants were fixed in glutaraldehyde followed by osmium tetroxide, dehydrated, and embedded in ERL epoxy resin (4). Thin sections were stained in alcoholic uranyl acetate and aq lead citrate and examined in a Zeiss EM-9A electron microscope.

Seedlings affected by WSSM such as those shown in Fig. 1 contained abundant pinwheel inclusions and rodlike particles (Fig. 2-A, B). Similar inclusions were occasionally found in symptomless plants grown in steamed or fumigated soil where disinfestation was not complete and where WSSM symptoms eventually developed. Similar viruslike entities were not found in plants grown in noninfested (nonwheat or autoclaved) soils.

Both particles and pinwheels were observed most frequently in mesophyll cells, but occurred also in the epidermis and vascular parenchyma. No inclusions of viral nature were found in sieve tubes. Inclusions were evident in cells from green and chlorotic portions of WSSM-affected leaves. In the latter, cells were often observed filled with debris that included pinwheel inclusions, viruslike particles, and disrupted organelles (Fig. 2-B).

The rodlike particles observed in diseased cells were 20-22 μ in diam. They occurred as aggregates in the cytoplasm or as spaced, single particles along the arms of pinwheels (Fig. 2-A). In comparison, particles of wheat streak mosaic virus which also affect wheat in Michigan (5) are 15-17 μ in diam and are not so aligned with pinwheel arms (2). While the length of the particles in WSSM-infected plants could not be readily determined in sectioned cells, the pinwheel inclusions observed are characteristic of those formed in plants infected with long flexuous viruses (1).

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