# Further Evidence for the Association of *Polymyxa graminis* with the Transmission of Wheat Spindle Streak Mosaic Virus

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## ABSTRACT

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Wheat spindle streak mosaic (WSSM), which is caused by wheat spindle streak mosaic virus (WSSMV), was detected in 36 of 76 Pennsylvania wheat fields surveyed in the spring of 1977 and 1978. The disease was identified by symptomatology, the temperature requirement for symptom development, and by an association with virus particles. Long, flexuous, rod-shaped virus particles measuring 14 nm wide and 600 to 925 nm long were detected in leaf dip preparations from plants that had developed characteristic WSSM symptoms at 5–15 C. Three zoosporic fungi, *Polymyxa graminis*, *Olpidium brassicae*, and *Lagena radicicola* were most commonly observed in the roots of both symptomatic and asymptomatic plants. The occurrence of WSSM in the field was correlated with the presence of *P. graminis*, but not *O. brassicae* or *L. radicicola*, suggesting that *P. graminis* plays a major role in the natural spread of WSSMV. The incidence of WSSM was not correlated, however, with the extent of root colonization by *P. graminis* WSSMV transmission occurred when test plants were inoculated with root washings and root pieces containing *P. graminis* from WSSMV-infected plants.

Wheat spindle streak mosaic (WSSM) of winter wheat (*Triticum aestivum* L. cm. Thell.) was first reported in Ontario, Canada, by Slykhuis in 1960 (11). Slykhuis (12) determined that the cause of this disease was a soilborne virus (WSSMV) with long, flexuous rod-shaped particles.

Early observations by Barr and Slykhuis (1,2) revealed the presence of various species of root-infecting fungi associated with WSSMV-infected wheat in Ontario. Four species of zoosporic fungi were identified as potential vectors of WSSMV: *Polymyxa graminis* Led. (Plasmodiophorales), *Olpidium brassicae* (Wor.) Dang. (Chytridiales), *Lagena radicicola* Vanterpool and Led. (Lagenidiales), and *Rhizophydium graminis* Led. (Chytridiales). Slykhuis and Barr (14) provided evidence indicating the transmission of WSSMV by *P. graminis*. Transmission occurred via the association of roots of test plants with the roots of plants infected with both *P. graminis* and WSSMV. However, the root-washing and root-piece transmission methods utilized by Brakke et al (3) and Rao et al (9) to demonstrate the transmission of soilborne wheat mosaic virus (SBWMV) by *P. graminis* were unsuccessful when attempted with WSSMV.

The only report of *P. graminis* and WSSMV in Pennsylvania was by Barr and Slykhuis (2) who detected their presence in soil samples from several wheat fields. Experiments reported here were conducted to determine the degree of correlation between the presence of WSSM in Pennsylvania wheat fields and those root-infecting fungi suspected of being vectors of WSSMV. Transmission experiments were also conducted utilizing root washings and root pieces containing *P. graminis* from WSSMV-infected plants.

## MATERIALS AND METHODS

**Maintenance of plants**. Winter wheat cultivar Redcoat was the test plant used throughout all transmission experiments. Plants inoculated in the transmission tests were transferred to a growth



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chamber maintained at 15 C with a 12-hr light period of approximately 10,000 lux to favor virus multiplication (13). After 60 days, the plants were maintained at 15 C during a 12-hr light period of approximately 8,000 lux, and at 5 C during a 12-hr dark period to induce WSSM symptom development (13).

**Field survey for WSSM.** Pennsylvania wheat fields were surveyed for WSSM and zoosporic fungi in the spring of 1977 and 1978. Plants were collected from 76 wheat fields representing 14 counties located throughout the state. Field-collected plants were transplanted into a steam-sterilized sand-soil-peat (1:1:1, v/v) mix, and maintained under the conditions described previously for symptom development. The development of WSSM symptoms (12) was recorded after 60–90 days.

**Indexing for virus.** Plants collected during the field survey that expressed characteristic WSSM symptoms were indexed by electron microscopy for the presence of virus particles reported to be associated with this disease (12). Leaf dips were prepared by touching a freshly cut edge of a symptomatic leaf to a drop of boiled distilled water on a Formvar coated grid for 5 sec. After 30 min the excess liquid was removed and a drop of 0.2% phosphotungstic acid solution, pH 7.0, was placed on the grid for 25 sec. A drop of octadecanol was added to the grid 15 sec before the excess liquid was removed. Grids were examined by using a Philips EM 300 electron microscope.

**Indexing for zoosporic fungi.** The root systems of plants collected from the field were washed in tap water and the roots nearest to the crown were cut into segments ~3 cm long and stained by boiling in 0.01% lactophenol acid-fuchsin for 2 min (7). Ten stained segments were randomly sampled from five plants per field sample and examined for the presence of *P. graminis*, *O. brassicae*, *L. radicicola*, and *R. graminis* by using a compound microscope at magnifications of ×100 and ×400.

TABLE 1. Incidence of wheat spindle streak mosaic (WSSM) and three zoosporic fungi in 76 Pennsylvania wheat fields in 1977 and 1978

| | Fields with WSSM | | Fields without WSSM | |
|---|---|---|---|---|
| Fungus | Incidence[y] | Incidence (%) | Incidence | Incidence (%) |
| *Polymyxa graminis*[z] | 34/36 | 94.4 a | 13/40 | 32.5 b |
| *Olpidium brassicae* | 27/36 | 75.0 a | 32/40 | 80.0 a |
| *Lagena radicicola* | 35/36 | 97.2 a | 34/40 | 85.0 a |

[y]The number of fields in which the fungus was detected divided by the number of fields surveyed.

[z]Values followed by the same letter, within the same row, are not significantly different ($P = 0.05$), according to a Clopper and Hartley (5) table which was used to construct confidence intervals for percentages.

TABLE 2. Transmission of wheat spindle streak mosaic virus (WSSMV) to healthy wheat seedlings via washings from roots infected by WSSMV and *Polymyxa graminis*

| Inoculum | Disease incidence[u] | Disease incidence (%) | *P. graminis* incidence[v,w] |
|---|---|---|---|
| 1-min root wash[x] | 0/36 | 0.0 | 17.2 b |
| 30-min root wash | 2/36 | 5.5 | 31.7 cd |
| 1-hr root wash | 0/36 | 0.0 | 1.1 a |
| 24-hr root wash | 2/36 | 5.5 | 41.7 d |
| 48-hr root wash | 6/36 | 16.6 | 24.4 bc |
| Water control[y] | 1/30 | 3.3 | 6.0 a |
| WSSMV-infectious soil[z] | 3/30 | 10.0 | 84.0 e |

[u]The number of plants with WSSM symptoms divided by the number of plants inoculated.

[v]The relative frequency of resting spores of *P. graminis* in the roots of each plant was expressed as a percentage: 0, no infection; 20, very light infection; 40, light infection; 60, moderate infection; 80, heavy infection; and 100, very heavy infection.

[w]Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's least significant difference test (6).

[x]Root washings were prepared by soaking the roots of WSSMV-infected plants for periods of either 1 min, 30 min, 1 hr, 24 hr, or 48 hr.

[y]Plants received tap water instead of root washings.

[z]Plants were grown in WSSMV-infectious soil.

**Root washing transmission test.** Root washings to be tested for transmission of WSSMV were prepared by immersing washed roots and crowns of WSSMV-infected plants in 400 ml of charcoal-treated distilled water at 15 C (3) for 1 min, 30 min, 1 hr, 24 hr, and 48 hr. Fifty-milliliter aliquots of these washings were added to each of six 10-cm-diameter clay pots, each containing six, 5-day-old Redcoat wheat seedlings growing in a steam-sterilized sand-soil (1:1) mix (4). The presence of WSSM symptoms was recorded after 60–90 days. At this time, six 3-cm root segments from each plant were stained and examined for the presence of zoosporic fungi. The relative frequency of *P. graminis* resting spores in the roots of each plant was expressed as percents: 0, no infection; 20, very light infection; 40, light infection; 60, moderate infection; 80, heavy infection; and 100, very heavy infection. The designation of a very heavy infection was used only when *P. graminis* resting spores were found in the majority of the cells of the root epidermis.

**Root piece transmission test.** Root segments 0.5–1.0 cm long from WSSMV-infected plants were used as inocula in these experiments. Only root segments containing *P. graminis* resting spores and apparently free of other fungi were selected. The root segments were stored under a layer of distilled water at 4 C to inhibit zoospore release (8). Approximately 100 root pieces were placed directly on the roots of six 3-day-old Redcoat seedlings and then covered with a steam-sterilized soil-sand (1:1, v/v) mix. Control treatments included root segments cut from the same WSSMV-infected source plant, but without detectable *P. graminis* resting spores, and seedlings grown in either WSSMV-infectious soil or steam-sterilized soil. The presence of WSSM symptoms and the amount of *P. graminis* infection after 60–90 days were recorded as described previously.

## RESULTS

**Detection of WSSM in Pennsylvania wheat fields.** The occurrence of WSSM in plants collected from the field was detected by growing the plants at a fluctuating temperature regime of 5–15 C to induce symptom development (13). Spindle-shaped chlorotic lesions appeared in the youngest leaves after 3–6 wk. Electron microscopic examination of leaf dip preparations from plants expressing symptoms revealed the presence of long, flexuous, rod-shaped virus particles measuring 14 nm × 600–925 nm. Of 76 fields from which plants were collected, 36 were found to have WSSM. These fields, which were located in 11 of 14 counties surveyed, were distributed throughout the state.

**Zoosporic fungi associated with WSSMV-infected wheat in Pennsylvania.** Three species of zoosporic fungi, *P. graminis*, *O. brassicae*, and *L. radicicola* were most frequently observed in the roots of both WSSMV-infected plants and symptomless plants. *Olpidium brassicae* and *L. radicicola* were present in the roots of

TABLE 3. Transmission of wheat spindle streak mosaic virus (WSSMV) to healthy wheat seedlings via *Polymyxa graminis*-infected root pieces from a WSSMV-infected plant

| Inoculum | Disease incidence[v] | Disease incidence (%) | *P. graminis* incidence[w,x] |
|---|---|---|---|
| Root pieces with *P. graminis* | 10/36 | 28.0 | 58.3 a |
| Root pieces without *P. graminis* | 2/36 | 5.5 | 49.4 a |
| WSSMV-infectious soil[y] | 0/19 | 0.0 | 76.8 b |
| Steam-sterilized soil[z] | 0/24 | 0.0 | 5.0 c |

[v]The number of plants with WSSM symptoms divided by the number of plants inoculated.

[w]The relative frequency of detecting *P. graminis* resting spores in the roots of each plant was expressed as a percentage: 0, no infection; 20, very light infection; 40, light infection; 60, moderate infection; 80, heavy infection; and 100, very heavy infection.

[x]Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's least significant difference test (6).

[y]Plants were grown in WSSMV-infectious soil.

[z]Plants were grown in steam-sterilized soil.

plants from a high percentage of the fields examined. No significant difference was found between the occurrence of *O. brassicae* and *L. radicicola* in fields with or without WSSM (Table 1). *Olpidium brassicae* was found in 75% of the fields with WSSM and in 80% of the fields in which WSSM was not detected. Similarly, *L. radicicola* was present in 97 and 85% of the fields with and without WSSM, respectively. *P. graminis*, on the other hand, was found significantly more often in fields with WSSM (Table 1). Ninety-four percent of the fields with WSSM also contained *P. graminis*, whereas this fungus was found in only 33% of the fields without WSSM. Thirty-seven fields surveyed had been sown with wheat at least once in the preceding 4 yr and 21 of these fields were found to have both WSSM and *P. graminis*. WSSM and *P. graminis* were absent in two fields that had been out of wheat production for over 13 yr while both *O. brassicae* and *L. radicicola* were present.

In general, the occurrence of WSSM was not correlated with the number of *P. graminis* resting spores found in individual root segments. When *P. graminis* was found in WSSMV-infected plants the root segments could contain from a few to numerous clusters of resting spores. Root segments from plants without WSSM symptoms exhibited a similar range of colonization, although the majority of the root segments were only lightly colonized.

**WSSMV transmitted via root washings from plants infected by WSSMV and *Polymyxa graminis*.** A low percentage (0–16%) of the wheat seedlings were infected following exposure to washings collected from infected roots which had been soaked in water for varying periods of time (Table 2). Three plants grown in WSSMV-infectious soil were also infected as was one plant in the water control group.

There was no apparent correlation between WSSMV transmission and the degree of *P. graminis* colonization (Table 2). For example, test plants grown in WSSMV-infectious soil had the highest level of *P. graminis* infection, but not the highest rate of WSSMV transmission. Test plants exposed to washings collected from either the 1 min or 48-hr soaking period had comparatively low levels of *P. graminis* infection, but WSSMV transmission only occurred following the prolonged soaking period.

**WSSMV transmitted via *Polymyxa graminis*-infected root pieces from WSSMV-infected plants.** WSSMV transmission was successful when root pieces containing *P. graminis* from a WSSMV-infected plant were placed on the roots of wheat seedlings (Table 3). Ten of 36 seedlings developed WSSM symptoms and all of these plants were found to be colonized by *P. graminis*. Nine of the 10 plants were also found to have a very light infection of *L. radicicola*. In addition, root segments from two plants contained three *R. graminis* sporangia and a root segment of one plant contained three sporangia of *O. brassicae*.

Plants infested with root pieces containing *P. graminis*, but which failed to develop WSSM symptoms, were found to be colonized by *P. graminis* to the same degree as plants that developed WSSM (*unpublished*). The majority of these plants also contained a light infection of *L. radicicola* and a few plants were lightly colonized by *O. brassicae*.

Two of the 36 plants that were infested with root pieces apparently free of *P. graminis* developed WSSM symptoms. Microscopic examination subsequently showed that the plants in this treatment were infected with *P. graminis* with a severity not significantly different from that of the plants inoculated with root pieces containing *P. graminis*. Several plants were also moderately colonized by *O. brassicae* and most had a light infection of *L. radicicola*.

## DISCUSSION

We found WSSM to be widespread in Pennsylvania wheat during the spring of 1977 and 1978 as judged by its occurrence in 36 of 76 fields located throughout the state. The disease was identified as WSSM in this study on the basis of symptomatology, the temperature requirement for symptom development, and by an association with virus particles. Symptoms of the disease were rarely observed on plants in the field during our survey, although it is not uncommon for WSSMV infection in wheat to go undetected in years when spring temperatures are not conducive for symptom expression (10). Consequently, it was necessary to subject the field-grown plants to a temperature regime known to promote WSSM symptom development (13). The symptoms that we observed on these plants were apparently the same as those described for WSSM as it occurred in Ontario (12). The fact that virus particles resembling WSSMV (15) in morphology and size could also be detected in these plants would further support the identity of the disease as WSSM.

The zoosporic fungi, *P. graminis*, *O. brassicae*, *L. radicicola*, and *R. graminis* have been suspected of being the vectors of WSSMV (1,2). In unifungal transmission tests, Slykhuis and Barr (14) clearly demonstrated that *P. graminis* was a vector of WSSMV. We have found *P. graminis*, *O. brassicae*, and *L. radicicola* to be most commonly associated with the roots of wheat plants in Pennsylvania. Of these, only the presence of *P. graminis* could be correlated with the incidence of the disease in the field which would suggest that this fungus plays a major role in the natural spread of WSSM in Pennsylvania.

We have shown that it is possible to transmit WSSMV to wheat seedlings via either root washings or root pieces containing *P. graminis* from WSSMV-infected plants as inoculum. The transmission rates observed in our experiments with either of these methods were generally low and poorly reproducible. This was best exemplified in the root washing experiment in which plants inoculated with root washings failed to become infected even though an uninoculated control plant inadvertently became infected, presumably from water-splashed inoculum. More importantly, the major shortcoming of the transmission experiments is that we were unable to obtain *P. graminis* to the exclusion of the other zoosporic fungi. The microscopic technique used to screen the inoculum root pieces was not sufficiently sensitive to detect trace levels of fungal propagules. Since all of the plants that became infected with WSSMV following artificial inoculation were shown to be infected with either *O. brassicae* or *L. radicicola*, we could not conclusively confirm *P. graminis* to be the vector.

Interestingly, there was no apparent correlation between the presence of WSSM in a plant and the degree to which the plant was colonized by *P. graminis*. Both naturally-infected plants collected from the field and artifically inoculated plants showed a broad range of infection by *P. graminis*. The fact that WSSMV transmission was largely independent of the vector inoculum dosage suggests that not all zoospores are infected or perhaps not all are equally competent in transmitting WSSMV.

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