

SHORT COMMUNICATION

PITHOMYCES CHARTARUM AS A PATHOGEN OF WHEAT**B. Tóth¹, M. Csósz¹, J. Dijksterhuis², J.C. Frisvad³ and J. Varga^{2,4}**¹ Cereal Research Non-profit Company, P.O. Box 391, H-6701 Szeged, Hungary² CBS Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands³ Center for Microbial Biotechnology, BioCentrum-DTU, Building 221, Technical University of Denmark, DK-2800 Kgs Lyngby, Denmark⁴ Department of Microbiology, Faculty of Sciences, University of Szeged, P.O. Box 533, H-6701 Szeged, Hungary**SUMMARY**

During routine surveys of wheat-growing (*Triticum aestivum* L.) areas of Hungary, symptomatic leaf samples were collected from different wheat cultivars. Macro- and micromorphological examinations of single-spore isolates showed some of them to belong to *Pithomyces chartarum* (teleomorph: *Leptosphaerulina chartarum*). Species assignment was confirmed by sequence analysis of the intergenic transcribed spacer region. *P. chartarum* isolates produced a range of secondary metabolites including gregatin, alternariol and alternariol monomethyl ether, but not sporidesmin, a mycotoxin responsible for photosensitisation and liver damage of grazing animals. Pathogenicity tests proved that *P. chartarum* can cause leaf damage to wheat. Disease symptoms were strikingly different in different wheat cultivars. This is the first report on pathogenicity of *P. chartarum* to wheat in Europe.

Key words: ITS, necrotrophic pathogen, pathogenicity, *Leptosphaerulina*, sporidesmin, wheat.

Leaf diseases of small grain cereals cause major yield losses throughout the world. These diseases may reduce yields directly and by predisposing wheat plants to other stresses, such as drought. Discoloration, spotting and blighting are symptoms commonly observed on wheat leaves. Causal agents are usually fungi, among which *Mycosphaerella graminicola* (anamorph: *Septoria tritici*), *Phaeosphaeria nodorum* (anamorph: *Stagonospora nodorum*), *Pyrenophora*, *Bipolaris* and *Alternaria*. In Hungary, the most important necrotrophic pathogens of wheat are *Pyrenophora tritici-repentis* and *Septoria tritici* (Csósz, 2005), although other fungi, including various *Alternaria* species and *Pyrenophora teres*, have been identified in various diseased wheat cultivars (Tóth *et al.*, 2007).

In 2005-2006, we examined the occurrence of necrotrophic pathogens on wheat, collecting samples of leaves with brownish necrotic lesions, with or without a

chlorotic ring, from different cultivars in several wheat-growing areas of Hungary. Single conidia were isolated from necrotic tissue fragments under a stereo microscope, and transferred to potato dextrose agar (PDA). Macro- and micromorphological examinations of single-spore isolates showed some of them to belong to *Pithomyces chartarum* (teleomorph: *Leptosphaerulina chartarum*; Roux, 1986), which was isolated from three leaf specimens of wheat cv. Bobino (PR 051-13) collected in 2006 at Debrecen.

Fungal colonies, first hyaline then dark grey (Fig. 1A), were fast growing on PDA and malt extract agar plates and produced dark brown multicellular conidia on small peg-like branches of the vegetative hyphae. Conidia were broadly elliptical, pyriform, verrucose, 20-25 µm long and 10-15 µm wide (Fig. 1B) and exhibited, as a distinctive feature, transverse and longitudinal septa. Low-temperature scanning electron microscopy showed the characteristic morphology of the large septated conidia (Fig. 1C) and revealed a complex ornamentation pattern on the cell wall (Fig 1C, inset). Electron microscopy was done with uncoated frozen samples and micrographs consisted of 30 averaged fast scans (SCAN 2 mode) in a JEOL 5600LV scanning electron microscope (JEOL, Tokyo, Japan) equipped with an Oxford CT1500 Cryostation.

To confirm the results of morphological examinations, fungal isolates were grown in liquid potato dextrose broth, and DNA was isolated with the MasterPure™ Yeast DNA Purification Kit (Epicentre Biotechnologies, Madison, WI, USA) according to the manufacturer's instructions. The intergenic transcribed spacer regions and the 5.8S rRNA gene (ITS region) were amplified using primers ITS1 and ITS4 (White *et al.*, 1990), and the amplified fragments were sequenced from both strands using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit. Sequences of the ITS region of the isolates were compared with those of *Leptosphaerulina* isolates from the Genbank database. Sequence alignments were made using CLUSTAL-X (Thompson *et al.*, 1997) and adjusted manually. Evolutionary distances between the sequences were calculated by Kimura's formula (Kimura, 1980) using the DNADIST of the PHYLIP program package (Felsen-

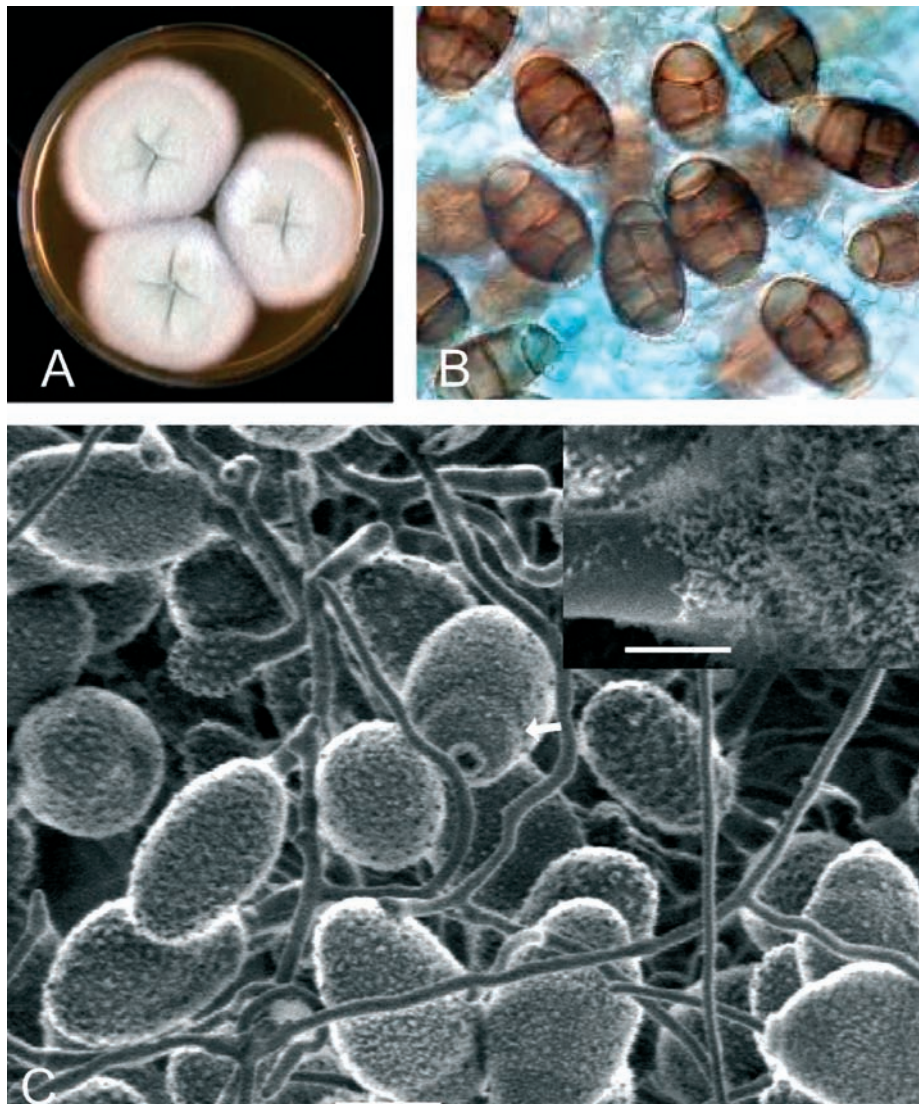


Fig. 1. A. *Pithomyces chartarum* colony on malt extract agar plate; B. Conidia under the light microscope; C. Low-temperature SEM micrograph showing conidia in different developmental stage and the aerial mycelium. The inset shows a detail of the surface ornamentation of conidia which contrasts with the smooth cell wall of the conidiferous pegs. Scale bar 2 μ m.

stein, 1995). Phylogenetic trees were constructed by the neighbour-joining method using the NEIGHBOR program of the PHYLIP package. Bootstrap values were calculated from 1000 replications of the bootstrap procedure using programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE (Felsenstein, 1995).

ITS sequences of the Hungarian isolates were identical to the comparable sequences of *L. chartarum*, clustering together in the phylogenetic tree (Fig. 2). The ITS sequence of isolate No. 79a was submitted to GenBank under the accession number EF489400.

Leptosphaerulina chartarum (teleomorphic state of *P. chartarum*) belongs to the family *Pleosporaceae*, order *Pleosporales* (Kodsueb *et al.*, 2006), and is cosmopolitan in distribution. This species can grow on dead grass material close to the soil (Sutton and Gibson, 1977). The fungal database lists 138 plant species from which *P. char-*

tarum was isolated (Farr *et al.*, 2007), including *Triticum aestivum* in Brazil and Tanzania, and *T. vulgare* in Africa (Ellis, 1960). This fungus, first observed in Europe in the sixties (Lacey and Gregory, 1962), has recently been recovered in Hungary from seeds of yellow oat-grass (*Trisetum flavescens*) and crested dog's-tail [*Cynosorus cristatus*; Varga *et al.* (2006)] and from leaves and seeds of smooth brome [*Bromus inermis*; Varga and Fischl (2006)], but has never been observed on wheat in Europe.

Several isolates of *P. chartarum* produce the mycotoxin sporidesmin, which is responsible for photosensitisation and facial eczema of sheep and other grazing animals (Thornton and Sinclair, 1960). The percentage of fungal strains producing this toxin varies in different countries. Toxigenic strains predominate in New Zealand and Australia, whereas non-toxic strains prevail in North and South America (Collin *et al.*, 1998). The first report of fa-

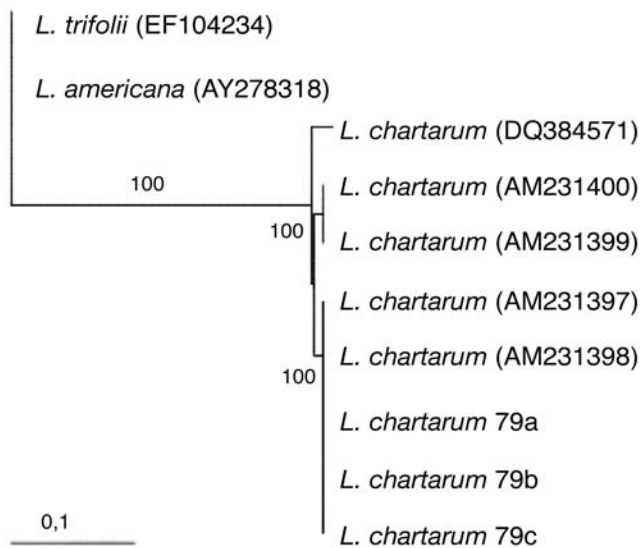


Fig. 2. Dendrogram constructed with ITS sequence data of *Leptosphaerulina/Pithomyces* species. Numbers above and on the left of the branches are bootstrap values.

cial eczema in Europe dates from 1984 (Bezille *et al.*, 1984) and since then only a few cases were reported from France, Portugal and The Netherlands (van Wuijckhuise *et al.*, 2006). Extrolite profiles of *P. chartarum* isolates were analysed by HPLC using alkylphenone retention indices and diode array UV-VIS detection as described by Frisvad and Thrane (1987), with modifications as described by Smedsgaard (1997). All isolates produced gregatin, alternariol and alternariol monomethyl ether, but not sporidesmin (not shown), contrary to *P. chartarum* isolates from The Netherlands, which synthesize also sporidesmin (Houbraken *et al.*, 2006).

Although several *Pithomyces* species are well-known plant pathogens including *P. sacchari*, *P. graminicola* and *P. maydicus* (Ellis, 1960), *P. chartarum* is primarily a saprophytic species, although it causes leaf spotting on various grasses including *Bromus inermis* (Eken *et al.*,

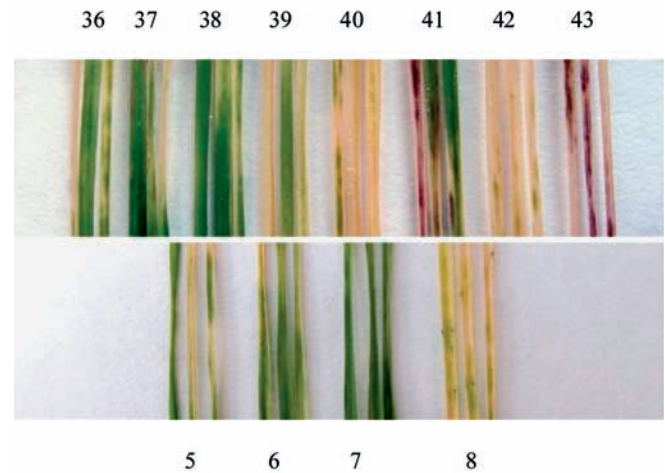


Fig. 3. Symptoms caused by *Pithomyces chartarum* on the leaves of different wheat cultivars and lines. Numbers correspond to cultivars/line numbers in Table 1.

2006), and glume blotch of rice and sorghum (Haware and Sharma, 1973; Ravise, 1957).

To determine the pathogenicity of *P. chartarum* to wheat, tests were conducted under greenhouse conditions inoculating the leaves of various wheat cultivars at the two leaf stage with conidial suspensions of 3×10^3 conidia ml^{-1} of the fungus. Twelve days after inoculation, necrotic lesions similar to those observed on naturally infected plants were produced (Fig. 3). Sporulation was observed after 12-15 days on infected leaves, from which the fungus was successfully reisolated. Disease symptoms were strikingly different in different wheat cultivars, suggesting that different levels of resistance exist in wheat against *P. chartarum* (Table 1). To our knowledge this is the first report on the occurrence and pathogenicity of *P. chartarum* to wheat in Europe.

Table 1. Reaction of different wheat cultivars and lines to infection by *Pithomyces chartarum* in greenhouse experiments.

No.	Wheat cultivar/line	Symptoms	Leaf spot (%)
36	9/05	Slight yellowing	29.8
37	18/05	Slight yellowing	26.0
38	28/05	Slight yellowing	25.7
39	22/05	Strong yellowing	21.0
40	11/05	Strong yellowing	23.2
41	GK Ati	Yellowing and discoloration	25.3
42	GK Csongrad	Strong yellowing	22.8
43	Jubilejnaja 50	Discoloration	28.4
5	Wattines	Slight yellowing	NT
6	Salamouni	Slight yellowing	NT
7	M3	Minute discoloration	NT
8	6B 662	Strong yellowing	NT

NT, not tested

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