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Monitoring of *Leptosphaeria maculans* and *L. biglobosa* ascospores around East Sudethian mountains – a joint initiative of Poland and the Czech Republic

Monitorowanie zarodników workowych grzybów *Leptosphaeria maculans* i *L. biglobosa* w rejonie Sudetów Wschodnich – wspólna inicjatywa Polski i Czech

Keywords: *Leptosphaeria maculans, L. biglobosa*, ascospore release, spore trap, molecular detection

A joint initiative of the Polish and Czech research institutes enabled monitoring of *Leptosphaeria maculans* and *L. biglobosa* ascospores around East Sudethian mountains — in the regions of intensive oilseed rape cultivation in both countries. Infection by both fungal species leads to blackleg or stem canker, causing considerable yield losses of brassicas. Daily concentrations of ascospores were observed from 1 September to 30 November 2008 at four sites, including two locations in Poland and two in the Czech Republic. The spore monitoring was done using Hirst type volumetric traps. The mean inoculum concentration was calculated on the basis of spore number on microscope slides containing cellophane tapes covered with a film of Vaseline, which enabled trapping and immobilization of spores. The species were identified based on Real-Time PCR data.

The first ascospores of *L. maculans* / *L. biglobosa* were detected in mid-September, and the maximum concentration of ascospores was observed from 2 to 19 days after the first spore release. At the earliest both parameters were observed at Šumperk, in the Czech Republic. The highest number of propagules per cubic meter ranged from 3 to 10 spores. Ascospores were present for 41.8% to 57.1% of days monitored. The inoculum of *L. maculans* was found less frequently, but the highest concentrations of DNA of this pathogen greatly outnumbered the values obtained for *L. biglobosa.* At both sites *L. biglobosa* was observed more frequently. The inoculum density of *L. maculans* and *L. biglobosa* in the autumn of 2008 was smaller as compared to the other seasons, what showed less risk of oilseed rape plant infection by the pathogens causing stem canker of brassicas over the period of this study.

Słowa kluczowe: *Leptosphaeria maculans, L. biglobosa*, uwalnianie zarodników, pułapka na zarodniki, detekcja molekularna

W okresie od 1 września do 30 listopada 2008 monitorowano stężenie zarodników workowych grzybów *Leptosphaeria maculans* i *L. biglobosa*, wywołujących suchą zgniliznę kapustnych. Badania

prowadzono w czterech lokalizacjach, w tym dwóch w Polsce (Charbielin, Sośnicowice) i dwóch w Republice Czeskiej (Opava, Šumperk), położonych na północ (Polska) i południe (Czechy) od Sudetów Wschodnich, w rejonach intensywnej uprawy rzepaku w obu krajach. W badaniach wykorzystano pułapki wolumetryczne na zarodniki typu Hirsta. Średnie stężenie dobowe inokulum obliczano na podstawie liczby zarodników na preparatach mikroskopowych zawierających taśmy celofanowe pokryte lepikiem, do którego przywierały zarodniki wychwycone przez pułapki. Monitoring prowadzono w trybie ciągłym, przy czym preparat mikroskopowy odpowiadał jednej dobie pracy pułapki. Przynależność gatunkową zarodników oznaczono metodą Real-Time PCR.

Pierwsze askospory *L. maculans/L. biglobosa* stwierdzono w połowie września, przy czym najwcześniej pojawiły się w Šumperku (10 września), w kolejnym dniu w Opavie, a najpóźniej w Charbielinie (15 września). Najwyższe stężenie zarodników workowych stwierdzono od 2 do 19 dni po ich pierwszej detekcji, ponownie najwcześniej w Šumperku, natomiast najpóźniej w Opavie. Na północ od pasma Sudetów Wschodnich największe stężenie wynosiło od 5 do 10 zarodników workowych w metrze sześciennym powietrza, natomiast na południe wartości te wynosiły od 3 do 4 zarodników/m^3. Askospory występowały od 41,8% monitorowanych dni (Opava) do 57,1% (Šumperk). Suma dobowych stężeń askospor wahała się od 31 (Šumperk) do 51 zarodników/m^3 powietrza (Opava). W porównaniu z pozostałymi latami badań — prowadzonych w tym regionie od 2005 roku — jesienią 2008 stwierdzono niewielkie stężenia zarodników *L. maculans* i *L. biglobosa*, świadczące o mniejszym zagrożeniu zdrowotności roślin rzepaku ze strony inokulum grzybów wywołujących suchą zgniliznę kapustnych.

Stężenia DNA inokulum badanych grzybów były znacznie wyższe w próbach zebranych w Charbielinie niż w Sośnicowicach (22-krotnie dla *L. maculans* i 7-krotnie dla *L. biglobosa*). W Charbielinie sumaryczne stężenie DNA grzyba *L. maculans* przekraczało 2,4-krotnie wartość stwierdzoną dla *L. biglobosa*, natomiast w Sośnicowicach wartości tego parametru były zbliżone. W obu przypadkach gatunek *L. biglobosa* obserwowano przez większą liczbę dni. Inokulum *L. maculans* stwierdzano rzadziej, lecz najwyższe stężenia DNA tego patogena znacznie przekraczały wartości uzyskane dla *L. biglobosa* (6,7-krotnie w próbach z Charbielina i 2,3-krotnie w Sośnicowicach).

Introduction

The Czech Republic and Poland are important producers of oilseed rape (*Brassica napus* L.) in Central Europe. In 2008, the annual production of oilseed rape in the Czech Republic was 1.048 thous. tonnes and in Poland it was 2.105 thous. tonnes, which together constituted 17.2% of the total oilseed rape production of the European Union (EU). In 2008, both countries cultivated, mainly, the winter type of this crop (99% in the Czech Republic and 98,3% in Poland). The mean yield collected in 2008 was 29,4 and 27,3 dt/ha respectively in the Czech Republic and Poland, which was less than the average yield recorded for winter oilseed rape in the EU. This is partly caused by less favourable growing conditions, but was due also to diseases decreasing total yield. Kazda et al. (2007) have demonstrated that highly intensive oilseed rape cultivation, exceeding 50% of oilseed rape crop in some areas of the Czech Republic, led to losses caused by pests and necessitated high costs of plant protection. On the other hand, in some years, the yield reduction on non-treated fields can escalate substantially. One of the major diseases responsible for substantial yield losses of rapeseed is phoma stem canker or

blackleg, caused by two fungal pathogens *L. maculans* and *L. biglobosa* (West et al. 2001, Fitt et al. 2006a). In recent years, the disease has reduced income by more than $1000 M per season, at current prices (Fitt et al. 2008).

In 2008, Polish and Czech research institutes undertook a joint initiative of monitoring *L. maculans* and *L. biglobosa* ascospores around East Sudethian mountains, based on the decision support systems developed in both countries (Jędryczka et al. 2004 and 2006, Plachká and Poslušná 2009). This geographic area is the region of intensive oilseed rape cultivation in both countries. The North Moravia zone accounts for 12% of the total annual production of oilseed rape in the Czech Republic, whereas the Lower Silesia and Opole regions account for 24% of the production obtained in Poland. The weather conditions of this agricultural region are conducive to the stem canker pathogens (Kaczmarek et al. 2010), resulting in infection levels that can lead to substantial yield losses. Monitoring of airborne primary inoculum of *Leptosphaeria* spp. by a combination of spore trapping and polymerase chain reaction (PCR)-based methods of molecular biology can help to pinpoint the crucial risk periods, when oilseed rape plants would be infected by ascospores that start the disease development (Petrie 1995, Guo and Fernando 2005). The aim of this study was also to compare the results obtained at the experiment sites located north (Poland) and south (the Czech Republic) of the Sudethian mountain chain.

Materials and methods

Location of the experiment

The experiment was done at four sites, including two in Poland:

1) Charbielin (N 50°20'37.2", E 17°25'49.1"),
2) Sośnicowice (N 50°16'19.2", E 18°37'31.2"),

and two in the Czech Republic:

1) Opava (N 49°55´47.582”, E 17°52´41.911”),
2) Šumperk (N 49°59′9.00″, E 17°00′20.00″).

The experiment sites were located north (Poland) and south (Czech Republic) of the East Sudethian mountains (Fig. 1).

Meteorological data

The meteorological dataset containing two basic weather data: mean daily temperature and rainfall was collected in 2008, between July (harvest) and November (the end of experiment) at daily intervals. Weather stations were located at experiment sites, with the exception of a synoptic station located in Otice near Opava (3 km).

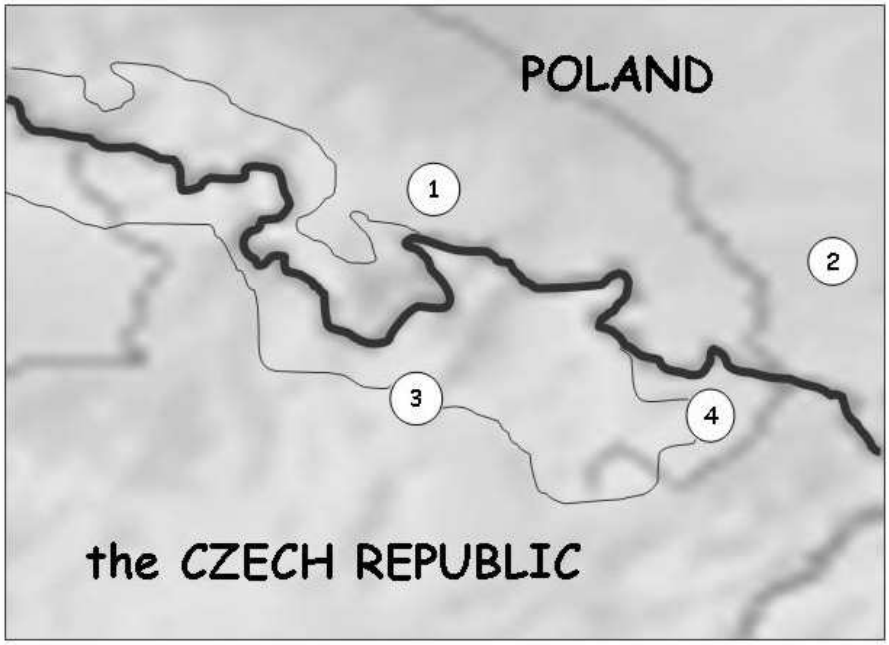


Fig. 1. The location of experiment sites: 1) Charbielin (PL), 2) Sośnicowice (PL), 3) Šumperk (CR), 4) Opava (CR). Thick line shows the Czech-Polish border, thin lines show the borderlines of Central and East Sudethian mountains — *Lokalizacja punktów doświadczalnych: 1) Charbielin (PL), 2) Sośnicowice (PL), 3) Šumperk (CR), 4) Opava (CR). Grubą linią zaznaczono granicę polsko-czeską, cienką linią zaznaczono zasięg Sudetów Środkowych i Wschodnich*

Sampling period

Monitoring of daily concentrations of *L. maculans* and *L. biglobosa* ascospores was done at daily intervals from 1 September until 30 November 2008. Molecular detection using Real-Time PCR was done for all samples collected in Poland.

Spore trapping method

The monitoring of airborne inoculum (ascospores) of *L. maculans* and *L. biglobosa* was done using Hirst type seven-day recording spore traps, which collect airborne particles on a wax-coated cellophane tape attached to a rotating drum. The wax coating comprised petroleum jelly (Vaselin) and paraffin wax, dissolved in hexane, according to the method published by Lacey and West (2006). The traps were surrounded by 0.35 dm^3 of oilseed rape stem debris infected by phoma stem canker in the previous season. The infected stubble was collected and kept under natural conditions of each experiment site.

The wax-coated tapes collected in the Czech Republic were mounted on to a microscope slide, stained with trypan blue and examined with a light microscope. The numbers of counted ascospores of *L. maculans* and *L. biglobosa* were transformed to daily ascospore concentration per 1 m^3 of air (Lacey and West 2006). The tapes collected in Poland were at first cut in half along their centrelines; then one half of the tape was used to prepare the microscope slide, stained and observed under a microscope. The concentration of *Leptosphaeria* spp. propagules in air samples was calculated from spore counts and the other half-piece of the tape was used for molecular examination using quantitative PCR.

Quantitative detection of *Leptosphaeria* spp. DNA from spore tapes

One half of each Melinex segment corresponding to a 24 h period was placed in a sterile 2 mL screw-topped microfuge tube with acid-washed glass beads (Sigma, UK) and extracted with CTAB (cetyltriammonium bromide = hexadecyltrimethyl ammonium bromide; Sigma, UK) buffer using a modification of the methods of Graham et al. (1994). Samples were subjected two 40 sec fast-prep cycles (Savant Instruments, Holbrook, New York, USA). The extract was incubated (30 min) at 70°C, centrifuged (15 min) at 14 000 *g* and extracted against an equal volume of a chloroform:isoamyl alcohol (24:1) mixture by vortexing. DNA was precipitated by incubation (1 h) at — 20°C with absolute ethanol (2 vols) and sodium acetate (pH 5, 3M, 0.1 vol) and centrifuged. The supernatant was discarded and DNA pellets were washed with ice-cold 70% ethanol, centrifuged, dried and dissolved in 100 µL 1 mM Tris-EDTA buffer.

For Real-time PCR a method described by Kaczmarek et al. (2009a) was used. To determine the relative amounts of *L. maculans* and *L. biglobosa* DNA in samples, quantitative PCR (qPCR) was done using the Sigma SYBR Green qPCR kit (Sigma, UK). Sterile deionised water was used as 'no template' controls in all qPCR reactions. Species-specific primers developed by Mahuku et al. (1996) for the identification of *L. maculans* and *L. biglobosa* were used. A standard 20-µL reaction volume consisted of 5 µL of DNA sample, 10 µL of SYBR Green Jump Start Ready Mix (Sigma, UK), 0.08 µL of Internal Reference dye (Sigma, UK), 0.6 µL (forward) and 054 µL (reverse) of 10 µM primers and 3.78 µL sterile deionised water. For this study, 10 µL assays were routinely done in duplicate in an ABI 7500 quantitative PCR system (Applied Biosystems, USA) for 2 min at 95°C followed by 38 cycles of 94°C for 15 s, 60°C for 1 min 95°C for 45 s. The increase influorescence from amplicons was recorded at 72°C during every cycle. To ascertain the specificity of the procedure, a dissociation curve was done after the final amplification cycle by heating samples at 95°C for 15 s, cooling to 60°C for 1 min and then heating to 95°C for 15 s. Fluorescence was measured continuously.

Statistical analyses

The amount of *Leptosphaeria* DNA on tapes was transformed (log10x+1). Log-transformed DNA yields were correlated against the numbers of ascospores on corresponding spore tapes using the GenStat statistical package, using the Pearson correlation coefficients (Payne et al. 2007).

Weather data

The weather parameters at four tested locations did not differ to a great extent (Table 1). The total rainfall yield from the beginning of July till the end of November corresponding to the period between the previous season's harvest of oilseed rape

Table 1

Weather conditions at experiment sites located in Poland and the Czech Republic in the autumn of 2008 — *Porównanie warunków pogodowych w miejscach doświadczalnych w Polsce i Republice Czeskiej jesienią 2008 roku*

Month *Miesiąc*	Poland — *Polska*		Czech Republic — *Republika Czeska*	
	Charbielin	Sośnicowice	Šumperk	Opava
Rainfall — *Opady* [mm]				
July — *Lipiec*	151.1	123.0	91.9	115.1
August — *Sierpień*	105.4	76.0	116.6	61.7
September — *Wrzesień*	60.6	102.2	22.7	76.5
October — *Październik*	58.6	36.9	23.5	23.4
November — *Listopad*	53.3	11.0	46.1	9.6
Rainfall [no. of days] — *Opady [liczba dni]*				
July — *Lipiec*	17	15	23	21
August — *Sierpień*	11	12	17	15
September — *Wrzesień*	15	12	14	16
October — *Październik*	15	10	13	18
November — *Listopad*	22	9	17	17
Mean daily temperature — *Średnia temperatura dobowa* [^{o}C]				
July — *Lipiec*	18.46	19.5	17.93	18.32
August — *Sierpień*	18.93	18.6	16.76	18.06
September — *Wrzesień*	10.76	12.9	12.28	12.75
October — *Październik*	10.78	10.3	8.22	10.01
November — *Listopad*	3.59	6.9	5.43	6.18
Cumulative monthly temperature — *Sumaryczna temperatura miesięczna* [^{o}C]				
July — *Lipiec*	572	566	556	568
August — *Sierpień*	587	560	453	560
September — *Wrzesień*	323	362	368	382
October — *Październik*	312	298	255	310
November — *Listopad*	108	138	163	186

and the end of the new crop's vegetative period before winter, ranged from 286.3 mm at Opava (CR) to 429 mm at Charbielin (PL). The average rainfall was 341 mm and a similar result was obtained in Sośnicowice (PL, 349.1 mm). July was the wettest month of the period studied, with 120.3 mm of rain on average. The average monthly rainfall were 89.9, 65.5, 35.6 and 30.0 mm respectively for August to November. For the summer months (July, August), the most humid location was Charbielin (256.5 mm) whilst the driest was Opava (176.8 mm). Charbielin was also the wettest location when September rains were added to the summer rainfall, and this trend remained until the end of the monitoring season. The mean number of rainy days over this period was 77.3 and ranged from 56 at Sośnicowice to 87 days at Opava. In general, the experimental sites in Poland were more humid than those in the Czech Republic.

The highest mean daily temperatures were recorded in July; the average value of this parameter was 18.55^{o}C, with the highest mean daily temperature of 19.5^{o}C at Sośnicowice (Poland) and the lowest (17.93^{o}C) at Šumperk (Czech Republic). The temperature readings for the months following this were gradually lower, with 18.09, 12.17, 9.83 and 5.53^{o}C in the succeeding months ofAugust to November respectively. On average, the mean daily temperature was 12.8^{o}C and ranged from 12.1^{o}C (Šumperk) to 13.6 ^{o}C (Sośnicowice). The highest cumulative temperature over the period of this study was 2006^{o}C and it was observed at Opava. The lowest value of this parameter was recorded at Šumperk (1795^{o}C). Average cumulative temperature was 1907^{o}C for Šumperk, and a similar result (1902^{o}C) was calculated for Charbielin.

For the entire period of this study, the experimental site at Opava was both the the driest and the hottest. Šumperk was also much drier than the average, and was also ranked as the coolest experiment site. In contrast, the two locations (Sośnicowice and Charbielin) within Poland were more humid and moderately warm.

Results

Effect of weather on ascospore release of *L. maculans* and *L. biglobosa*

The cumulative rainfall necessary for the fungal fruiting bodies of the generative stage to be formed and to mature, ranged from 131 to 175 mm and occurred over 22 to 33 days (Table 2). The cumulative temperature recorded over the period from pseudothecial maturation on oilseed rape stubble to the production of the first ascospores was approximately 1000^{o}C. The production and release of mature ascospores needed a few to several rain events (9–13) with considerable rainfall, reaching 43 to 73 mm of rain. In this case 100^{o}C to 200^{o}C were needed for the pseudothecia to attain full maturation of fruiting bodies and ascospore release.

Table 2

Weather parameters connected with the maturation of the primary inoculum of *Leptosphaeria maculans* and *L. biglobosa*
Porównanie parametrów pogodowych związanych z dojrzewaniem inokulum pierwotnego L. maculans i L. biglobosa

Period *Okres*	Weather parameter *Parametr pogodowy*		Poland *Polska*		Czech Republic *Republika Czeska*	
			Charbielin	Sośnicowice	Šumperk	Opava
From harvest (mid July) to the first ascospore detection *Od żniw (połowa lipca) do pierwszej detekcji askospor*	cumulative rainfall [mm] *sumaryczne opady*		165.6	175.4	131.5	131.1
	cumulative no. of rainy days *sumaryczna liczba dni deszczowych*		22	23	33	30
	cumulative temperature [^{o}C] *sumaryczna temperatura*		1005.4	1089.6	917.7	1052
From harvest (mid July) to the maximum concentration of the ascospores *Od żniw (połowa lipca) do najwyższego stężenia askospor*	cumulative rainfall [mm] *sumaryczne opady*	peak 1 peak 2	205.8 232.8	249.1	131.5	188
	cumulative no. of rainy days *sumaryczna liczba dni deszczowych*	peak 1 peak 2	30 44	32	33	42
	cumulative temperature [^{o}C] *sumaryczna temperatura*	peak 1 peak 2	1115.8 1388.5	1180.1	951.1	1253.3
From the first to the maximum ascospore release *Od detekcji pierwszych askospor do ich największego stężenia*	cumulative rainfall [mm] *sumaryczne opady*	peak 1 peak 2	43 70	73.7	0	56.9
	cumulative no. of rainy days *sumaryczna liczba dni deszczowych*	peak 1 peak 2	9 23	9	0	13
	cumulative temperature [^{o}C] *sumaryczna temperatura*	peak 1 peak 2	118.8 391.9	99	33.3	201.3

Table 3

Characteristics of the primary inoculum of *Leptosphaeria maculans* and *L. biglobosa* evaluated in the autumn of 2008 (microscope observations) — *Charakterystyka inokulum pierwotnego L. maculans i L. biglobosa oznaczanego jesienią 2008 roku (obserwacje mikroskopowe)*

Parameters of the primary inoculum *Parametr inokulum pierwotnego*		Poland *Polska*		Czech Republic *Republika Czeska*	
		Charbielin	Sośnicowice	Šumperk	Opava
First ascospore release *Pierwsza detekcja*	date — *data*	15.IX	14.IX	10.IX	11.IX
	amount of spores per 1 m^3 *liczba askospor w 1 m^3*	0.69	0.14	0.28	0.28
Maximum ascospore release *Masowa detekcja*	date – the first peak *data – pierwszy szczyt*	28.IX	25.IX	12.IX	30.IX
	date – the second peak *data – drugi szczyt*	24.X	–	–	–
	amount of spores per 1 m^3 *liczba askospor w 1 m^3*	4.86	9.58	2.64	3.75
No. of days from the first to the max. spore release *Liczba dni od pierwszej detekcji do masowej*		13	11	2	19
Percent of days with ascospores *Procent dni z askosporami*		50.5%	54.9%	57.1%	41.8%
Accumulative concentration of spores in the autumn *Sumaryczne stężenie askospor w okresie jesiennym*		49.16	45.69	30.69	51.18

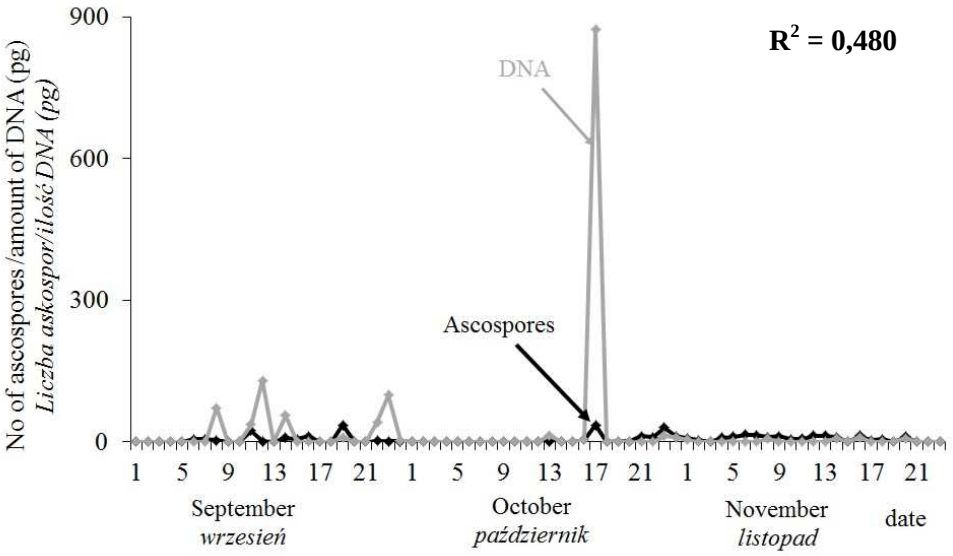
Concentration of *L. maculans* and *L. biglobosa* in the air samples

Based on spore detection with the light microscope, it was found that the earliest release of the first ascospores of *L. maculans* /*L. biglobosa* took place in the Czech Republic on 10–11 September. In Poland, the same process was observed and a few days later (14–15 September) (Table 3). The number of spores observed at this time was very small, falling below 1 per a cubic meter of the air. The highest concentration of ascospores was observed from 2 to 19 days after the detection of the first ascospores of *L. maculans* and *L. biglobosa*. Again the earliest observation was made in Šumperk, but the latest in Opava. In the north of the East Sudethian Mountains, the highest number of spores per cubic meter ranged from 5 to 10 spores, whereas in the south it was only 3 or 4 spores/m^3 of the air. At Charbielin two peaks of the maximum ascospore concentration were found: first at the end of September and then again one month thereafter. Ascospores were present for about half of the period of the study, and ranged from 41.8% of monitored days (Opava) to 57.1% (Šumperk). The cumulative daily concentrations of ascospores varied from 31 spores/m^3 of the air (Šumperk) to 51 spores/m^3 (Opava).

The Pearson correlation coefficient between the ascospore numbers and log-transformed DNA yields on corresponding spore tapes was 0.480 for Charbielin and 0.570 for Sośnicowice (Fig. 2ab). At both locations DNA of *L. maculans* and *L. biglobosa* was monitored on alternate days (Fig. 3ab).

The DNA concentrations of the two *Leptosphaeria* species were much higher in the samples originating from Charbielin than from Sośnicowice (22 times for *L. maculans* and 7 times for *L. biglobosa*). At Charbielin the sum concentration of *L. maculans* DNA was 2.4 times higher than determined for *L. biglobosa*. At Sośnicowice, however, both values for both parameters were similar. At both sites, the species *L. biglobosa* was observed for a greater number of days. Although *L. maculans* inoculum was found less frequently, the higher concentrations of DNA from this pathogen than from *L. biglobosa* were detected (6.7 times in the samples originating from Charbielin and 2.3 times at Sośnicowice). Both the first detection of *Leptosphaeria* spp. DNA and the detection of the maximum amount of DNA of each species were observed later than for the cumulative number of the ascospores of both species counted together (Table 4).

a



b

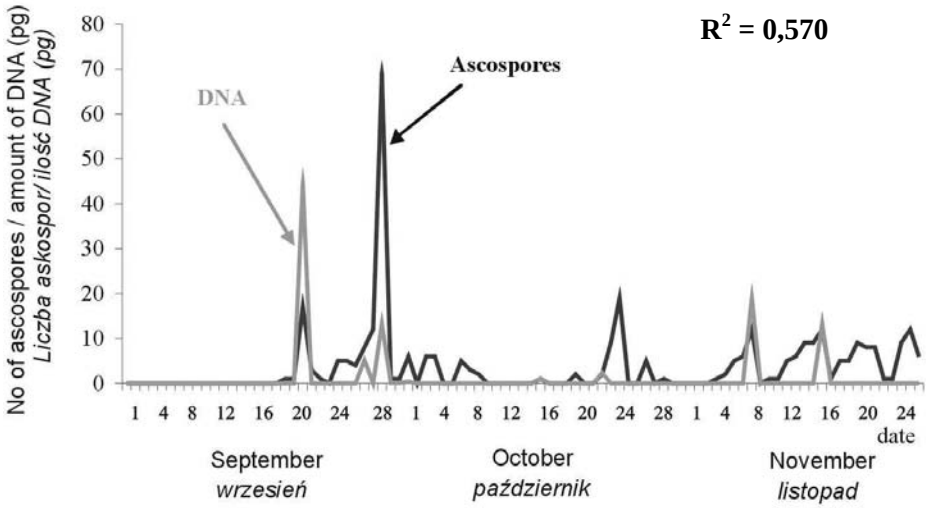


Fig. 2. Fluctuations in the number of ascospores (black line) and amount of DNA evaluated with quantitative PCR (grey line) of *Leptosphaeria maculans* and *L. biglobosa* at Charbielin (a) and Sośnicowice (b) — *Zmiany liczby askospor (czarna linia) i ilości DNA oznaczonego na podstawie ilościowego PCR (szara linia) grzybów Leptosphaeria maculans i L. biglobosa w Charbielinie (a) i Sośnicowicach (b)*

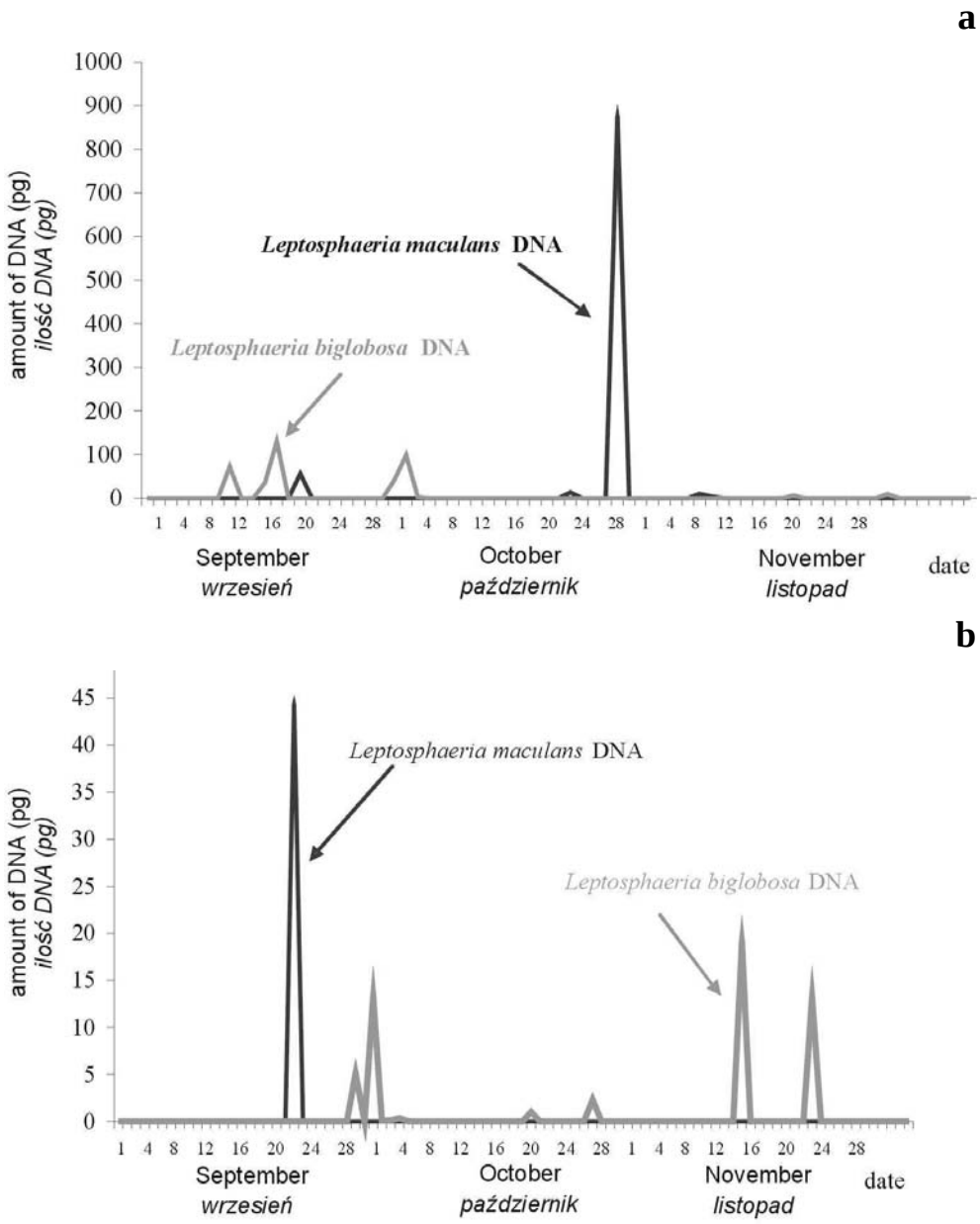


Fig. 3. Fluctuations in the quantities of DNA determined by qPCR from airborne propagules of *Leptosphaeria maculans* (black line) and *L. biglobosa* (grey line). DNA amount (pg) in Charbielin (a) and Sośnicowice (b) — *Zmiany ilości DNA (pg) zawartego w zarodnikach grzybów Leptosphaeria maculans (czarna linia) i L. biglobosa (szara linia*) *oznaczonego przy pomocy ilościowego PCR dla prób pobranych w Charbielinie (a) i Sośnicowicach (b)*

Table 4

Characteristics of the primary inoculum of *Leptosphaeria maculans* and *L. biglobosa* observed in autumn 2008, evaluated using quantitative PCR — *Charakterystyka inokulum pierwotnego Leptosphaeria maculans i L. biglobosa obserwowanego w 2008 roku (ilościowy PCR)*

Parameters of the primary inoculum *Parametry inokulum pierwotnego*			Experiment site *Lokalizacja doświadczenia*	
			Charbielin	Sośnicowice
Leptosphaeria maculans	first ascospore release *pierwsza detekcja*	date — *data*	23.IX	16.IX
		amount of DNA in the sample (pg) *ilość DNA w próbie (pg)*	56	44.3
	maximum ascospore release *masowa detekcja*	date — *data*	24.X	16.X
		amount of DNA in the sample (pg) *ilość DNA w próbie (pg)*	873	44.3
	no. of days from the first to maximum DNA detected *liczba dni od pierwszej detekcji do masowej*		34	0
	percent of days with DNA detected *procent dni, w których wykryto DNA*		7	1.1
	cumulative concentration of DNA (pg) *sumaryczne stężenie DNA (pg)*		956.19	44.3
Leptosphaeria biglobosa	first ascospore release *pierwsza detekcja*	date — *data*	17.IX	23.IX
		amount of DNA in the sample (pg) *ilość DNA w próbie (pg)*	72	5.13
	maximum ascospore release *masowa detekcja*	date — *data*	5.X	6.XI
		amount of DNA in the sample (pg) *ilość DNA w próbie (pg)*	98.7	19
	no. of days from the first to maximum DNA detected *liczba dni od pierwszej detekcji do masowej*		18	44
	percent of days with DNA detected *procent dni, w których wykryto DNA*		11.3	9.9
	cumulative concentration of DNA (pg) *sumaryczne stężenie DNA (pg)*		395.48	54.43

Discussion

Fluctuations in the concentrations of airborne propagules of *L. maculans* and *L. biglobosa* in the region located north of the Sudethian mountains, on the territory of Poland, have been studied since 2005 (Kaczmarek et al. 2010). This study has shown that the propagules of this species complex were less frequent in the autumn of 2008 when compared to the previous years which had greater numbers of airborne ascospore effecting greater risk of oilseed rape plant infection by the pathogens causing stem canker of brassicas over a similar period of study (Baranyk 2009a, b). Such considerable differences in the composition and the development of fungal inoculum were also found in some previous studies (Jędryczka et al. 1999, Huang et al. 2005). The experiments done by the independent research groups from Czech and Polish institutes have shown very similar results from experimental sites within the same geographic region. Some of the spore traps used for the monitoring of *L. maculans* and *L. biglobosa* ascospores in Poland are located close to the border with the Ukraine, Czech Republic and north Germany (Jędryczka et al. 2004 and 2006). It is highly probable that the information obtained from these spore traps would be of great decision-making use, not only for the local surroundings but also for some more distant locations.

Out of the experimental sites that were used in this study, the region of Šumperk was the most favourable for the development of phoma stem canker infections of oilseed rape plants in the season of study. The first ascospore release was observed very early in the season and the maximum concentration of the propagules of *L. maculans* and *L. biglobosa* was observed soon, thereafter. Moreover, the percent of days with ascospore presence in the air samples was the highest among all regions of study. Weather conditions that were condicive for the disease were also recorded at the two locations in Poland. Moreover the concentration of the airborne fungal propagules at both Polish sites was twice as high as at the Czech locations. Higher humidity and moderately high temperatures are more suitable for the development of the disease (Zhou et al. 1999, Toscano-Underwood et al. 2003). In contrast, the region of Opava was less conducive for the stem canker. There was a lower percent of days with inoculum in the air, the tardiest mass ascospore release and small concentration of *L. maculans* and *L. biglobosa* ascospores in the air at this site.

PCR-based molecular detection of airborne propagules of *Leptosphaeria* spp. was successfully done using volumetric spore samplers (Calderon et al. 2002, Stachowiak et al. 2005, Kaczmarek et al. 2008, 2009a, b) utilizing methods that are similarly reported for the detection of other pathogens, such as *Monilia fructicola* (Luo et al. 2007). In the current study, we have demonstrated that molecular detection using Real-Time PCR can be used to monitor the risk of oilseed rape infection by the stem canker disease organisms. Smaller numbers of samples with

L. maculans and *L. biglobosa* DNA may either bedue to low spore concentrations detected on tapes or could suggest thatthe SYBR Green-based qPCR method had a low resolution. Nevertheless, molecular detection by qPCR had the additional advantage of indicating which *Leptosphaeria* species were present at particular sites and identifying the periods of time, thereby allowing for greater precision at selecting the appropriate dosage of a fungicide for disease control. It was already proved that fungicides have different efficacies on *in vitro* spore germination and mycelial growth of *L. maculans* and *L. biglobosa* (Eckert et al. 2010). Both fungal pathogens often coexist within certain regions and over similar periods of time, and can colocalise on the same host plants (Jędryczka 2007, Fitt et al. 2006b). The knowledge on pathogen population structures and the ratio between the species, may serve as an additional tool, leading to more precise advice on chemical products used for the protection of oilseed rape against stem canker.

This paper has concentrated on the detection of *L. maculans* and *L. biglobosa*, with little interest on polymorphisms within these species. Genetic differentiation of *L. maculans* may be further studied by assessing alleles for mating types (Cozijnsen and Howlett 2003), avirulence genes and races (Balesdent et al. 2006; Stachowiak et al., 2006, Van de Wouw et al. 2010) as well as mini- and microsatellites (Eckert et al. 2005; Hayden et al. 2007). Minisatellite markers have recently shown great potential in studies of *L. maculans* isolate variation from Poland (Jędryczka et al. 2010). High fertility of the sexual stage of this fungal species suggests equal ratios of both mating types. The most valuable and informative fungal markers, among the ones listed above, are avirulence genes. They show the potential of *L. maculans* inoculum to infect particular cultivars and genotypes of oilseed rape. The erosion of a host-plant resistance arising from the presence and expression of virulent alleles of the pathogen is of great concern to producers of oilseed rape as well as breeders of the crop. The reports of short-term duration of specific resistance genes, such as *Rlm1* (Rouxel et al. 2003), *B. rapa* var. *sylvestris*-derived resistance genes (Hua Li et al. 2003) or *Jlm1* (Brun et al. 2000) are quite well documented. The phenomenon reported in *B. napus* could be likened to the one resulting from the epidemiological model of rice blast resistance breakdown of resistant rice cultivars (Kiyosawa 1982). The same problem was described in real field situations (McDonald and Linde 2002, Khang et al. 2008) and the best solution to obtain durable resistance was to combine specific R genes with quantitative resistance (Brun et al. 2010).

Life cycles of plant pathogens do not only reflect substantial differences between the experiment sites, but could also be modified and altered in response to quite subtle weather parameters. Careful observations of particular weather data and — to greater extent — the combination of these parameters, give rise to successful protection of agricultural crops. Weather differences affected the maturation and development of *L. maculans* and *L. biglobosa*, studied as both a species complex and separate species.

Conclusions

1. The data obtained based on monitoring of the ascospores of *L. maculans* and *L. biglobosa* using a spore sampler can be extrapolated to a bigger region.
2. The knowledge of weather influence on the pathogen life cycle can greatly help in effective protection of the agricultural crop.
3. Joint efforts in monitoring of the inoculum density of plant pathogens allow to better control the disease in a certain geographic region. They can be undertaken both at national and international scale.

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