

Occurrence and pathogenicity of *Pythium* spp. in seedling roots of winter rye

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Abstract. Seedlings of winter rye collected from yellowing patches during October to November 1985—1987 showed oospores of *Pythium* species in apparently healthy as well as in discolored roots. Examination of 1550 root pieces of rye on CMA yielded fungi belonging to 35 genera. The most commonly isolated ones were *Fusarium* spp, *Penicillium* spp, *Mucor* spp, *Mortierella* spp. and *Cladosporium* spp. *Pythium* spp. were isolated from 35 root pieces on CMA. Identified species were *P. splendens*, *P. irregulare*, *P. dissimile*, a species resembling *P. aristosporum* and a species resembling *P. ultimum*. In *in vitro* and *in vivo*, tests on the cereals winter rye, spring wheat, oats and barley the pathogenicity of some *Pythium* isolates varied from high (*P. splendens*, *P. irregulare*) to moderate (*P. irregulare*, *P. dissimile*) and low (a species resembling *P. ultimum*).

Index words: *Pythium*, pathogenicity, *in vitro*, *in vivo* winter rye, spring wheat, oats, barley

Introduction

Pythium species are common in agricultural soils and they parasitize a wide range of hosts (DOMSCH et al. 1980). All spring and winter small grains and forage grasses are susceptible to root rot caused by one or several *Pythium* species acting singly or in combination (WIESE 1977).

Root rot on rye caused by species of *Pythium* has been reported only in a few cases. The species *Pythium aphanidermatum* (Edson) FITZ (SECHLER & LUKE 1967) and *P. myriotylum* Drechs. (LITRELL & McCARTER

1970, MITCHELL 1975) have caused seedling damping-off and root rot in the southern parts of the U.S.A.

Several species of *Pythium* cause a browning root rot in barley. The disease has been encountered in the U.S.A. (BRUEHL 1955, KILPATRICK 1968, MATHRE 1982, BRATOLOV-EANU and WALLACE 1985), Canada (McKEEN 1977), Argentina (FREZZI 1956), England (WALLER 1979) and Australia (DEWAN and SIVASITHAMPARAM 1988). *P. arrhenomanes* Drechs., *P. aphanidermatum*, *P. graminicola*

Subr., *P. irregulare* Buisman, *P. splendens* Braun, *P. tardicrescens* Vanterpool and *P. volutum* Vanterpool & Truscott are the most important species reported to be pathogenic on barley.

Browning root rot of wheat also has a world-wide distribution, and it has caused damage to wheat seedlings in the U.S.A. (KILPATRICK 1968, CHAMSWARNG and COOK 1985), England (WALLER 1979), Austria (GLAESER 1979) and Australia (DEWAN and SIVASITHAMPARAM 1988). More than ten species of *Pythium* have been reported to cause root rot, seed rot and damping-off in wheat seedlings. The most extensively documented wheat pathogens are *P. arrhenomanes*, *P. aphanidermatum*, *P. graminicola*, *P. myriotylum* Drechs. and *P. volutum* (WIESE 1977).

In oats, root rot has been caused by the species *P. debaryanum* Hesse (WELCH 1945), *P. aphanidermatum* and *P. splendens* (KILPATRICK 1968).

In autumn 1984, patches of yellowed and stunted rye plants were observed in rye fields in southern Finland (BREMER and VESTBERG 1986). Electron microscopy revealed two types of virus particles in the leaves and roots of the yellowed rye seedlings. No virus vector, e.g. *Polymyxa graminis* Ledingham, was observed, only indications of fairly abundant occurrence of *Pythium* spp in roots of yellowed rye seedlings.

The aim of this study was to isolate and to identify species of *Pythium* from roots of rye seedlings. Introductory pathogenicity tests were conducted with some isolates on rye, barley, oats and winter wheat.

Materials and methods

Sampling and sample treatment

Seedling samples were collected from yellowing patches of winter rye fields during October to November 1985—87 and in May 1987 in southern and central Finland. The localities numbered 12, 23, 9 and 19 in autumn

1985, autumn 1986, autumn 1987 and spring 1987, respectively.

The roots were rinsed thoroughly in tap water. One half of the root sample was examined with a compound microscope. From the other half of the sample, discoloured pieces (approx. 0.5 cm long) of fine roots, including healthy and discoloured ones, were transferred to water agar (WA) without any surface sterilization or antibiotic treatment. The fungi were transferred from the WA to corn meal agar (CMA) for identification.

Studies on Pythium strains

Morphological characteristics of the strains of *Pythium* spp. were determined on 4-week cultures on CMA and on 9-day cultures in water. The water cultures were established by transferring a small piece of culture (1 cm in diameter) on CMA beneath an autoclaved piece of young rye leaf, cv 'Dan Kowskie Zlote' into a Petri dish containing autoclaved water. The water consisting of "pond water" and distilled water (1:1) (Van der PLAATS-NITERINK 1981) was changed twice daily. On these occasions sexual and asexual structures of *Pythium* isolates were distinguished on the piece of rye.

Pathogenicity of Pythium strains in vitro

The pathogenicity of four *Pythium* strains (P1, P2, P3, P9) isolated from winter rye was tested *in vitro* in four replicates on barley cv 'Kymppi', oats cv 'Hankkijan Vouti', spring wheat cv 'Tähti' and winter rye cv 'Dan Kowskie Zlote'. A system of hanging file folders made of filter paper was arranged in a water bath with 5 cm of water at the bottom. Due to water suction the filter paper remained wet when the water level was maintained at 5 cm. In each folder five surface sterilized seeds (1 min in 1 % NaOCl) were applied between the two layers, 2 cm from the top. Inocula of *Pythium* (1 cm in diameter), 1-month culture on CMA, were placed 2 cm

beneath the cereal seed. Paper clips were used to bring the two layers of the hanging folder close to each other. The whole experiment consisted of six water baths with 16 hanging file folders in each. After three days of incubation in darkness the baths were placed into a growth chamber with a 16-h daylength and a light intensity of approx 5000 lux. The shoot lengths were measured seven days after onset of the experiment. Final observations of root and shoot lengths, disease symptoms and infection of *Pythium* were made four days later. Disease symptoms in shoots and roots of seedlings were estimated visually on a scale from 0 to 2:

- 0 = Healthy
- 1 = Moderately discolored
- 2 = Highly discolored

The disease index (DI) of a treatment was calculated as the mean of altogether 20 seedlings belonging to four replicates.

Pathogenicity of *Pythium* strains *in vivo*

The pathogenicity of three *Pythium* isolates (P2, P9, P17) was tested *in vivo* in six replicates on the same species and cultivars of cereals as in the *in vitro* experiment. Surface sterilized seeds (1 min in 1 % NaOCl) were pregerminated for five days at room temperature (about 22°C) until the seedling was approx. 4 cm long; equally long seedlings were thereafter planted into white plastic pots (3 dl, 5.5 cm deep) at a depth of 2 cm, five in each. The pots were filled with a sand-vermiculite-leca gravel mixture (1:1:1). The inoculum, a piece of *Pythium* culture on CMA (1 cm in diameter, 14-day old culture) was placed 1 cm beneath the seed. The pots were fertilized with the slow-release fertilizer osmocote, 2 kg/m³, and were kept in a glass house at a temperature of about 20 ° C throughout the experiment. Supplementary light was given to get a daylength of 16 h.

The experiment started on the 19th of October, 1987, and ended eight days later, when

the disease symptoms in roots and on leaves, as well as seedling lengths and dry weights of above ground plant parts and roots were determined.

The disease symptoms in roots were estimated visually in the same way as in the *in vitro* experiment. The DI of a treatment was calculated as the sum of disease symptoms in all seedlings in that treatment, altogether 30 seedlings.

Statistical analysis

Statistical analysis of the results of the pathogenicity experiments was done using the analysis of variance. Means were compared with Duncan's multiple range test.

Results

Direct microscopy of seedling roots of rye

Although all seedling samples of rye collected in autumn 1985—1987 were taken from yellow patches in the field, the majority (70.5 %) of samples contained apparently healthy roots (Table 1). Highly discolored roots were found in five samples out of 44 samples. Oospores of *Pythium* were detected in healthy as well as in moderately or highly discolored roots. However, severe infection of *Pythium* was found only in samples with highly discolored roots. On the other hand, no oospores of *Pythium* were detected in two samples with highly discolored roots (Table 1).

Including the seedling samples collected in spring 1987, which makes a total of 63 samples studied by direct microscopy, the samples containing *Pythium* spp. in roots numbered as follows:

No infection of <i>Pythium</i> detected: 34 samples				
Mild	»	»	: 20	»
Moderate	»	»	: 7	»
Severe	»	»	: 2	»

Table 1. Occurrence of oospores of *Pythium* in roots of rye seedlings collected from 44 localities in October—November 1985—87.

Root symptoms	Number of samples	Number of samples with <i>Pythium</i> oospores			
		Severity of infection			
		0	+	++	+++
Healthy roots	31	15	12	2	0
Moderately discolored roots	8	4	2	2	0
Highly discolored roots	5	2	0	1	2
	44	21	14	5	2

0 = No oospores detected

+ = A few » »

++ = A moderate number of oospores detected

+++ = Large numbers of oospores detected

Fungi identified on CMA

From seedling samples collected in the autumn of 1985 and 1986 and in spring 1987 altogether 1550 root pieces were examined on CMA for fungi. Fungi belonging to 35 genera were found (Table 2).

Among 884 root pieces studied from the autumn samples of 1985 *Fusarium* spp (84 pieces), *Penicillium* spp. (65 pieces) and *Mucor* spp. (45 pieces) were the most common fungi. In 1986, 423 samples were studied and the most common fungi were the same as in 1985, i.e. *Fusarium* spp. (31 pieces), *Penicillium* spp. (25 pieces) and *Mucor* spp. (24 pieces). The result for 243 root pieces evaluated in spring 1987 was *Mortierella* spp. (79 pieces), *Cladosporium* (76 pieces) and *Fusarium* spp. (74 pieces).

The number of fungi per root piece averaged 0.4 and 0.3 for autumns 1985 and 1986 respectively, while in spring 1987 there were 2.1 fungi per root piece.

Pythium spp. were identified in altogether 27 root pieces, i.e. in five pieces in autumn 1985, 13 pieces in autumn 1986 and nine pieces in spring 1987.

Studies on Pythium spp

Twenty-seven fungal isolates on CMA were

identified as *Pythium* spp. Eight of these representing several species of *Pythium* were obtained in pure culture (Table 3).

Pathogenicity of Pythium strains in vitro

The "hanging file folder" method was successful. Infection of *Pythium* was noticed in all cereals tested. Of the isolates, P1 (*P. splendens*) caused very extensive infection in all cereals, 87.5 % of seedlings in this treatment being affected. On the other hand, P3 (isolate resembling *P. ultimum*) caused very low or no infection at all. The isolates P2 (*P. irregulare*) and P9 (*P. dissimile*) gave rise to infection in about 50 % of the seedlings (Table 4).

Oats was the healthiest cereal, infection occurring in 37.5 % of seedlings, while spring wheat was the most extensively infected, (60.3 %).

Host specificity was observed especially for isolates P2 and P3. P2 infected winter rye by 67 %, while it caused no infection in barley. P3 caused no infection in oats but a slight or moderate infection in barley (Table 4).

Out of four isolates tested, only P1 caused a significant reduction in shoot and root length (Table 5). It also caused more leaf symptoms than the control treatment. Inverse-

Table 2. Fungi isolated from roots of rye collected in autumn 1985 and 1986 and in spring 1987.

Fungus	Number of isolations			
	1985	1986	1987	Total
<i>Absidia</i> sp.	3		2	5
<i>Acremonium</i> spp.	22		36	58
<i>Alternaria alternata</i> (Fr.) Keissler	1	5	4	10
<i>Aspergillus</i> sp.	1			1
<i>Aureobasidium pullulans</i> (de Bary) Arnaud	1			1
<i>Broomella acuta</i> Shoem. & E. Müll.			3	3
<i>Chaetomium globosum</i> Kunze ex Steud.	2			2
<i>Cladosporium</i> spp.	11	9	76	96
<i>Cochliobolus sativus</i> (Ito & Kuribayashi) Drechsler ex Dastur	10		5	15
<i>Coniocytrium</i> sp.		4		4
<i>Cylindrocarpon</i> spp.	3	1	8	12
<i>Dendryphion nanum</i> (Nees ex Gray)			1	1
<i>Doratomyces</i> sp.	1	1		2
<i>Epicoccum purpurascens</i> Ehrenb. ex Schlecht.	6	2	8	16
<i>Fusarium avenaceum</i> (Fr.) Sacc.	34	9		43
<i>F. culmorum</i> (W.G. Sm.) Sacc.	21	6		27
<i>F. graminearum</i> Schwabe		2		2
<i>F. oxysporum</i> Schlecht.	3	1		4
<i>F. sambucinum</i> Fuck.	4			4
<i>F. solani</i> (Mart.) Sacc.	1			1
<i>Fusarium</i> sp.	21	13	66	100
<i>Geotrichum</i> sp.	1		1	2
<i>Gliocladium</i> sp.	2	6	1	9
<i>Humicola fuscoatra</i> Traaen			5	5
<i>Mortierella</i> spp.	25		79	104
<i>Mucor</i> spp.	45	24	52	121
<i>Papulaspora</i> sp.	19	5	10	34
<i>Penicillium</i> spp.	65	25	41	131
<i>Pestalotia</i> sp.	1			1
<i>Phoma</i> spp.	20		9	29
<i>Pythium</i> spp.	5	13	9	27
<i>Rhizoctonia solani</i> Kühn	10			10
<i>Rhizopus nigricans</i> Ehrenb.	2		4	6
<i>Scopulariopsis brevicaulis</i> (Sacc.) Bain	1			1
<i>Sporotrix schenckii</i> Hektoen & Perkins			12	12
<i>Torula herbarum</i> Pers. ex Gray		1	3	4
<i>Trichocladium asperum</i> Harz			6	6
<i>Trichoderma viride</i> Pers. ex Gray	26	8	61	95
<i>Ulocladium consortiale</i> (Thüm.) Simmons	2		6	8
<i>Verticillium</i> sp.	16		3	19
<i>Zygorhynchus</i> sp.	1		9	10
Total number of root pieces examined	884	423	243	1550

ly, isolate P9 gave rise to the highest root symptom index, causing brown roots especially in barley. Both P9 and P1 increased the severity of root damage.

Pathogenicity of Pythium strains in vivo

Isolate P9 significantly decreased shoot dry weight, while the isolates P2 and P17 (*P. ir-*

regulare) caused an increase as compared to the control. In root dry weight there were no decreases due to *Pythium* isolates, but P17 significantly increased root dry weight (Table 6).

All isolates slightly decreased seedling length, isolates P17 and P9 significantly so, as compared to the control.

The leaves and seedling bases showed no symptoms.

Table 3. Morphological characteristics of *Pythium* isolates from rye seedlings.

Characteristics of isolate	Isolate	Tentative name
Growth 13.4 mm/day on CMA. Oogonia 34.3 µm (26.2—43.6). Several in clusters. Older oogonia and oospores somewhat pigmented. Hyphal swellings. Oospores 26.3 µm (22.5—35.9). Antheridia broad, sac-like, generally 2—5/oogonium. No growth on rye leaf in water. No asexual structures.	P1	<i>P. splendens</i>
Growth 26.8 mm/day on CMA. Oogonia 22.0 µm (19.4—25.2), terminal or intercalary, of varying appearance, with one or a few finger-like projections. Oospore 19.2 µm (15.5—21.3). Antheridia 1—2/oogonium, mostly arising from the same hypha as the oogonium. Scanty growth on leaf of rye in water. No asexual structures.	P2, P10 P17	<i>P. irregulare</i>
Hyphal growth 13.5 mm/day on CMA. Small oogonia with thin wall. No antheridia or rarely 1/oogonium, big. Oospores 13.7 µm (8.7—18.4). Scanty hyphal growth on rye leaf in water. Sporangia irregular, subglobose forming complex structures. No zoospores observed.	P9	<i>P. dissimile</i>
Hyphal growth 27.1 mm/day on CMA. Oogonia 25.0 µm (17.2—29.4). Antheridia almost thread-like with long stalk, several/oogonium, difficult to observe. Oospores 22.6 µm (19.4—26.7). Sickle-shaped appressoria at bottom of Petri dish, in clusters. Moderate growth on leaf of rye in water. No asexual structures.	P13, P15	<i>Pythium</i> sp. Resembling <i>P. aristosporum</i>
Growth 15.4—17.5 mm/day on CMA. Oospores 18.8 µm (15.8—20.7) with a thick wall. Antheridia seldom visible, with a long bent and irregular stalk, 1/oogonium. Club-shaped appressoria at bottom of Petri dish. Good growth on rye leaf in water. No asexual structures.	P3	<i>Pythium</i> sp. Oospore resembling <i>P. ultimum</i>

Table 4. Percentage of cereal seedling roots infected by *Pythium* in the *in vitro* experiment.

Cereal	Percentage of roots infected				
	Pythium isolates				
	P1	P2	P3	P9	Mean
Barley	92	0	25	75	48.0
Oats	75	50	0	25	37.5
Spring wheat	83	75	8	75	60.3
Winter rye	100	67	17	33	54.3
Mean	87.5	48.0	12.5	52.0	

Seedlings showed few shoot and root symptoms in the *in vivo* experiment. The disease severity index of roots (maximum 60) was calculated as the sum of DI of 30 seedlings. Isolate P17 had a somewhat higher root DI than the control and the other *Pythium* isolates, 3.8 as compared to 2.6, 2.8 and 2.5 for the control, P2 and P9, respectively. Barley had on average the most diseased roots, while spring wheat had the healthiest ones (Table 6).

Discussion

The aim of this investigation was to study the occurrence and the importance of *Pythium* spp. in roots of rye seedlings. Because *Pythium* spp. are often relatively infrequently isolated from surface sterilized roots (WALLER 1968), an isolation method using no chemical compounds or antibiotics was chosen. The method yielded 35 genera of soil borne fungi. Most of them were classified as common saprophytic soil fungi, while other genera such as *Fusarium* spp., which was found quite frequently, and *Pythium* spp. often act as pathogens or minor pathogens in cereal roots (SALT 1979).

With the exception of *P. dissimile*, all the *Pythium* species identified, i.e. *P. splendens*, *P. irregulare*, *P. dissimile*, a species resembling *P. aristosporum* and a species resembling *P. ultimum* occur in roots of cereals in various parts of the world (CHAMSWARNG & COOK 1985 DEWAN & SIVASITHAMPARAM 1988, KILPATRICK 1968 SINGLETON & ZIV 1981).

Table 5. The effect of inoculation with strains of *Pythium* isolated from winter rye on shoot and root length, shoot and root symptoms in four cereal crops *in vitro*.

Cereal	Treatment				
	Control	Inoculation with <i>Pythium</i>			
		P1	P2	P3	P9
	<i>Shoot length</i>	<i>Difference from control</i>			
Barley	20.1 ^a	-1.4 ^a	+0.2 ^a	-0.1 ^a	-0.5 ^a
Oats	18.3 ^a	-4.3 ^b	-0.8 ^a	-0.8 ^a	-0.9 ^a
Spring wheat	21.4 ^a	-4.6 ^b	+0.6 ^a	+0.6 ^a	-0.3 ^a
Winter rye	17.8 ^a	-2.7 ^a	+1.7 ^a	+0.4 ^a	-1.3 ^a
Mean	19.4 ^a	-3.3 ^b	+0.4 ^a	+0.2 ^a	-0.7 ^a
	<i>Root length</i>	<i>Difference from control</i>			
Barley	14.7 ^a	-2.2 ^a	-0.7 ^a	0.0 ^a	-0.9 ^a
Oats	9.4 ^{ab}	-1.0 ^b	-0.4 ^{ab}	+1.0 ^a	-0.1 ^{ab}
Spring wheat	15.1 ^a	-1.8 ^a	0.0 ^a	0.0 ^a	-2.9 ^a
Winter rye	10.8 ^a	-0.4 ^a	-0.6 ^a	-0.6 ^a	-1.2 ^a
Mean	12.5 ^a	-1.8 ^b	-0.4 ^a	+0.1 ^a	-1.1 ^{ab}
	<i>Shoot DI</i>	<i>Difference from control</i>			
Barley	0.0	+0.1	0.0	0.0	0.0
Oats	0.0	+0.2	0.0	0.0	0.0
Spring wheat	0.0	+0.4	+0.1	0.0	+0.1
Winter rye	0.3	+0.8	-0.1	+0.2	+0.3
Mean	0.06	+0.31	+0.02	+0.07	+0.10
	<i>Root DI</i>	<i>Difference from control</i>			
Barley	0.1	+0.8	+0.2	+0.2	+1.4
Oats	0.2	+0.4	-0.2	+0.1	-0.1
Spring wheat	0.1	-0.1	+0.1	+0.1	+0.5
Winter rye	0.2	+0.2	-0.1	-0.1	0.0
Mean	0.15	+0.31	-0.04	+0.08	+0.44

* Values in rows marked with the same letter do not differ significantly at $p=0.05$.

Direct microscopy revealed easily oospores of *Pythium* in fine roots of rye seedlings, but a lower percentage of *Pythium* species was obtained on CMA and only a few isolates could be studied in pure culture. This indicates that on CMA the growth of the *Pythium* was disturbed or even overgrown by the saprophytic fungi, and some kind of surface sterilization

of root pieces could have increased the number of isolates obtained in pure culture.

Oospores of *Pythium* were detected in brown as well as in apparently healthy roots, a fact which supports the findings of WALLER (unpublished, ref. SALT 1979). This also suggests that in the roots of rye seedlings *Pythium* may rather be a minor pathogen than a

Table 6. The effect of inoculation with three strains of *Pythium* isolated from winter rye on shoot and root dry weight, seedling length and root disease index (DI) in four cereal crops *in vivo*.

Cereal crop	Treatment			
	Control	Pythium strain		
		P2	P9	P17
	<i>Shoot DM, g</i>	<i>Difference from control</i>		
Barley	0.50 ^b	+ 0.20 ^a	- 0.05 ^b	+ 0.11 ^a
Oats	0.43 ^a	+ 0.06 ^a	- 0.9 ^b	+ 0.08 ^a
Spring wheat	0.49 ^a	0.00 ^a	- 0.11 ^b	0.00 ^a
Winter rye	0.45 ^{ab}	+ 0.07 ^a	- 0.06 ^b	+ 0.01 ^{ab}
Mean	0.47 ^b	+ 0.08 ^a	- 0.08 ^c	+ 0.05 ^a
	<i>Root DM, g</i>	<i>Difference from control</i>		
Barley	0.21 ^a	+ 0.02 ^a	- 0.01 ^a	+ 0.05 ^a
Oats	0.18 ^a	- 0.04 ^a	- 0.01 ^a	+ 0.02 ^a
Spring wheat	0.21 ^{ab}	- 0.06 ^b	- 0.04 ^b	+ 0.09 ^a
Winter rye	0.16 ^{ab}	+ 0.02 ^{ab}	- 0.02 ^b	+ 0.06 ^a
Mean	0.19 ^b	- 0.02 ^b	- 0.02 ^b	+ 0.05 ^a
	<i>Seedling length, cm</i>	<i>Difference from control</i>		
Barley	37.6 ^a	- 0.7 ^a	- 0.5 ^a	- 1.2 ^a
Oats	35.4 ^a	+ 1.0 ^a	- 1.1 ^a	- 1.5 ^a
Spring wheat	43.9 ^a	- 2.7 ^b	- 2.4 ^b	- 2.5 ^b
Winter rye	34.2 ^{ab}	+ 0.9 ^a	+ 1.0 ^{ab}	- 2.2 ^b
Mean	37.8 ^a	- 0.04 ^{bc}	- 1.3 ^{bc}	- 1.9 ^c
		<i>Root DI</i>		
Barley	5	5	5	7
Oats	2	3	2	4
Spring wheat	2	1	0	3
Winter rye	2	2	3	2
Mean	2.6	2.8	2.5	3.8

* Values in rows marked with the same letter do not differ significantly at $p = 0.05$.

real damage causing pathogen. This role of *Pythium* was supported also by the findings in the *in vivo* and *in vitro* pathogenicity experiments, in which the pathogenicity of some *Pythium* isolates varied from pathogenic to

beneficial. This kind of variation in pathogenicity is commonly documented (DEWAN and SIVASITHAMPARAM 1988, KILPATRICK 1968, SINGLETON and ZIV 1981).

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Ms received April 6, 1990

SELOSTUS

Rukiin oraiden juurissa esiintyvät *Pythium*-sienet ja niiden patogeenisuus viljalajeille

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Etelä-Suomen ruispelloilla oli syksyllä 1984 monin paikoin kellastuneiden oraiden muodostamia laikkuja. Kellastuneiden oraiden juurissa havaittiin kahdenlaisia virus-huikkasia. Samanaikaisesti etsittiin rukiin juurista virus-vektoreiksi sopivia sieniä. Niitä ei kuitenkaan löytenyt.

Sen sijaan juurissa oli usein havaittavissa *Pythium*-sienen rakenteita.

Rukiin oraita kerättiin kellastuneista peltokohdista vuosina 1985—1987. Keruupaikkoja oli syksyllä 1985 12, syksyllä 1986 23, syksyllä 1987 9 ja keväällä 1987 19. Osa

näytteiden juurista tutkittiin suoraan stereomikroskopilla ja osa juurista laitettiin maissiagarille. *Pythium*-sienten rakenteita esiintyi sekä terveen- että sairaannäköisissä juurissa. Kuitenkin hyvin runsaita *Pythium* esiintymiä löytyi ainoastaan voimakkaasti tummuneista juurista. Maissiagarilla esitetyt sienet kuuluvat 35 sienisukuun. Näistä suurin osa lienee saprofyyttisiä maasieniä. *Pythium*-sientä eristettiin 27:stä juurenpalasta. Lajit olivat *P. splendens*, *P. irregulare*, *P. dissimile*, *P. aristosporum* ja muistuttava laji sekä *P. ultimum* ja muistuttava laji.

Neljän viljalajin (ruis, kevätvehnä, ohra, kaura) pato-

geenisuustesteissä tulokset olivat vaihtelevia. *In vitro* testissä *P. splendens* oli hyvin patogeeninen kaikille viljalajeille, kun taas *P. ultimum* ja muistuttava laji ei juuri infektoinut. *In vivo* testissä *P. irregulare* hieman lisäsi juurten tautisuusindeksiä.

Tutkimus osoitti, että *Pythium*-sieniä esiintyy melko yleisesti rukiin juurissa. Sienten todellista merkitystä on kuitenkin vaikea päätellä. Todennäköisesti ne kuuluvat siihen suureen maasienten ryhmään, jotka ovat taudinaiheuttajia vain tietyissä sienille edullisissa olosuhteissa, ovat n.s. "minor patogeeneja".