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First Report of the Natural Occurrence of the Teleomorph of *Leptosphaeria maculans* on Oilseed Rape and Airborne Dispersal of Ascospores in Hungary

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Abstract

Leptosphaeria maculans (anamorph *Phoma lingam*) causes blackleg disease of oilseed rape. The teleomorph stage of the blackleg pathogen on oilseed rape in Hungary is reported for the first time. Airborne ascospores of the pathogen were monitored in the infected source area using a portable Hirst-type spore trap in different horizontal and vertical sampling points. Meteorological conditions influencing airborne spore dispersal of the fungus were analysed. Airborne ascospores of pathotypes A/Tox⁺ and B/Tox⁰ groups of *L. maculans* were identified using a modified germination test. A mixed population of *L. maculans* A/Tox⁺ and B/Tox⁰ groups were found in Hungary. The occurrence of the fungus on a wide range of its wild cruciferous hosts appears to be an important factor in the outbreak of the disease on oilseed rape. High relative humidity, rainfall, melting snow and moderate wind were the most important factors in the dissemination of the ascospores.

Introduction

Leptosphaeria maculans (Desm.) Ces. & de Not. [anamorph *Phoma lingam* (Tode:Fr.) Desm.] causes phoma stem canker (blackleg) on oilseed rape (*Brassica napus* ssp. *Oleifera*) in many areas of the world (Guo et al., 2005). The disease is of major economic importance in the main oilseed rape-growing countries. The pathogenic population has been shown to contain at least two different species, A or Tox⁺ (Howlett, 2004) and B or Tox⁰ (Shoemaker and Brun, 2001). The group A/Tox⁺ can be divided into pathogenicity groups (PG) 2, 3 and 4 and group B/Tox⁰ into PG1 (Kuusk et al., 2002). Renewed interest of the epidemiology of the pathogen, which took account of the species complex concept, has clearly identified the aggressive canker forming A/Tox⁺ group as the main causal agent of

the stem canker symptoms responsible for the yield losses on oilseed rape crops.

At first, the teleomorph of the fungus was collected by Hollós in 1913 from Hungary (near Kecskemét) (Hollós, 1913). *Phoma lingam* (Tode) Desm., anamorph state of *L. maculans* has been found recently on seeds of oilseed rape in Hungary (Jakabné et al., 1997). Although the teleomorph stage of the fungus was known in the Carpathian Basin almost 100 years ago, it was not reported from oilseed rape.

The objective of this study was to: (i) report the first occurrence of the teleomorph stage of the blackleg fungus on oilseed rape in Hungary and (ii) follow up the early steps of the ascospore dispersal using aerobiological methods in the source area of its first observation.

Materials and Methods

Fungal collection and identification

Crop debris of oilseed rape was collected into plastic bags from the investigated area (Ikervár, west Hungary). No tillage applications was performed in the 30 ha of the infected field, thus it was covered with several plant residues and volunteer plants before 11 November 2003, and thereafter minimum tillage was applied to the field. The samples were examined for pseudothecia. Mean length and width of spores, asci and ostiola were measured under Olympus BX51 phase contrast microscope (Olympus Optical Co., Ltd., London, UK) with 800× magnification. The fungus was identified according to Müller (1950) and Shoemaker and Brun (2001). Frequency of infection (infection on 100 leaves) and degree of infection (percentage infected leaf surface) were determined in a neighbouring oilseed rape field investigating 3 × 50 leaves (Aponyiné, 1997) in various distances (10–500 m) from the source area (18 November 2003).

Air sampling

A portable VPPS 1000 Hirst-type air sampler (Hirst, 1952; Lanzoni Co. Ltd, Bologna, Italy) was used to register concentration of airborne ascospores. The spore trap was placed in various vertical (10 and 100 cm above ground level) and horizontal (0–1000 m) distances from the infected area. A total of 62 samples were taken between 22 October 2003 and 22 December 2003, and between 27 April 2004 and 9 November 2004. Airborne spores were captured in the afternoon (13:00–15:00 hours), in the downwind direction; time of sampling was limited to 30 min. An Olympus BX51 microscope was used to scan the total area of the silicone-coated microscopic slide to determine spore concentration in the air (ascospore/m³). The airborne spores were identified using herbarium specimen previously collected from the infected area. Meteorological factors were measured [wind speed (m/s), wind direction, temperature (°C), precipitation (binary data) and relative humidity (%)]. Spearman's correlation analysis was applied to clarify the connection between spore number and weather variables.

Germination test

Identification of the pathotypes (A/Tox⁺ and B/Tox⁰ groups) of *L. maculans* in air samples was performed by employing the germination test method described by Huang et al. (2001) with some modification (Fig. 1). Air samples were scanned under a 200× object lens for the ascospores of *L. maculans* adhered to the silicone coating of the microscopic slide in the spore trap. A 3.5-mm-wide block of 2% distilled water agar was cut and mounted over the total area of the air sample. Ascospores of *L. maculans* were incubated at 15°C for 48 h. A few drops of lactophenol with cotton blue dye were added to the sample, allowing them to infiltrate the agar block. A cover slip was placed on top of the agar block, and pressed gently to flatten the agar to facilitate the microscopic study with 400× object lens. The outflow of agar margins were also recovered and scanned for germinating spores. For the identification of the pathotypes A/Tox⁺ and B/Tox⁰ group of *L. maculans* in stem samples, we employed the germination test method described by Huang et al. (2001) without any modifications.

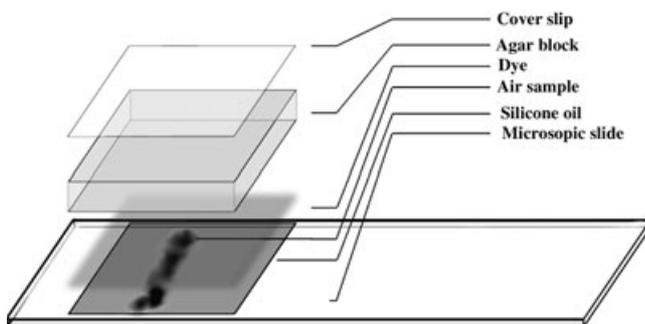


Fig. 1 A method to identify the pathotypes of *Leptosphaeria maculans* from air samples (modified from the method described by Huang et al., 2001)

Results

Pseudothecia of *L. maculans* were first observed on stem residues on 15 October 2003 and at the end of September 2004. Ascospores were long fusoid, smooth, brownish, straight to slightly curved, 76.25–47.5 × 67.75–6.25 μm, transversely five septate, with guttule(s) per cell, with large (6–7 μm) globoid appendages (Fig. 2). Bitunicate asci were eight-spored, 150–162.5 × 13.75–15 μm, ostiole 130 μm long and 130 μm wide.

Aerobiological monitoring was initiated on 22 October 2003, and the first ascospores were detected in the air samples on 30 October in high concentration (2400 spore/m³). The spore concentration correlated significantly with environmental data (Table 1). Ascospore concentration peaks were observed when relative humidity was high (98%). When the saturation of air was low (<79%), or temperatures were low (<3°C) or high (>17°C) the traps were ascospore-free. Spores were captured from the air after wetting by free water from melting snow and after rain showers. High relative humidity without rainfall appeared to be sufficient to develop high spore numbers. The influence of weather variables on the concentration of airborne ascospores was mainly rainfall, temperature and relative humidity. The number of spores was not affected by wind speed and by minimum tillage.

The leaf spots appeared extensively on oilseed rape plants near the infected area on 11 November 2003, 12 days after the first observation of ascospores in the trap. The average spore concentration decreased in the air exponentially according to the horizontal distance at the different sampling points. Similar trend was documented for the degree of infection caused by the primary infection by the ascospores. The frequency of symptoms decreased exponentially with horizontal

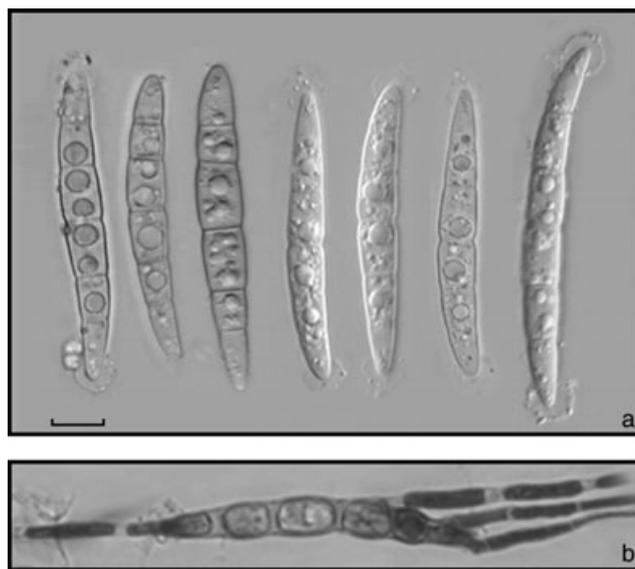


Fig. 2 Ascospores of *Leptosphaeria maculans* from stem residues of Hungarian oilseed rape. (a) Germinating ascospore from air samples and (b) bar = 10 μm

Table 1
Spearman's correlation coefficients (r_s) between environmental and meteorological factors on the spore concentration of *Leptosphaeria maculans*

Parameters	r_s
Distance	-0.239 ^{NS}
Tillage	0.126 ^{NS}
Rainfall	0.280*
Relative humidity	0.323*
Temperature	0.220 ^{NS}
Temperature < 3°C	-0.342**
Temperature 4–15°C	0.298*
Temperature > 16°C	-0.075 ^{NS}
Wind speed	0.118 ^{NS}
Wind direction (N)	0.204 ^{NS}
Wind direction (S)	-0.115 ^{NS}
Wind direction (E)	-0.126 ^{NS}
Wind direction (W)	-0.039 ^{NS}
Wind direction (NE)	0.001 ^{NS}
Wind direction (NW)	-0.067 ^{NS}
Wind direction (SE)	0.353**
Wind direction (SW)	0.056 ^{NS}

* r_s is significant at the 0.05 level (two-tailed).

** r_s is significant at the 0.01 level (two-tailed).

NS, r_s is not significant.

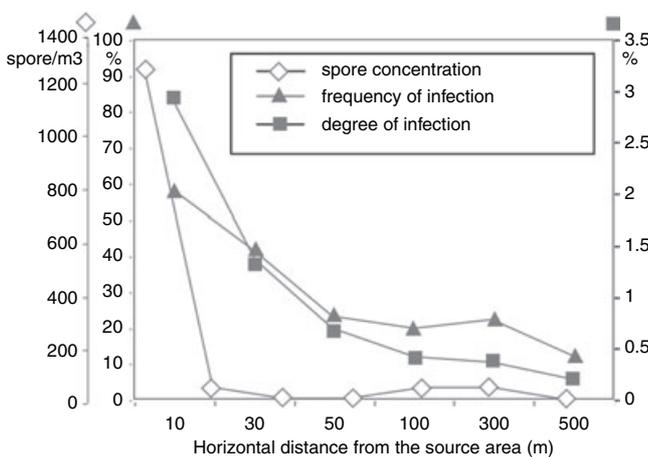


Fig. 3 Ascospore concentration of *Leptosphaeria maculans*, frequency of infection and degree of infection of oilseed rape plants in horizontal distances from the inoculum source

distance as well (Fig. 3). Close to the source area (within 10 m) the degree of infection was high (30%); however, 500 m from source, it was only 2%. Near to the source (within 10 m horizontal distance), spore concentration was higher at 10 cm, than at 100 cm vertical height (Fig. 4). Further from the source (over 10 m horizontal distance), airborne spore numbers reached relatively high counts at 100 cm vertical height, but were relatively low near the ground level (at 10 cm vertical height).

Eighty-four percentage of airborne ascospores of *L. maculans* germinated at 15°C after 48 h of incubation. Germ tubes originated from interstitial cells or terminal cells of ascospores. The average number of germ tubes produced from ascospores was 5. Maximum number of germ tubes per spore was 9. Germ

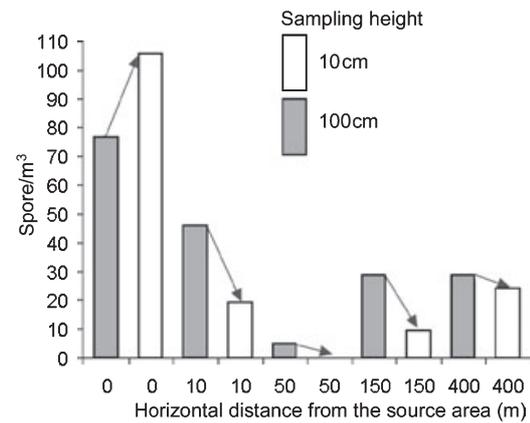


Fig. 4 Ascospore concentration of *Leptosphaeria maculans* in two different heights from ground level (10 and 100 cm). Arrows indicate the decrease of spore numbers between the companion air samples of the same time and point

tubes originating from the central cells of ascospores were 35% longer in the broadest part on the first germ tube cell, than on the other germ tubes. Hyphae from A/Tox⁺ group ascospores grew tortuously with extensive branching, whilst those from B/Tox⁰ group ascospores were predominantly long and straight with little branching.

Both A/Tox⁺ and B/Tox⁰ groups of *L. maculans* were found on the plants and in the air samples; however, the spore number of the B/Tox⁰ group reached a higher concentration. On plants, however, B/Tox⁰ group was less frequent according to the symptoms and the results of the germination test.

Discussion

Our findings showed that a mixed population of *L. maculans* A/Tox⁺ and B/Tox⁰ groups are present in Hungary. The teleomorphic stage of the blackleg pathogen on oilseed rape is reported for the first time in Hungary. Blackleg disease of oilseed rape plants caused by the fungus *L. maculans* is regarded to be common on its wild and cultivated plant hosts (Hollós, 1913: *Alliaria petiolata*, *Syrenia cana*, *Turritis glabra*; Urbizsy, 1941: *Erysimum effusum*; Jakabné et al., 1997: *Brassica geminifera*, *Sinapis arvensis*; Gönczöl and Révay, 2001: *Euphorbia* sp.; Magyar and Tóth, 2003: *E. odoratum*). It is our opinion that the blackleg inoculum attacking oilseed rape in areas of Hungary should originate from the large background of alternative cruciferous hosts. However, further studies are needed to identify the pathotypes of *L. maculans* isolated from alternative hosts.

As our results show, minimum tillage (which is the preferred tillage method in recent times) cannot significantly reduce the number of airborne spores. A study carried out in Canada showed the importance of rotation and tillage (conventional) practices in reducing blackleg inoculum (Guo et al., 2005). The risk of an outbreak of an epidemic should dramatically increase when crop residues remain on the soil surface. The data presented by Rouxel et al. (2003) demonstrated

the high evolutionary potential of *L. maculans* populations to adapt novel resistance genes. The sexual stage on crop residues is of primary importance for the blackleg pathogen, for recombination and increasing virulence characteristics and as the primary source of inoculum. The development of minimum tillage practices has direct consequences on the evolution of populations of the blackleg pathogen.

According to West et al. (2001), the greatest risk of infection is within 500 m of the source. In our experiment, only a small number of ascospores (0.2–0.4% of the total ascospore concentration) travelled a considerable long (> 500 m) distance. In a study carried out in western Canada, where canola (oilseed rape) is the major oilseed crop, maximum infection through ascospores was restricted to < 50 m (Guo and Fernando, 2005). Our findings are most closely related to the study by Guo and Fernando (2005).

In Canada, spore discharge was mainly during June and July, between 13 and 18°C (Guo and Fernando, 2005). In England and Poland ascospores were first released in late-September and late-October, after rain and when temperature had decreased to below 15°C. The majority of spores were detected in the period from late-October to late-December (West et al., 2002). In northern Germany ascospore discharge starts in September or October, and reaches maximum 1 or 2 months later (Thürwächter et al., 1999). In Hungary, spore season of *L. maculans* starts at late-October, with very high concentration of spores (when temperature had decreased to below 17°C). In Poland, phoma leaf spots on oilseed rape plants appeared 1 month after spores were first detected. However, in Hungary leaf spots appeared 12 days after registration of first airborne spores. The temperature seems to be a critical factor in spore discharge and infection.

The phoma stem canker epidemics are generally initiated by airborne ascospores released from pseudothecia after wetting by rain (Salam et al., 2003; Guo and Fernando, 2005; Huang et al., 2005), or dew (McGee, 1977). In Hungary, ascospore release may be increased by melting snow as well. Several methods have been proposed, or used, for forecasting the risk of development of epidemics of stem canker (West et al., 2001; Calderon et al., 2002; Aubertot et al., 2004). Air sampling combined with germination test has the potential to detect and quantify airborne inoculum of pathotypes A/Tox⁺ and B/Tox⁰ groups of the blackleg pathogen.

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