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**DEVELOPMENT OF THE PERFECT STAGE
OF *LEPTOSPHAERIA MACULANS* AND *L. BIGLOBOSA*
UNDER VARIABLE WEATHER CONDITIONS
OF POMERANIA IN 2004–2008**

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Abstract

The ascomycete fungi *Leptosphaeria maculans* and *L. biglobosa* cause phoma stem canker and stem lesions, which account for economic loss in oilseed rape in Poland and worldwide. The aim of this study was to investigate the development of the sexual fruiting bodies and ascospore release of these pathogen complex in Radoštowo located in Pomerania (north Poland), one of the regions of the most intensive oilseed rape cultivation in Poland. Monitoring of pseudothecial maturation and ascospore release was done over five consecutive autumn periods from the year 2004 to 2008, under variable weather conditions. The parameters were interdependent, but differed greatly between seasons. Lengthy durations of pseudothecial maturation coincided with delayed ascospore release. Conversely, early and short maturation process led to early detection of ascospores. The slowest rate of pseudothecial maturation was observed in 2005, which coincided with the smallest number of rainy days in August. In contrast, the fastest pseudothecial maturation, as well as the earliest and most intensive release of ascospores was noted in 2007, when regular rainfall events were observed throughout the whole summer. In 2006 and 2008 small amounts of daily mean ascospores in air samples were detected, which coincided with little rainfall in July, when pseudothecia are formed on oilseed rape stubble. The influence of weather conditions on ascospore release and its relevance to aerobiological surveillance and disease management are discussed.

Key words: ascospore release, oilseed rape, phoma stem canker, pseudothecia maturation, spore trapping

Introduction

Phoma stem canker and stem lesions are caused by the ascomycetes *Leptosphaeria maculans* and *L. biglobosa* (Shoemaker and Brun 2001). In the current taxonomic classification the fungi belong to the class Dothideomycetes and the order Pleosporales (Barbee 2001). Both species affect oilseed rape and account for substantial annual yield loss in Poland (Frencel et al. 1991) and worldwide (Zhou et al. 1999, Fitt et al. 2006).

Ascospores, the primary inoculum of these pathogens (Bokor et al. 1975, West et al. 1999) are formed within asci and released from maturing pseudothecia (ascocarps) on the exposed woody remains of the infected stubble of the previous season's crop (McGee 1977). The rate of pseudothecial maturation and ascospore release during the intercrop season depends on weather conditions (Toscano-Underwood et al. 2003) and varies with geographical location (Bokor et al. 1975, McGee 1977, Rempel and Hall 1993, Khangura et al. 2001) and years (Petrie 1995). In western Canada a developmental course that is similar to that of Australia occurs in June after the long, cold winter months (Kharbanda 1993). However, in western and central Europe pseudothecial maturation and ascospore release start at the end of August and may last throughout the autumn months (Gladders and Symonds 1995, Thürwächter et al. 1999, Aubertot et al. 2004, Kaczmarek and Jędrzycka 2008). The most damaging stem-base (crown) cankers originate from cotyledon and leaf lesions produced on young plants (West et al. 2001).

Monitoring both the presence of mature pseudothecia on oilseed rape stubble and the concentration of ascospores in air samples enable the identification of periods of early disease onset and assist in decisions about the optimal time for fungicide application (Gladders et al. 1998, Jędrzycka et al. 2006). In Poland, such monitoring was established in the autumn of 2004 and currently comprises 10 ecologically different locations equipped with volumetric spore traps (Burkard Manufacturing Ltd., Rickmansworth, UK and Lanzoni S.r.l., Italy). In addition, pseudothecial development is evaluated at 44 sites distributed across the country. Monitoring is performed within the System for Forecasting Disease Epidemics (SPEC) programme (Jędrzycka et al. 2008).

This paper reports results obtained from experiments conducted over five consecutive autumn seasons at a site located in the region of Pomerania, differing in weather conditions. The assessment that was undertaken involved investigations of growth and development of the pseudothecia of *L. maculans* and *L. biglobosa*, collecting of air samples containing trapped spores and estimation of ascospore release by light microscopic examinations.

Materials and methods

Location of the experiment

Stubble and air samples were collected from the Experimental Station for Variety Testing in Radostowo (N 53°59'27.2", E 18°43'59.6"), near Tczew in Pomerania, Poland.

Meteorological data

Temperature and rainfall data were recorded daily from the beginning of July to the end of November each year at the experiment site (Table 1).

Sampling periods

Monitoring of pseudothecial maturation and ascospore release was done from 2004 until 2008. Samples were collected on a weekly basis from 1st September until 30th November.

Method of evaluation of pseudothecia maturation

Infected oilseed rape stubble was collected after harvest in Radostowo every summer from 2004 to 2008. Stubble fragments were maintained outdoors, on soil surface under natural weather conditions. The process of pseudothecial maturation was monitored at weekly intervals. Six to ten stem fragments with signs of fruiting bodies were collected and observed with a light microscope (Zeiss Axiostar, Germany) at 100× magnification. At first, based on morphology, each fruiting body was ascribed to one of the two forms produced during the life cycle of species of *Leptosphaeria* – either pycnidia, the asexual conidiomata containing pycnidiospores or pseudothecia, the sexual ascocarps containing ascospores. In total, 4200 fruiting bodies were examined and classified during the whole period of the experiment. Pseudothecia were further divided into five classes of maturation (A to E) using a method described by Kaczmarek and Jędryczka (2008).

Method of air-borne ascospore collection

Ascospore release from stubble of the previous season's crop stubble was monitored using a seven-day recording volumetric Burkard spore trap (Burkard Manufacturing, Rickmansworth, UK). This Hirst-like trap that collects air-borne particles on a wax-coated Melinex tape, which is attached to a slowly rotating drum. Tapes were coated with 5:1 mixture of petroleum jelly (Vaseline, Unilever Ltd, London, UK) and paraffin wax, dissolved in hexane (Airborne... 1995) and were replaced every seven days and after a full revolution of the drum (McCartney et al. 1997). They were cut into 48 mm sections, each representing a 24-h period and each piece was cut in half lengthwise, along the centerline in the direction of

rotation. One half was mounted on to a microscope slide, stained with 0.1% Trypan Blue in lactophenol and examined with a light microscope (Zeiss Axiostar, Germany) at 200× magnification. The number of spores on whole tape was multiplied by a conversion factor to obtain the abundance of spores per 1 m³ of air sampled. The corresponding half-piece of the tape was placed in a 1.5 ml microfuge tube and stored at –20°C for DNA extraction and further analysis using either traditional, end-point or quantitative Real-time PCR (Kaczmarek et al. 2008).

Results

Maturation of pseudothecia

In all seasons of the study, samples of oilseed rape stubble contained all developmental stages of the fruiting bodies of *L. maculans* and *L. biglobosa*. These were pycnidia, immature pseudothecia (class A), pseudothecia with developing asci (class B), pseudothecia with developing ascospores (class C), mature pseudothecia with most of asci containing eight fully developed ascospores (class D) and empty pseudothecia (class E).

In 2004, the first fully mature pseudothecia were found on 19 September and they constituted 2.2% of the whole pool of fruiting bodies (Fig. 1). By the following week this proportion had increased to more than 10 times (29.8%). From the beginning of October the ratio of fully mature pseudothecia remained at 50% and decreased only at the end of November (33.3% class D pseudothecia on 17 November). Over the whole autumnal season, the mean ratio of fully developed pseudothecia was 37.3% for 2004.

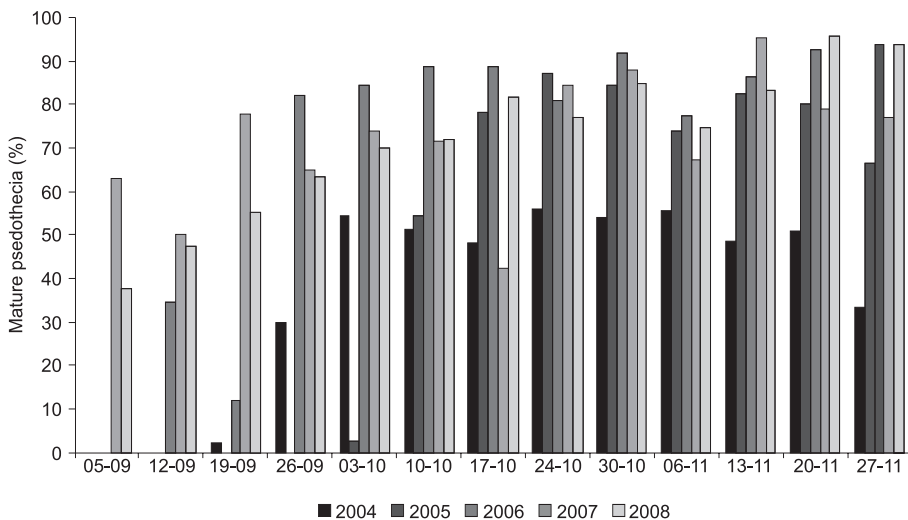


Fig. 1. The proportion of mature pseudothecia (class D) in a total pool of fruiting bodies of the fungi *Leptosphaeria maculans* and *L. biglobosa* in autumn 2004–2008 in Radostowo (Pomerania)

In the following autumn, fully developed pseudothecia were not observed on stubble samples until the beginning of October 2005. In that year, the fewest number of rainy days was noted from mid-July to mid-September (Table 1), which is, historically, the usual period for maximal production of *L. maculans* and *L. biglobosa* pseudothecia in Poland. Nevertheless, two weeks thereafter, fully mature pseudothecia increased in frequency to above 50% of all fruiting bodies. From the second half of October class D (fully developed) pseudothecia constituted about 80% of fruiting bodies and their number decreased to 66.7% by the end of November 2005 (Fig. 1). The mean proportion of class D pseudothecia was 46.9% of the total sum of all fruiting bodies.

Table 1

Weather data for summer and autumn months at Radostowo (Pomerania)

Year	Month	Rainfall (mm)		Rainy days			Temperature (°C)	
		mean	sum	%	number		mean	sum
					in the first half of the month	in the second half of the month		
2004	July	3.52	109.1	41.9	8	5	16.22	502.9
	August	3.12	96.7	41.9	3	10	18.20	567.2
	September	0.98	29.5	40.0	3	8	13.55	406.4
	October	2.25	69.9	58.1	5	13	9.19	284.9
	November	1.06	31.7	40.0	4	8	3.35	100.5
2005	July	2.31	71.7	41.9	2	11	18.76	581.7
	August	2.52	78.2	32.3	8	2	16.60	514.7
	September	1.41	42.4	20.0	3	3	15.03	451.0
	October	0.53	16.5	25.8	0	8	8.35	258.8
	November	0.71	21.4	16.7	3	2	3.25	97.4
2006	July	1.44	44.5	22.6	3	4	20.61	639.0
	August	3.92	121.5	64.5	12	8	16.75	519.3
	September	0.96	28.9	23.3	5	2	15.50	464.9
	October	1.17	36.2	29.0	4	5	10.21	316.6
	November	1.66	49.8	36.7	9	2	5.76	172.8
2007	July	2.88	89.4	58.1	10	8	17.60	546.0
	August	1.52	47.2	41.9	5	8	18.13	562.3
	September	1.90	57.0	40.0	8	4	13.01	390.2
	October	1.46	45.3	38.7	7	5	7.88	244.2
	November	0.73	21.9	40.0	7	5	1.94	58.3
2008	July	3.24	100.4	35.5	7	4	18.00	558.0
	August	5.34	165.5	71.0	11	11	17.54	543.8
	September	1.46	19.0	43.3	6	7	12.53	350.9
	October	3.59	53.8	48.4	5	10	9.51	275.7
	November	3.22	41.9	43.3	3	10	5.01	140.4

In 2006 a large number of fully mature pseudothecia were found within two weeks from the start of this season and this stage of pseudothecial development accounted for more than 80% of all fruiting bodies by the end of September. Following this, the proportion of class D pseudothecia ranged between 80% and more than 90% over the study period.

In the autumns of 2007 and 2008 there was a significant increase in the rate of development of the perfect stage of *L. maculans* and *L. biglobosa* compared to other years. This phenomenon was the most intensive in 2007 when rainfall in summer and early autumn months (July to mid-September) was observed regularly (Table 1). High numbers of fully mature pseudothecia were found in all samples at the beginning of September: 63% in 2007 and almost 40% in 2008 (Fig. 1). In the last three seasons of the study (2006–2008) the average numbers of class D pseudothecia consistently exceeded 70%. High rate of pseudothecia maturation coincided with high number of rainy days from mid-July to mid-September: ranging between 29 and 32 days.

Ascospore release

The release of ascospores was assessed with a spore trap by calculating the average spore concentration per cubic meter of the air sampled. There were seasonal differences in the timing of ascospore release, the date and number of the maximum concentration of spores in air samples as well as the number of days with trapped ascospores.

The highest concentration of ascospores was observed in 2004 reaching 122 spores per 1 m³ of the air sampled on 16 October (Fig. 2). Ascospore release pattern showed only one peak during that season. First ascospores were detected one

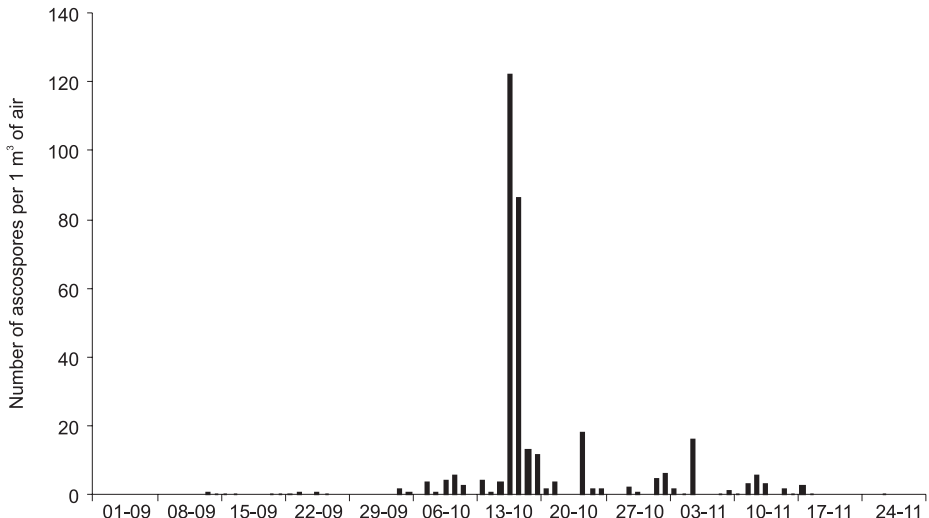


Fig. 2. Pattern of *Leptosphaeria maculans* and *L. biglobosa* ascospore release in Radostowo (Pomerania) – autumn 2004

Table 2

Parameters of *Leptosphaeria maculans* and *L. biglobosa* ascospore release in Radostowo (Pomerania) in 2004–2008

Parameter	Year				
	2004	2005	2006	2007	2008
Date of the first ascospore detection	13 Sept.	9 Oct.	19 Sept.	3 Sept.	8 Sept.
Date of the detection of maximum ascospore concentration	16 Oct.	5 Nov.	26 Nov.	28 Sept.	29 Oct.
The highest daily mean concentration of ascospores in 1 m ³ of air	122	96	10	65	15
Number of days with ascospore detection in the air	47	36	53	67	54
Number of days with ascospore concentration > 10 per 1 m ³	6	8	1	17	2
Number of days with ascospore concentration > 25 per 1 m ³	2	4	0	6	0
Number of days with ascospore concentration > 50 per 1 m ³	2	2	0	2	0
Sum of daily mean ascospore concentrations in 1 m ³ of air	342	323	81	508	90

month earlier in September 2004. Ascospores were present in the air for 47 days, amounting to 52% of the season. The sum of daily mean ascospore concentrations was 342 spores in 1 m³ of the air (Table 2).

The latest date for the first detection of ascospores was observed in autumn 2005, when these spores were first observed on 9 October (Table 2). There were two smaller peaks of ascospores (on 16 October: 14 ascospores per 1 m³ and 23 October: 57 ascospores per 1 m³) before the highest number of ascospores was released (Fig. 3) on 5 November in that season. However, the daily mean concentration of spores was high (96 spores per 1 m³). The sum of daily mean ascospore concentrations was comparable to that observed in 2004 (Table 2).

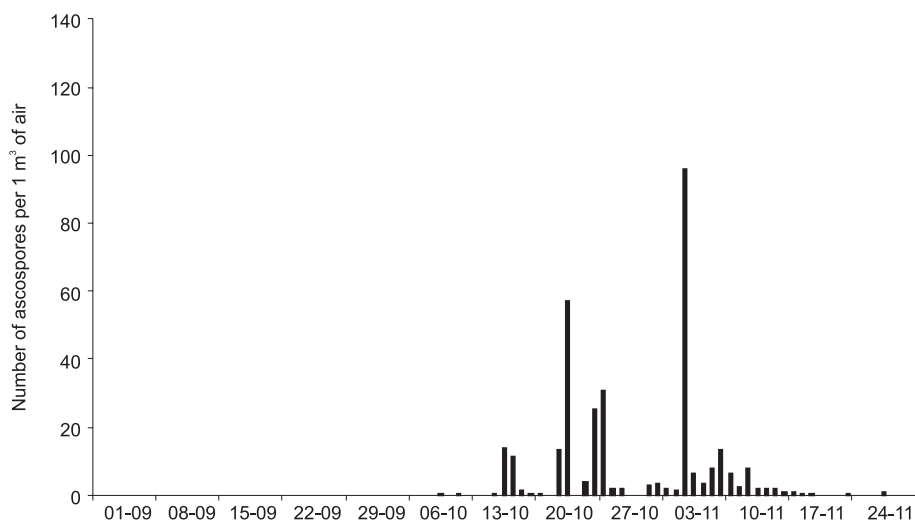


Fig. 3. Pattern of *Leptosphaeria maculans* and *L. biglobosa* ascospore release in Radostowo (Pomerania) – autumn 2005

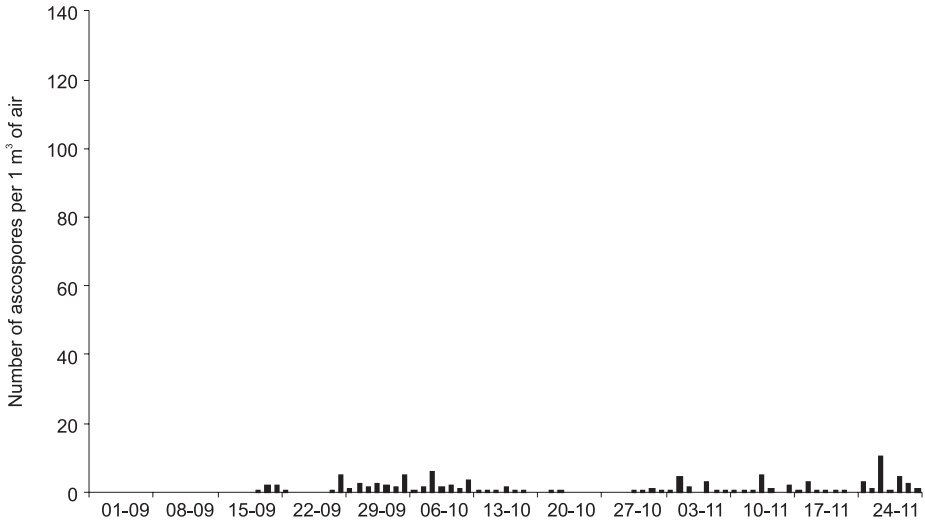


Fig. 4. Pattern of *Leptosphaeria maculans* and *L. biglobosa* ascospore release in Radostowo (Pomerania) – autumn 2006

In next two subsequent seasons the ascospore release was at two extremes; very late and low in 2006 (Fig. 4) and very early and high in 2007 (Fig. 5). Maximum daily mean concentration of ascospores, observed on 26 November, was only 10 spores per 1 m³ of air in 2006 whereas in 2007 the highest number (65 spores per 1 m³) of air-borne ascospores was found at the end of September. Moreover, in 2007 the first ascospore release was also observed earliest in the five-year duration of this study (Table 2). In that year there was the highest number as well as propor-

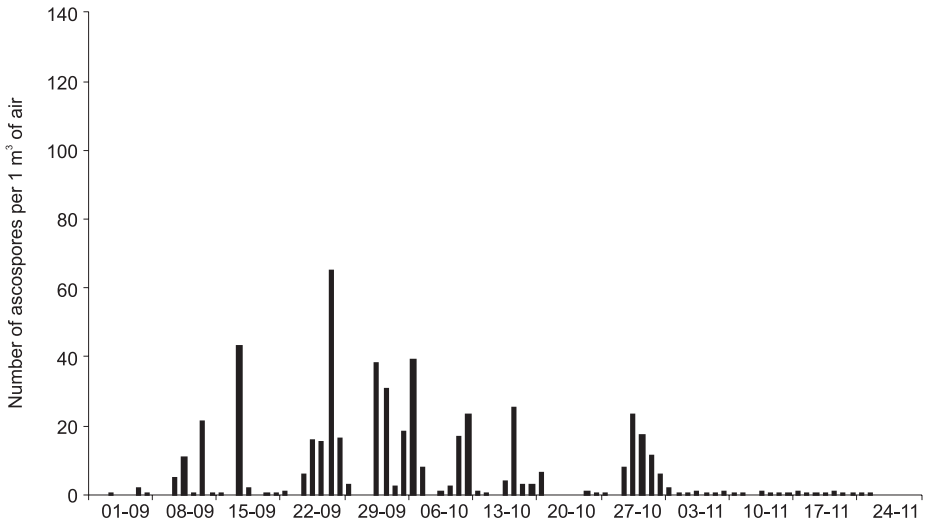


Fig. 5. Pattern of *Leptosphaeria maculans* and *L. biglobosa* ascospore release in Radostowo (Pomerania) – autumn 2007

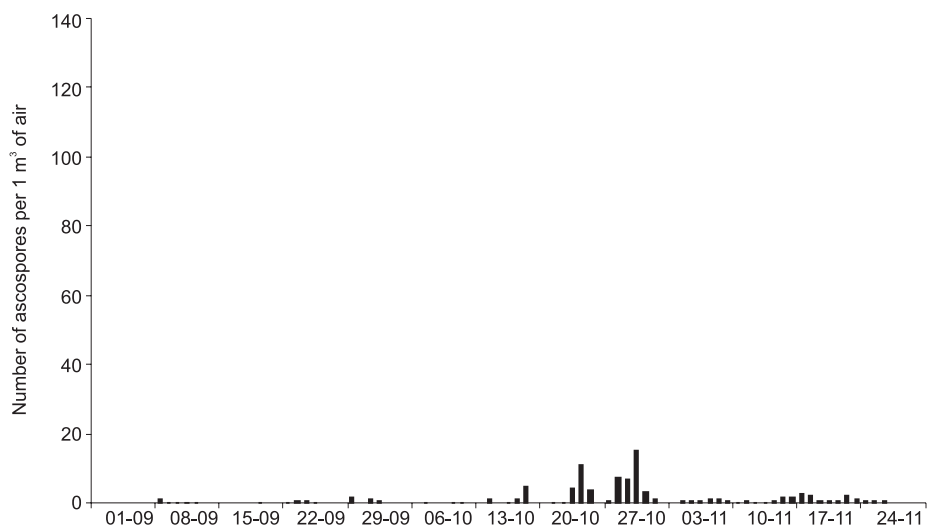


Fig. 6. Pattern of *Leptosphaeria maculans* and *L. biglobosa* ascospore release in Radostowo (Pomerania) – autumn 2008

tion of days with ascospores present in the air (67 days and 74% respectively). Moreover, the highest number of days with high concentrations of ascospores was also recorded for 2007 as there were 17 days (28%) with more than 10 ascospores per 1 m³ of air and six days (10%) with more than 25 ascospores per 1 m³ of air. In two days, the number of ascospores exceeded 50 per 1 m³ of air sampled. High ascospore release in 2007 was the consequence of high pseudothecial production, coinciding with frequent rain events over summer months (Table 1). By contrast, in 2006 there was only one peak day when there were more than 10 ascospores per 1 m³ of sampled air and no day, whatsoever, with air-borne ascospore concentration exceeding 25 or 50 in a 1 m³ (Fig. 5). Ascospore release from *L. maculans* and *L. biglobosa* pseudothecia in the autumn 2008 was comparable to that of autumn 2006 as both years witnessed little rainfall in July (Table 1). The average daily sum and peak ascospore concentrations in both years were also similar (Table 2), although days with higher numbers of spores were observed about one month earlier in 2008 (Fig. 6) than in 2006 (Fig. 4).

Discussion

Availability of primary inoculum of a virulent pathogen for dispersal is of great importance at the onset of plant disease epidemics. Thus the current study on *L. maculans* and *L. biglobosa* life cycles concentrated on ascospore-containing pseudothecia, rather than on pycnidia, which contain asexual pycnidiospores. These conidia constitute the secondary inoculum in the development of phoma stem canker epidemics. However, as they are splash dispersed, they are trans-

ported over a relatively shorter range. Ascospores are wind-dispersed, instead, and are consequently transported over significantly greater distances (Hall 1992, West et al. 1999, Guo and Fernando 2005). They incite earlier symptoms and establish more severe infections than pycnidiospores (West et al. 2001, Huang et al. 2005). The most epidemiologically damaging stage of pseudothecial development is class D, which contains fully developed ascospores and the rate and timing of attainment of this developmental stage may vary with cropping seasons and climate (West et al. 2002, Toscano-Underwood et al. 2003).

Rain events coinciding with full maturation of fruiting bodies trigger ascospore release (West et al. 2002). This process is observed for numerous ascospores that develop under similar conditions. For practical reasons that are connected with forecasting the risk of disease development in economically important *Brassica* crops such as oilseed rape, the process has been subjected to mathematical modelling (Salam et al. 2003, Aubertot et al. 2006, Dawidziuk et al. 2006).

In the current study the development of pseudothecia of *L. maculans* and *L. biglobosa* varied greatly from year to year. In the first two years of this study (2004 and 2005) the first fully mature pseudothecia were observed on winter oilseed rape stubble in mid-September and became more frequent towards the beginning of October. Rate of development was slow and it took two–three more weeks for the fully mature pseudothecia to constitute more than 50% of the entire pool of fruiting bodies formed on the stubble sampled. Slow maturation rate of pseudothecia coincided with the most dry summer period after harvest, starting in mid-July and ending in mid-September. In 2006, however, numerous class D pseudothecia were observed in mid-September, and in the following two years, fully developed pseudothecia were found on stubble at the beginning of the sampling season. These findings imply increasingly high probabilities for very early infection of the cotyledon and leaves of oilseed rape seedlings and young plantlets, leading to severe phoma stem canker symptoms at flowering and crop maturity (West et al. 2001). In the final three years of this study (2006–2008), fully mature pseudothecia exceeded 90% of all fruiting bodies, by the end of October or in the November of each autumn. The fastest rate of pseudothecial maturation occurred in 2007, when the amount of rainfall over July and August was high and rain events were regular. By contrast, the slow pseudothecial maturation that was observed in 2005 coincided with the lowest number of rain events in the August of that year.

Release of *L. maculans* and *L. biglobosa* ascospores is consequent upon pseudothecial maturation and occurs in concomitance with periods of rainfall (West et al. 2002, Salam et al. 2003, Dawidziuk et al. 2006). A similar interdependence of rainfall, maturation of pseudothecia and earliness of ascospore release in *Leptosphaeria* species was also observed in this study. Prolonged pseudothecial maturation, such as was observed in the autumn of 2005, resulted in very late ascospore release. Conversely, early and speedy pseudothecial maturation led to early detection of ascospores, usually in early September, while seedling and juvenile oilseed rape plants were at their most vulnerable to the disease. In 2006 and 2008 the amounts of daily mean number of ascospores that were captured in air samples were smaller than was observed in the other years of this study. This phenomenon coincided

with little rainfall in July, when, historically, pseudothecia are usually formed on Polish oilseed rape stubble. Prolonged dryness of stems could have strongly decreased the number of pseudothecia produced.

Taken together, abundant rainfall in July enhanced the production of pseudothecia on stems of oilseed rape in Poland. Regular rain events over the summer months would greatly speed up the process of pseudothecial maturation. High concentrations of *L. maculans* and *L. biglobosa* ascospores in the air emanate from the large quantities of fertile pseudothecia formed on stems and the optimal time of their peak release into the air depends on the speed of pseudothecial maturation. Knowledge about the development of perfect stages of fungal pathogens generates information about the abundance and timing of primary inoculum release and enhances both aerobiological and disease surveillance. Thus, epidemiological studies of the type that was conducted in this study play a very important role in the proper formulation and timing of plant protection practices deployed in field conditions.

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Streszczenie

ROZWÓJ STADIUM DOSKONAŁEGO *LEPTOSPHERAERIA MACULANS* I *L. BIGLOBOSA* W ZRÓŻNICOWANYCH WARUNKACH POGODOWYCH NA POMORZU W LATACH 2004–2008

Grzyby *Leptosphaeria maculans* i *L. biglobosa* są przyczyną suchej zgnilizny kapustnych – groźnej choroby rzepaku w Polsce i na świecie. Celem badań było określenie szybkości rozwoju stadium doskonałego kompleksu tych patogenów w Stacji Doświadczalnej Oceny Odmian Radostowo, zlokalizowanej na Pomorzu, stanowiącym jeden z najbardziej intensywnych regionów uprawy rzepaku w Polsce. Monitorowanie dojrzewania pseudotecjów i uwalniania zarodników workowych prowadzono przez pięć kolejnych sezonów jesiennych (2004–2008), znacznie zróżnicowanych pod względem warunków pogodowych. Wartości obu parametrów były współzależne, lecz znacznie różniły się w poszczególnych latach. Stężenie zarodników workowych w powietrzu było silnie uzależnione od szybkości dojrzewania pseudotecjów. Wczesne i szybkie dojrzewanie owocników stadium

doskonałego wiązało się z wczesną detekcją zarodników workowych. Długi czas dojrzewania pseudotecjów był powiązany z późnym uwalnianiem askospor. Najwolniejszy przebieg dojrzewania pseudotecjów miał miejsce w 2005 roku, kiedy odnotowano najmniejszy odsetek dni deszczowych w sierpniu. Najszybsze dojrzewanie pseudotecjów oraz najwcześniejsze uwalnianie i największe stężenie zarodników workowych odnotowano w 2007 roku, kiedy w letnich miesiącach występowały regularne opady deszczu. W latach 2006 i 2008, charakteryzujących się wyjątkowo suchym lipcem, stwierdzono najmniejszą liczebność askospor w próbach powietrza, zarówno pod względem sumy zarodników uwolnionych w danym sezonie, jak i ich średnich stężeń dobowych.

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