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OCCURRENCE OF *LEPTOSPHAERIA MACULANS* AND *L. BIGLOBOSA* IN OILSEED RAPE LEAVES WITH DIFFERENT SYMPTOMS OF STEM CANKER

Z. Karolewski, D. Walczak, T. Kosiada and D. Lewandowska

Abstract

In the field experiment in 2004/05 and 2005/06 with winter oilseed rape cultivars 'Capitol', 'Darmor', 'Eurol' and 'Falcon' the assessment of symptoms occurring on leaves caused by *Leptosphaeria* spp. was done. It was difficult to diagnose the species of *L. maculans* and *L. biglobosa* basing on symptoms occurring on oilseed rape leaves. Despite the division of spots into seven types, none of the types was caused by one pathogen species only. Different symptom types of stem canker on leaves were caused by each pathogen – *L. maculans* or *L. biglobosa,* however, in various proportions. From leaves with type 1 ("typical" of *L. maculans*) 26% of isolates belonged to *L. biglobosa*, whilst from those with type 3 ("typical" of *L. biglobosa*) 37% of isolates belonged to *L. maculans*.

Key words: *Phoma* leaf spot, stem canker, blackleg, disease assessment, resistance, oilseed rape

Introduction

Stem canker (= *Phoma* leaf spot, blackleg) is an important disease of oilseed rape (*Brassica napus*) and some other *Brassica* species in Europe, North America and Australia (West et al. 2001, Fitt et al. 2006, Sprague et al. 2006). Yield losses caused by the disease in oilseed rape crops reached in the UK 56 mln in 2002, 30 mln in Australia in 1999 and 36.8 to 147 mln in France (Fitt et al. 2006, Khangura and Barbetti 2001). Before 2001 one species – *Leptosphaeria maculans* was known to cause the disease, however, divided into two groups named 'A' (aggressive, highly virulent, Tox^+) and 'B' (non-aggressive, weakly virulent, Tox^0) (Williams and Fitt 1999). The differences in pigment production, growth rate,

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pathogenicity, toxin production, molecular patterns and inability of crossing between the two groups decided about dividing the pathogen into two different species – *L. maculans* for A-group and *L. biglobosa* for B-group (Shoemaker and Brun 2001). The co-existence of both species was found in France, Germany, Poland, Czech Republic and Hungary, however, the spread of *L. maculans* from Western to Eastern Europe, where *L. biglobosa* was predominant in the past, has been observed (Fitt et al. 2006, Jędryczka et al. 1994, Karolewski 1999, Karolewski et al. 2002, Szlávik et al. 2003, West et al. 2001).

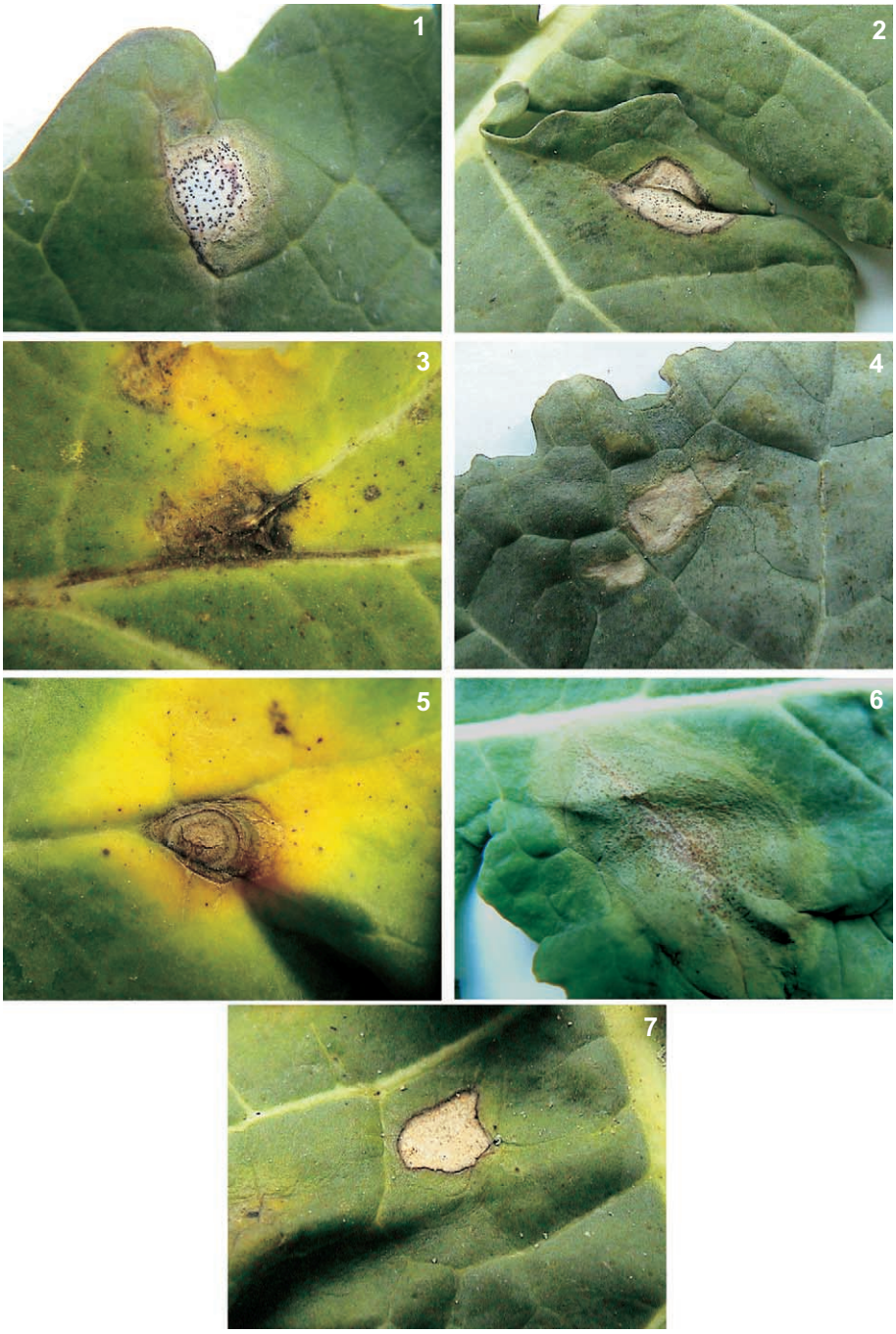
There is information about differences between symptoms caused by *L. maculans* and *L. biglobosa* on oilseed rape plants, however, there is unanimity between authors. Brun et al. (1997) distinguished two groups of lesions on oilseed rape leaves: typical – ash-grey with many pycnidia, and atypical – which were often darker than typical, without or with fewer pycnidia, with or without chlorosis around lesions. Toscano-Underwood et al. (2001) described symptoms on leaves caused by infection with ascospores of *L. maculans* as pale grey/green lesions with many pycnidia, whilst those caused by ascospores of *L. biglobosa* as lesions with a dark margin and light brown centre, with few pycnidia. Three types of lesions suggested Jędryczka (2006). Typical spots were beige with light green margin and with many pycnidia. Mediate spots were light green or beige with dark margin, smaller than typical ones and with fewer pycnidia. Atypical spots were small, dark, sometimes light green but without pycnidia.

Moreover, little is known about resistance of oilseed rape cultivars to *L. biglobosa*, whilst both types of resistance to *L. maculans* – polygenic and race-specific ones – were found (Rouxel et al. 2003, Delourme et al. 2004). Despite the occurrence of two species of fungi causing stem canker in Poland, on the list of winter oilseed rape cultivars there is only information concerning susceptibility of cultivars to the disease, not to either of the two different pathogens (Borys 2006). It might be caused by difficulties in distinction between both pathogens during field assessments based on the disease symptoms.

The aim of the work was to investigate, whether it is possible to distinguish *L. maculans* and *L. biglobosa* on the basis of symptoms appearing on winter oilseed rape leaves.

Materials and methods

Field experiments were done in the 2004/05 and 2005/06 growing seasons in The August Cieszkowski Agricultural University experimental farm in Złotniki. Seeds of four cultivars were sown on 27 August 2004 or 30 August 2005 (80 seeds per 1 m^2) in plots (7.5 × 3.5 m) arranged in four replicated blocks. Three cultivars possessed resistance genes to *L. maculans*: 'Capitol' – *Rlm 1*, 'Eurol' – *Rlm 2*, 'Falcon' – *Rlm 2* and *Rlm 4*, whilst cv. 'Darmor' did not possess any resistance gene (Rouxel et al. 2003). Stem debris with visible symptoms of *Leptosphaeria* spp. infestation, were collected as a source of inoculum from winter oilseed rape field cv. 'Marita'



Phot. 1. Types (1–7) of symptoms on winter oilseed leaves infected by *Leptosphaeria* spp. and *Alternaria* spp. (description – in Table 1) (photo by Z. Karolewski)

located in Złotniki at harvest of the previous vegetation seasons. After sowing ten 20-cm-long stem pieces of infected debris were spread on every plot. Plants were not sprayed with fungicides during vegetation.

Leaves with symptoms of stem canker were collected four times (ca 20 leaves of each cultivar). Assessments were done at growth stages BBCH 39, 55, 65, 75 (Mrówczyński and Pruszyński 2006). Symptoms appearing on leaves were divided into seven types (Phot. 1). Fungi were isolated from leaf pieces (ø 1 cm) with lesions disinfected for 1 min in 1% NaClO on PDA medium. The pathogen diagnostics was done using morphological features: shape and size of conidia and the presence of brown pigment in the medium after 14 days of maintaining the colonies at 20°C (Karolewski 1999, Williams and Fitt 1999).

Results

All seven types of symptoms (Table 1) were found on all four cultivars, however, the frequency of the particular type occurrence varied (Tables 2, 3). In both years spots of type 1 were found most often (177), whilst spots of type 7 were found the rarest (34). The total percentage of successful isolations made from leaves with symptoms was 66 in 2005 and 55 in 2006. The lowest percentage of successful isolations was found for type 7 – 42 (mean for two years), in comparison to isolations made from other types, where it varied between 57 and 64.

The percentage of isolates obtained from winter oilseed rape leaves differed and depended on types of symptoms and date of sampling (Fig. 1). 100% of *L. maculans* isolates were only found when isolations were made from leaves with type 6 at BBCH 39 and from leaves with type 7 at BBCH 65. 100% of *L. biglobosa* isolates were only found when isolations were made from leaves with type 4 at BBCH 75. *Alternaria* spp. isolates were obtained only from leaves with type 5 of symptoms at all sampling dates. Percentage of isolates belonging to the genus *Alternaria* varied between 45 and 63, depending on date of sampling. The highest mean percentage

Table 1

Symptoms of stem canker on winter oilseed rape leaves

Type of symptoms	Description
1	Light spot without a brown margin, black pycnidia visible
2	Light spot surrounded by a brown margin, black pycnidia visible
3	Dark (brown) spot surrounded by chlorosis, black pycnidia present but usually fewer than in type 1 and 2
4	Light spot without a brown margin, pycnidia not present
5	Dark brown spot, with concentric rings, pycnidia not present
6	Green spot without a brown margin, black pycnidia visible
7	Light spot surrounded by a brown margin, pycnidia not present

Table 2

Number of isolates of *Leptosphaeria maculans*, *L. biglobosa* and *Alternaria* spp. obtained from winter oilseed rape leaves (Złotniki 2005) (mean of four assessments done at BBCH 39, 55, 65, and 75)

Type of symptoms	Number of isolations		'Capitol'			'Darmor'			'Eurol'			'Falcon'		
	total	positive	Lm	Lb	Alt	Lm	Lb	Alt	Lm	Lb	Alt	Lm	Lb	Alt
1	93	56	11	3	0	15	4	0	12	2	0	8	1	0
2	98	66	10	9	0	11	4	0	11	3	0	14	4	0
3	71	50	2	12	0	6	6	0	3	3	0	5	13	0
4	36	21	3	3	0	6	3	0	3	3	0	0	0	0
5	45	32	1	2	6	4	1	6	1	1	4	2	0	4
6	44	28	0	0	0	10	3	0	1	0	0	12	2	0
7	19	7	0	0	0	4	0	0	3	0	0	0	0	0

Lm – *L. maculans*, Lb – *L. biglobosa*, Alt – *Alternaria* spp.

Table 3

Number of isolates of *Leptosphaeria maculans*, *L. biglobosa* and *Alternaria* spp. obtained from winter oilseed rape leaves (Złotniki 2006) (mean of four assessments done at BBCH 39, 55, 65, and 75)

Type of symptoms	Number of isolations		'Capitol'			'Darmor'			'Eurol'			'Falcon'		
	total	positive	Lm	Lb	Alt	Lm	Lb	Alt	Lm	Lb	Alt	Lm	Lb	Alt
1	84	52	7	5	0	8	5	0	10	6	0	9	2	0
2	76	40	5	8	0	2	0	0	5	5	0	8	7	0
3	70	40	1	8	0	5	4	0	5	6	0	5	6	0
4	25	15	0	4	0	0	4	0	0	2	0	0	5	0
5	43	22	1	1	2	1	1	3	1	1	4	2	4	1
6	40	20	5	2	0	4	3	0	2	1	0	2	1	0
7	15	8	1	3	0	0	0	0	2	1	0	1	0	0

Lm – *L. maculans*, Lb – *L. biglobosa*, Alt – *Alternaria* spp.

of *L. maculans* isolates was 75, when isolations were made from leaves with type 7 of symptoms, 64 from leaves with type 1 and 61 when isolations were made from leaves with type 2. The highest mean percentage of *L. biglobosa* isolates was 64, when isolations were made from leaves with type 4 of symptoms, and 63 when isolations were made from leaves with type 2.

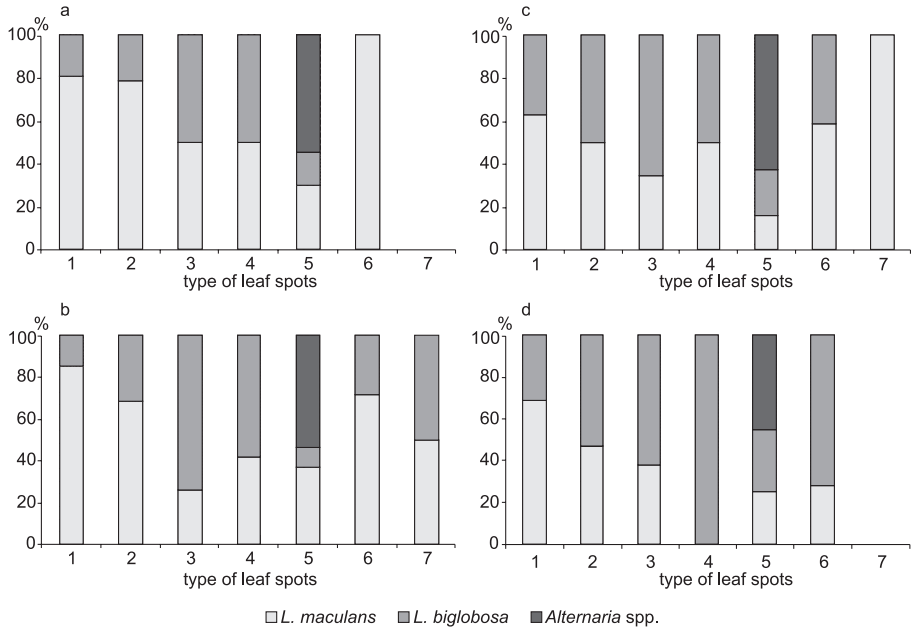


Fig. 1. Percentage of *Leptosphaeria maculans*, *L. biglobosa* and *Alternaria* spp. isolates obtained from winter oilseed rape leaves at BBCH 39 (a), BBCH 55 (b), BBCH 65 (c), BBCH 75 (d); mean for four cultivars: 'Capitol', 'Darmor', 'Eurol', 'Falcon' and two seasons: 2004/05 and 2005/06

Discussion

Results obtained in this research showed that it is difficult to diagnose the species of *L. maculans* and *L. biglobosa* – on the basis of symptoms occurring on oilseed rape leaves. Despite the division of spots into seven types, none of the types was a source of one pathogen species only. From leaves with symptoms of type 1 (light spots without a brown margin, black pycnidia visible), which were similar to those described by Toscano-Underwood et al. (2001) as typical of *L. maculans*, 26% of *L. biglobosa* isolates were obtained. In comparison, from leaves with symptoms called "atypical" (Brun et al. 1997), similar to type 3 (brown spots surrounded by chlorosis with few pycnidia), 37% of *L. maculans* isolates were obtained. The share of *L. biglobosa* isolates from spots of symptom types 1, 2, 3, 4, 5 and 6 increased as plant vegetation advanced. This confirms previous results: in Poland *L. biglobosa* is predominant on winter oilseed rape at late stages of plant development (Karolewski et al. 2002). Jędryczka (2006) suggested that maturation of *L. biglobosa* pseudothecia in autumn may be inhibited under Polish conditions, so major infection at that time is caused by *L. maculans*. The temperature of 10°C is less favourable for *L. biglobosa* than for *L. maculans* (Toscano-Underwood et al. 2003).

It seems that different symptom types of stem canker on leaves might be caused by each pathogen – *L. maculans* or *L. biglobosa*, despite the fact that some tendencies

were observed. Usually from leaves with type 1 of spot ("typical") *L. maculans* was isolated, but just in 74%, whilst from type 3 ("atypical") *L. biglobosa* was isolated just in 63%. These results did not confirm other authors' suggestions that it was possible to distinguish *Leptosphaeria* species by the assessment of leaf symptoms. It causes severe problems in breeding of resistant oilseed rape cultivars to both pathogens and in monitoring resistance durability in the field. The *in situ* diagnostics of *L. maculans* and *L. biglobosa* should be supplemented with other laboratory methods available.

Streszczenie

WYSTĘPOWANIE *LEPTOSPHAERIA MACULANS* AND *L. BIGLOBOSA* NA LIŚCIACH RZEPAKU OZIMEGO Z RÓŻNYMI OBJAWAMI SUCHEJ ZGNILIZNY KAPUSTNYCH

W doświadczeniu polowym wykonanym w latach 2004/05 i 2005/06 z czterema odmianami rzepaku ozimego: 'Capitol', 'Darmor', 'Eurol' i 'Falcon' prowadzono obserwacje występowania na liściach objawów suchej zgnilizny kapustnych powodowanej przez *Leptosphaeria* spp. Odróżnienie gatunków sprawców – *L. maculans* od *L. biglobosa* – na podstawie objawów chorobowych było znacznie utrudnione. Pomimo wyodrębnienia siedmiu typów objawów, nie stwierdzono, aby którykolwiek z nich był powodowany tylko przez jeden gatunek patogenu. Zaobserwowano natomiast, że każdy z typów objawów choroby może być wywołany przez obydwa gatunki, choć w różnych proporcjach. Z plam na liściach opisywanych w literaturze jako typowe dla *L. maculans* (typ 1.) izolowano w 26% *L. biglobosa*, podczas gdy z plam uważanych za typowe dla *L. biglobosa* (typ 3.) uzyskiwano w 37% gatunek *L. maculans*. Wyniki wskazują, iż identyfikacja sprawców suchej zgnilizny kapustnych jedynie na podstawie objawów chorobowych występujących na liściach rzepaku ozimego może być obarczona dużym błędem, dlatego diagnozowanie tych patogenów powinno być uzupełnione innymi dostępnymi metodami.

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