Further observations on zoosporic fungi associated with wheat spindle streak mosaic virus[1]

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folymyxa graminis was generally found on the roots of wheat plants grown in soil samples collected from wheat fields in southern Ontario, Indiana, Kentucky, Pennsylvania, and Arkansas where wheat spindle streak mosaic virus (WSSMV) was found or suspected to be present. It was not found in soil from a field where wheat has not been grown, or in any of 21 samples of soil from fields of winter wheat in southern Alberta where there was no evidence of WSSMV. ***Lagena radicicola, Olpidium brassicae, Rhizophydium graminis,*** and several ***Pythium*** spp. were found in the soils from Alberta as well as from most of the other locations.

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On a generalement decele la presence de ***folymyxa graminis*** sur les racines de plants de ble provenant d'echantillons de sol preleves d'emblavures du sud de l'Ontario, de l'Indiana, du Kentucky, de la Pennsylvanie et de l'Arkansas où on a constaté ou soupconne la presence du virus de la mosaïque striee fusiforme (WSSMV). Le sol provenant d'un champ non affecte a la culture du ble en était exempt, de même que les 21 echantillons provenant de champs de ble d'hiver du sud de l'Alberta ou on n'a pu deceler la presence du virus. ***Lagena radiciola, Olpiduim brassicae, Rhizophydium graminis,*** et plusieurs espèces de ***Pythium*** ont ete observees dans les sols provenant de l'Alberta et de la plupart des autres endroits.

Earlier experiments indicated that wheat spindle streak mosaic virus (WSSMV) is transmitted by a soil-borne agent that passes readily through fine screens (44 μm), is persistent in soils even when dry, but which can be transported via soil water (Slykhuis 1970). These observations and the examination of roots of diseased wheat plants indicate that natural transmission is by zoosporic fungi (Barr and Slykhuis 1969). ***Polymyxa graminis*** Ledingham (1939) (Plasmodiophorales), because of its spectacular presence in the roots of diseased wheat plants in Ontario and its association with wheat (soil-borne) mosaic virus in the USA (Rao and Brakke 1969), has been a prime suspect as vector of WSSMV. However, other zoosporic fungi are also generally found on the roots of diseased wheat plants and must be considered as possible carriers of the virus. One of these, ***Olpidium brassicae*** (Wor.) Dang. (Chytridiales), is a vector of several other plant viruses (Teakle 1969). It occurs on the roots of many species of plants and although there are many physiological strains (Garrett and Tomlinson 1967, Barr and Kemp 1975), most isolates from wheat grew on lettuce but not on cabbage and only one grew on both lettuce and cabbage. ***Lagena radicicola*** Vanterpool and Led. (Lagenidiales) was reported on cereals in eastern as well as western Canada (Truscott 1933, Vanterpool 1930). Although initially it was not detected in roots of wheat infected with WSSMV in Ontario (Barr and Slykhuis 1969), later observations showed that it was usually present. This fungus has also been found in the roots of barley in England (MacFarlane 1970). ***Rhizophydium graminis*** Led. (Chytridiales) is also found on wheat roots. It differs from the preceding fungi by producing epibiotic, but not endobiotic, zoosporangia and resting spores on root hairs and epidermal cells. The zoosporangia dehisce to release zoospores and the resting spores soon become separated from the host leaving no observable bodies on the roots, hence this fungus is easily overlooked. Several ***Pythium*** spp., some of which may produce zoospores, are also found frequently in the roots of wheat plants and have been considered as possible vectors of soil-borne viruses. Unlike the other zoosporic fungi considered here, ***Pythium*** spp. are readily cultured on artificial media and so are readily isolated in pure culture; however in roots they may cause necrosis and hence are less likely to be able to transmit a virus.

The following is a report of the zoosporic fungi found on the roots of wheat plants grown in samples of soil tested for WSSMV from collections made in Ontario from 1972 to 1975, and also in samples obtained from Alberta, Arkansas, Indiana, Kentucky, and Pennsylvania.

Methods

Most samples of soil from Ontario were collected during surveys for wheat spindle streak mosaic in May each year from 1972 to 1975. In 1974, additional collections were made in October from fields selected in May,

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Table 1. Wheat spindle streak mosaic virus and root fungi on wheat grown in seven samples of soil from each of eight locations in Ontario, 1974

Collection no.	No. of plants with WSSM/no. tested*	Number of collections (out of 7) yielding fungi				
		Polymyxa graminis	*Lagena radicicola*	*Olpidium brassicae*	*Rhizophydium graminis*	*Pythium*
5	7/66 & 881147	7	3	7	5	7 *aristosporum, arrhenomanes*
10	8/60	6	6	4	5	6 *aristosporum, tardicrescens, volutum*
13	10/72 & 80/143	7	6	6	4	5 *arrhenomanes, paroecandrum, vanterpooli, volutum*
17	8/71	5	7	0	7	7 *aristosporum, arrhenomanes, ultimum*
33	28/76 & 721148	4	6	3	6	4 *aristosporum, arrhenomanes, ultimum*
54	22/69	5	6	1	2	2 *ultimum*
57	5/66	4	6	3	5	4 *aristosporum, arrhenomanes, volutum*
70	7/64 & 49/139	2	7	6	7	7 *arrhenomanes, vexans*

* Tests were made with composite soil samples from individual collections.

and six samples were collected from marked locations in each field for testing separately. All other samples were composites from locations where the disease was most prevalent in each field. The samples were dug by trowel to a depth of about 10 cm and usually included roots of diseased wheat plants. Most samples were from areas of southern Ontario where wheat is grown most intensively, but some collections were from eastern Ontario, including plots at the Central Experimental Farm, Ottawa, where wheat was grown every third year since about 1952 to 1967, then annually to increase the WSSMV infectivity of the soil.

Other samples of soil tested included collections from fields in which wheat was suspected or known to be affected by WSSMV. Samples were received from J.P. Jones, Department of Plant Pathology, University of Arkansas, Fayetteville, Arkansas, 72701; A.O. Jackson, Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907; T.P. Pirone, Department of Plant Pathology, University of Kentucky, Lexington, Kentucky 40506; and S.H. Smith, Department of Plant Pathology, Pennsylvania State University, University Park, Pennsylvania 16802. Some samples were provided from Alberta by U.J. Pittman, Research Station, Agriculture Canada, Lethbridge, including samples from a field in which winter wheat was grown twice every three years from 1951 until the samples were collected in 1974. Other samples from Alberta were collected from farm fields with long histories of winter wheat in short rotations including barley or oats and summerfallow.

The presence of WSSMV in soil samples was tested by sowing Kent wheat in 7.5-cm pots of each sample, growing at 15°C with about 15,000 lux of light 12 h per day for 3 weeks, then replanting in 10 cm pots of sterile soil or sand and growing at 10°C for about 3 months for symptoms to develop (Slykhuis 1973, 1976).

Table 2. Fungi in the roots of wheat plants grown in soil from fields at Ottawa with and without a crop history of wheat

Fungi	Incidence of fungi	
	24 samples from a field of wheat highly infectious with WSSM virus	12 samples from an adjacent field that has not grown wheat
Polymyxa graminis	20124	0/12
Lagena radicicola	24	12
Olpidium brassicae	10	5
Rhizophydium graminis	4	8
Number of samples with		
Pythium spp. in roots	24	6
Py. aristosporum	4	0
Py. tardicrescens	9	0
Py. volutum	10	4
Other *Pythium* sp.	2	2

To determine the presence of fungi, Kent wheat was sown in about 2 cm of the test soil placed on 4 cm of sterile quartz sand in 7.5-cm pots and grown at 15° or 20°C with 15,000 lux of light 16 h per day for 3 weeks. Roots that grew into the sand were cleaner and easier to examine unstained with phase contrast illumination than roots in soil. However, it was found in 1974 that longer incubation times were desirable for easier observations of ***P. graminis,*** hence for later tests, after an initial examination as described above, the plants were repotted in sterile sand, watered with 0.3- or 0.5- strength Hoagland's solution and grown a further 3 weeks under similar conditions, then the roots were reexamined for fungi.

Pythium spp. were isolated by placing pieces of washed roots on nutrient agar containing 200 ppm neomycin sulfate. The isolates were grown on V-8 juice agar (2.4% V-8 vegetable juice in 1% agar without $CaCO_3$), or on autoclaved hemp seed added to fresh, sterile water. Isolates were also plated on homemade potato dextrose agar and tested for growth at 25°, 30° and 35°C. The identity of each species was verified in culture.

Results

Fungi in roots of wheat grown in Ontario soils infectious with WSSMV

The roots of wheat were examined after growing for 3 weeks in soil samples from 53 fields in which WSSMV was prevalent when the samples were collected in May 1972, 1973, or 1974. O. ***brassicae*** was found in 46, L. ***radicicola*** in 45, ***P. graminis*** in 40, and ***Rhizophydium graminis*** in 32 of the soils tested. In three samples of soil from wheat fields in which WSSMV was not detected, only *O. **brassicae*** and L. ***radicicola*** were found.

Pythium aristosporum Vanterpool, ***Py. arrhenomanes*** Dreschsler, ***Py. tardicrescens*** Vanterpool and ***Py. volutum*** Vanterpool & Truscott were frequently isolated from wheat grown in Ontario soils and were associated with root necrosis of pot-grown plants. Also isolated from wheat roots but less frequently than the aforementioned species were ***Pythium aphanidermatum*** (Edson) Fitzpatrick, ***Py. paroecandrum*** Dreschsler, ***Py. torulosum*** Coker & Patterson, ***Py. ultimum*** Trow, ***Py. vanterpooli*** Kouyeas & Kouyeas and ***Py. vexans*** de Bary. ***Py. graminicola*** Subramaniam, sensu stricto, was not found; however, morphological and physiological characteristics of some isolates identified as ***Py. aristosporum*** overlapped the characteristics of ***Py. graminicola.***

Examinations were made of wheat roots grown 3 weeks in sand following 3 weeks growth in seven samples of soil from each of eight fields in southern Ontario were WSSMV was prevalent in 1974. One sample tested was a composite of six samples collected in May. The other six samples were collected in October from the same locations as the samples collected in May. Each of the zoosporic fungi, including L. ***radicicola,*** O. ***brassicae, P. graminis, Rhizophydium graminis*** and one or more species of ***Pythium,*** was found on the roots of wheat grown in some of the samples from each field. Some were found in all samples from some fields but none was found in all samples from all of the fields (Table 1). There was no apparent correlation between the presence of any fungus and the numbers of test plants developing WSSMV.

Table 3. Incidence of wheat spindle streak mosaic virus and root fungi on wheat grown in samples of **soil** from Alberta and the United States

Virus and fungus isolated	Number of samples yielding virus and fungi				
	Alberta	Arkansas	Indiana	Kentucky	Pennsylvania
WSSM virus	0	0	1	3	2
Polymyxa graminis	0	1	1	3	2
Lagena radicicola	14	1	1	3	2
Olpidium brassicae	14	1	1	3	2
Rhizophydium graminis	4	0	0	2	1
Pythium aristosporum	0	1	1	0	0
Pythium arrhenomanes	5	0	0	0	1
Pythium tardicrescens	3	0	0	0	0
Pythium torulosum	0	0	1	1	0
Pythium volutum	5	0	0	1	0
Other *Pythium* spp.	1	0	0	1	0
Number of samples tested	21	1	1	3	2

Zoosporic fungi in infectious and noninfectious soils in adjacent fields at Ottawa

Tests were made for the presence of fungi in 24 samples of infectious soil collected throughout plots at the Central Experimental Farm, Ottawa, in which wheat had been grown every third year from 1952 to 1967, then annually until 1975, and in 12 samples of noninfectious soil from plots 100-150 m distant, in a field with similar soil in which wheat had not been grown for at least 15 years (Table 2). ***P. graminis*** was found in 20/24 samples from the infectious field but in none from the other. Also, ***Pythium aristosporum*** and ***Py. tardicrescens*** were found in some samples from the infectious field but in none from the other. ***L. radicicola*** was found in all samples from both fields, and the other fungi were found in some **of** the samples from each field.

Zoosporic fungi in relation to the presence of WSSMV in soils from different regions of Canada and the USA

Tests with samples of soil from Arkansas, Indiana, Kentucky, and Pennsylvania, where WSSMV-like symptoms were found on wheat, confirmed the presence of WSSMV in all except the sample from Arkansas. ***P. graminis, L. radicicola, O. brassicae*** and one or more species of ***Pythium*** were present in all these samples, and ***Rhizophydium graminis*** was present in some.

WSSMV was not detected in any of the 21 samples from the winter-wheat-producing areas of southern Alberta, which are about 2500 km from the nearest known sites of WSSMV. However, all groups of the zoosporic fungi under consideration, except ***P. graminis,*** were found in a number of the samples.

Discussion

Although ***Olpidium brassicae, Rhizophydium graminis*** and several species of ***Pythium*** were found on the roots of some of the wheat plants grown in samples of soil from wheat fields in which WSSMV was known to be present, ***Polymyxa graminis*** and ***Lagena radicicola*** were most commonly found in soils infectious with the virus. The occasional failure to find either of the latter fungi on roots of plants grown in virus infective soil could have resulted, not from the absence of the fungi in the soil, but from their suppression by other organisms in the soil complex under the conditions of the test. These fungi were difficult to find or absent in roots heavily invaded by ***Pythium*** spp., most of which caused some necrosis of wheat roots, and therefore are not likely to be vectors of the virus.

L. radicicola was present but ***P. graminis*** was not found on the roots of wheat plants grown in soil from a field at the Central Experimental Farm, Ottawa, where cereals have not been grown for at least 15 years. However, ***P. graminis*** was most abundant in another field in which WSSMV was prevalent after many successive crops of wheat. It appears that frequent cropping to wheat favors the buildup of WSSMV and ***P. graminis*** in soils in Ontario. However, neither ***P. graminis*** nor WSSMV was detected in samples of soil from fields in southern Alberta with long histories of frequent cropping to wheat.

The above results support the suggestion that transmission of WSSMV from soil occurs only when ***Polymyxa graminis*** is present.

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