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Pyrenophora teres, an Agent Causing Wheat Leaf Spot

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Abstract—In 2007–2008, the barley net blotch agent *Pyrenophora teres* was found to infect spring wheat in northwestern Russia, causing symptoms similar to wheat tan spot caused by *P. tritici-repentis*. The frequency of occurrence of *P. teres* on spring wheat cultivars was 12–29%. *P. teres* isolates were more virulent to some wheat cultivars than *P. tritici-repentis* ones. *P. teres* was not found on wheat in the south of Russia (Krasnodar krai, Dagestan).

Key words: fungi, *Pyrenophora teres*, *P. tritici-repentis*, wheat.

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Fungi of the genus *Pyrenophora* are causative agents of cereal leaf blotch. The species *P. teres* Drechsler [anamorph: *Drechslera teres* (Sacc.) Shoemaker] causes barley net blotch, while *P. tritici-repentis* (Died.) [anamorph: *D. tritici-repentis* (Died) Shoemaker] causes wheat tan spot. The first epiphytoty of tan spot was observed in Krasnodar krai in the 1980s [1]. The disease is presently widespread in Russia, including in northwestern regions [2]. The fungus *P. teres* is a common barley pathogen found practically everywhere barley is cultured. Both fungal species induce formation of necrotic spots on leaves surrounded by a chlorotic zone and produce exotoxins involved in development of the symptoms of the disease.

In the course of investigation of the structure of the *P. tritici-repentis* population collected in various zones of Russia in relation to virulence, together with this fungus, another one was observed, which had not previously been found on this cereal culture. It differed from *P. tritici-repentis* in conidial morphology and was initially designated as *Pyrenophora* sp. Species identification of these isolates was necessary, as well as comparative investigation of two fungi, including spore morphology, pathogenicity to wheat, and DNA markers.

MATERIALS AND METHODS

Leaves with symptoms of wheat tan spot were collected in 2005–2008 in various geographical points in Russia, Canada, Finland, and Kazakhstan (Table 1). On Russian territory leaf samples were collected in the State Station of Cultivar Testing.

Since the material was intended for isolation of *P. tritici-repentis*, leaves with typical symptoms of tan spot were collected, i.e., those with small light-brown or yellow spots with a dark-brown dot in the center. According to our observations, the probability of isolating a fungal culture from such spots is higher than from large yellow spots.

Leaf fragments (about 3 × 3 mm) including part of the infection spot and green tissue were sterilized with 0.1% $AgNO_3$ solution for 40 s and placed on V-4 agar medium in glass Petri dishes [3]. The plates were illuminated with LE-30 UV lamps and, on the fourth day, were transferred to a refrigerator at about 10°C to induce conidia formation. Individual conidia were then transferred to fresh V-4 medium with a needle in order to obtain single-spore cultures.

For determination of capacity of the *Pyrenophora* isolates for synthesis of Ptr ToxA, Ptr ToxB, and Ptr ToxC exotoxins in order to determine their race, a Canadian differential set was used containing the wheat variety Glenlea and lines 6B365 and 6B662 [4]. For the study of virulence, a set of 18 varieties of winter and spring wheat differing in their resistance to *P. tritici-repentis* was used. The procedure of virulence determination based on the size of necrotic and chlorotic spots formed after inoculation of seedling leaves was described in [3]. The isolates inducing the reaction type above 2/2 (necrosis/chlorosis) were classified as virulent and those with the reaction type below 2/2 as avirulent. In the present work, the isolates of the pathogens were compared according to manifestations of the necrosis reaction.

For PCR analysis, the isolates were used obtained from wheat leaves collected in 2007 in various regions of the Russian Federation (Table 2). DNA was isolated from 7- to 10-day cultures according to Bulat et al. [5]. Five random primers were used for PCR: OPA-08

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Table 1. Wheat leaf samples affected with spots

Sampling site	Wheat	Sampling year
Dagestan, Derbent	Winter and spring	2005–008
Krasnodar krai	Winter	2005–2008
Northwest Batetsky, Novgorod oblast	Winter and spring	2005–2008
Gatchina, Leningrad oblast	Spring	2006–2008
Pskov GSU, Pskov oblast	"	2005–2008
Pavlovsk, Leningrad oblast	Winter	2005–2008
Omsk	Spring	2007
Kazakhstan, near Alma-At	Winter	2006
Finland, Yokioinen, and vicinity of Turku	Spring	2007, 2008
Canada	"	2006

(5'-GTGACGTAGG-3'), OPA-09 (5'-GGG-TAACGCC-3'), OPA-10 (5'-GTGATCGWAG-3'), OPB-11 (5'-GTTTCGCTCC-3'), and OPI-09 (5'-TGGAGAGCAG-3') (Orlon Technologies, United States) and the AS15inv universal primer (5'-CAT-TGCTGGCGAATCGG-3') [6]. PCR with random primers was carried out on a MyCycler™ amplifier (Bio-Rad) under the following conditions: for 4 min at 95°C); primer annealing, 45 s at 37.5°C; and 45 cycles of DNA synthesis, 90s at 72°C (during the last cycle, 10 min at 72°C). PCR with the universal primer (30 cycles) was carried out under the following conditions: denaturation, 50 s at 92°C (denaturation during the first cycle was carried out for 3 min at 94°C); primer annealing, 70 s at 50°C; and DNA synthesis, 60s at 72°C (during the last cycle, 3 min at 72°C).

The reaction mixture (25 μl) contained 10- to 50-ng genomic DNA, ~1× PCR buffer, 0.4 mM dNTP mixture, 10 pmol primers, and 0.5 U *Taq* polymerase. Amplification products were separated by electrophoresis in 1.7% agarose gel at 100 V for 3 h in 1× TBE. The gels were stained with ethidium bromide.

Table 2. Fungal isolates used for PCR analysis

Species	Name of the isolate	Geographic origin
P. triticirepentis	P1, P2, P3, P14	Gatchina, Leningrad oblast
	3D	Dagestan, Derbent
	4, 6, 8	Krasnodar krai
P. teres	Ter181	Leningrad oblast
Pyrenophora sp.	P6, P9, P10, P15, P16, P18	Gatchina, Leningrad oblast

Note: Isolate Ter181 was allocated from barley.

DNA markers of the isolates were represented as a binary matrix, with 1 signifying the presence of a marker and 0 its absence. Only polymorphic amplification products reproducible in two or three repetitions were used for genetic typing. The matrix was used to construct a similarity tree by the unweighted pair group with arithmetic mean method. Genetic relations were determined by the neighbor-joining method. The trees were constructed using the TREECON v.1.3.b software package [7]. The bootstrap method was used for statistical treatment of the nodes.

RESULTS AND DISCUSSION

The appearance of the primary symptoms caused by *P. tritici-repentis* and by *Pyrenophora* sp. was similar (Fig. 1). Morphologically, the fungus corresponded to the species *P. teres* [8–11]. Conidia of the isolates of the tentative *P. teres* were straight, cylindrical, with rounded ends, subhyaline or straw-yellow, and smooth, with one to ten (usually four to six) pseudosepta. Conidia on conidiophores were located singly or in chains. A whorled location of conidia at the tip of the conidiophore and elongation of the terminal conidial cell into a conidiophore with formation of two or three secondary conidia were observed. Unlike *P. tritici-repentis*, stimulation with decreased temperature was not required to obtain conidia of *P. teres*; conidia were formed already while the plates with wheat leaf fragments or fungal cultures were illuminated with UV lamps.

To confirm classification of the isolates as *P. teres*, molecular genetic typing was used with subsequent

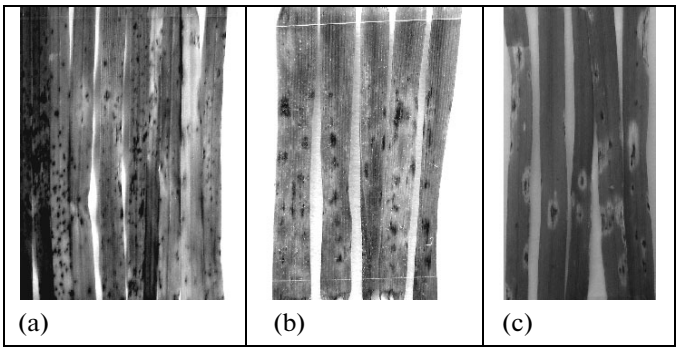

Fig. 1. Wheat leaves infected by *P. teres* isolated from wheat (a), *P. teres* isolated from barley (b), and *P. tritici-repentis* (c).

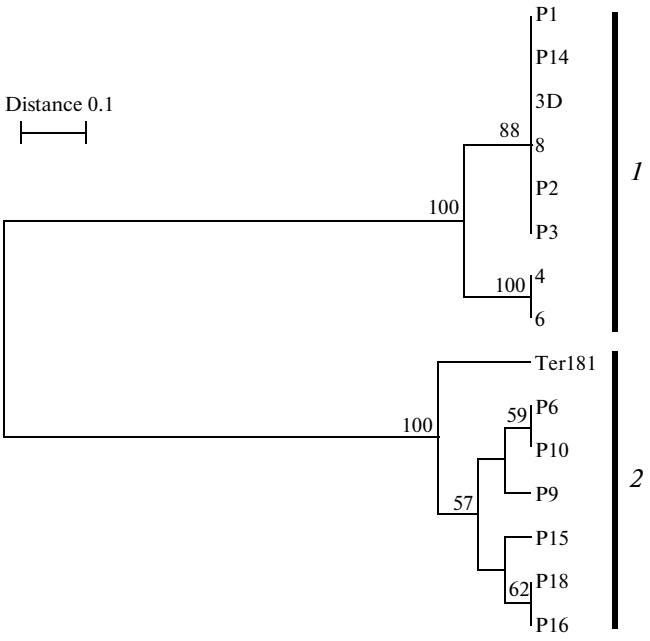

Fig. 2. Genetic relationship of *Pyrenophora* isolates. Numerals at branching points indicate bootstrap values for 100 repeats. Isolates of *P. teres* (*1*) and *P. tritici-repentis* (*2*).

determination of genetic relationship. RAPD genetic typing of the isolates listed in Table 2 produced a binary matrix including 60 characteristics obtained with six primers. This matrix was used to construct a genetic affinity tree (Fig. 2).

The isolates formed two clusters at high bootstrap values. Cluster 1 comprised *P. tritici-repentis* isolates, while cluster 2 contained *Pyrenophora* sp. isolates and *P. teres* Ter181. The genetic difference between the clusters exceeded 80%, which is characteristic of species differences. Thus, the isolates of cluster 2 belonged to the same species, *P. teres*. Intraspecific differences between *P. tritici-repentis* isolates, also at high bootstrap values, probably resulted from their different geographical origin.

Throughout the four years of investigation, only *P. tritici-repentis* isolates were obtained from herbarium samples of wheat leaves collected in Dagestan and Krasnodar krai; no *P. teres* isolates were obtained (Table 3). They were not found also among the isolates obtained from the samples collected from winter wheat in Alma-Ata (2006), from spring wheat in Canada (2006), and from spring wheat in Omsk (2007). In 2007 and 2008, *P. teres* isolates were recovered with high frequency (29 and 12%, respectively) in spring wheat leaf samples collected in northeastern Russia (Table 3). *P. teres* isolates were obtained from spring wheat leaves collected in Finland in 2007.

P. teres constituted 22–100% of *Pyrenophora* isolates obtained from wheat leaves in the northwestern region (Table 4). Almost all isolates were obtained from spring wheat leaves, except for the isolates from the leaves of winter wheat grown in the Gatchina (Leningrad oblast) in 2008.

The virulence of single-spore isolates of *P. tritici-repentis* and *P. teres* to 25 varieties of winter and spring wheat was determined. The average scores for development of necrotic and chlorotic spots on inoculated leaves are presented in Table 5. In 11 tester varieties, development of necrosis was lower after inoculation with *P. teres* than with *P. tritici-repentis*. However, on the varieties Asiago, Komadi 3, Payne, Tara-

Table 3. Frequency of occurrence of *Pyrenophora teres* (Pt) isolates on wheat

Year	Derben		Krasnodar krai		Northwest		Finland		Omsk		Alma-Ata		Canada	
	Total	Pt (%)	Total	Pt (%)	Total	Pt (%)	Total	Pt (%)	Total	Pt (%)	Total	Pt (%)	Total	Pt (%)
2005	26	0	58	0	47	0	–	–	–	–	–	–	–	–
2006	35	0	38	0	36	0	–	–	–	–	25	0	20	0
2007	4	0	43	0	55	29	19	21	10	0	–	–	–	–
2008	32	0	62	0	64	12	19	0	–	–	–	–	–	–

Table 4. Frequency of occurrence of *Pyrenophora teres* (Pt) isolates on wheat in northwestern Russia

Year	Way of life	Batetsky, Novgorod oblast		Gatchina, Leningrad oblast		Pskov Pskov oblast		Pavlovsk Leningrad oblast	
		Total	Pt (%)	Total	Pt (%)	Total	Pt (%)	Total	Pt (%)
2007	Spring	7	100	14	50	9	22	–	–
	Winter	25	0	–	–	–	–	–	–
2008	Spring	16	50	–	–	6	0	–	–
	Winter	22	0	7	28	–	–	26	0

sovskaya 29, 181-5, Glenlea, Katepwa, and 6B365, development of necrosis after inoculation with *P. teres* was equal to or higher than that caused by *P. tritici-repentis*. Varieties Tarasovskaya 29, Glenlea, and Katepwa were more susceptible to both *P. teres* and *P. tritici-repentis*. Unlike *P. tritici-repentis, P. teres* isolates induced almost no formation of chlorotic spots on wheat varieties.

Table 5. Characterization of *Pyrenophora teres* and *P. tritici-repentis* by infection type

Wheat line, variety	Necrosis (average per isolate)		Chlorosis (average per isolate)	
	P. teres	*P. tritici-repentis*	*P. teres*	*P. tritici-repentis*
Allies	2.2	2.6	0.4	2.3
Norin 58	1.3	2.3	0.2	2.2
Riley 67	1.0	2.0	0	1.4
Satsukei 86	1.7	2.5	0.3	1.9
Asiago	1.7	1.7	0.3	0.8
Clark	1.1	1.3	0.5	1.8
Hokkai 252	1.4	2.4	0.5	1.9
Komadi 3	2.7	2.7	0	1.7
Dartajnan	1.5	2.4	0.4	1.6
Payne	2.3	1.6	0	0.4
Tarasovskaya 29	3.3	3.0	0.7	1.9
181-5	1.7	1.4	0.1	0.3
Glenlea	2.9	3.0	1.1	2.6
Katepwa	3.0	3.1	0.9	2.5
6B365	2.1	1.6	1.5	2.0
6B362	2.4	2.7	0.5	1.1
Salamouni	1.8	2.2	0.2	1.2
M3	0.7	1.1	0	0.6
Numbers of isolates	17	38	17	38

According to the race identification key accepted for *P. tritici-repentis* [12], *P. teres* isolates belonged to races 2 and 4 (Table 6). Race 2 produces the Ptr ToxA toxin, which induces formation of necrotic spots on the Glenlea differentiator variety, and does not produce toxins Ptr ToxB and Ptr ToxC, which are responsible for chlorosis on differentiators 6B365 and 6B662. Race 4 does not produce toxins. *P. teres* is known to form a toxin similar or identical to Ptr ToxA [13]. In *P. tritici-repentis*, this toxin is a 13.2-kDa protein and is controlled by one gene in toxin-producing isolates [14–16].

To our knowledge, this is the first Russian report concerning pathogenicity of *P. teres* for wheat. Other reports exist on *P. teres* occurrence on wheat. This species has been isolated from wheat in Canada and other regions [17, 18]. It was recently found in wheat crops in Hungary with a frequency of up to 9% [19].

Our investigation suggests the following conclusions.

(1) *P. teres*, the barley net blotch agent, may be a wheat pathogen causing symptoms similar to those caused by *P. tritici-repentis*. The similarity of the symptoms may be explained by production of the same toxin Ptr ToxA by both *P. teres* and *P. tritici-repentis*.

(2) *P. teres* occurs mainly in northwestern Russia. It is usually associated with spring wheat and almost never occurs on winter wheat. This may result from the combination of the phenological stages of barley and wheat, which promotes transition of the pathogen from one host to another. In the case of winter wheat, which in the northwest ripens earlier than spring wheat, the phases probably do not coincide. *P. teres* was not found in samples of the populations collected

Table 6. Race affiliation of *Pyrenophora teres* and *P. tritici-repentis* isolates from wheat leaves

Year	Species of the pathogen	Number of isolates	Race, %							
			1	2	3	4	5	6	7	8
2007	*P. teres*	14	0	71	0	29	0	0	0	0
	P. tritici-repentis	40	30	53	2	10	0	0	0	5
2008	*P. teres*	9	0	44	0	55	0	0	0	0
	P. tritici-repentis	27	48	37	4	7	0	0	0	4

from North Caucasus winter wheat (Krasnodar krai, Dagestan).

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