

Influence of meteorological parameters on *Leptosphaeria maculans* and *L. biglobosa* spore release in central and eastern Poland

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Abstract

Leptosphaeria maculans and *L. biglobosa* are fungal pathogens able to cause allergic reactions in humans and infect plants of *Brassica* species. The rate of their development and subsequent spore release depend on weather conditions. The aim of this paper was to pinpoint the exact meteorological conditions triggering the release of *L. maculans* and *L. biglobosa* ascospores in central and eastern Poland. Multiple regressions indicated that the frequency and amount of rainfall over short periods were important in mediating spore release. The first ascospore event depended mainly on the number of rainy days during the first 10 days of July and the cumulative precipitation during July and September. The most important variables for maximum spore release were cumulative rainfall in the beginning of July and the end of September, as well as the number of days with precipitation events in the first 10 days of August. The results highlighted for the first time the importance of the days preceding the collection of oilseed rape plants from the field. Higher moisture content of senescing but still living stems play a crucial role in the early start of the ascospore season and the maximum release of ascospores. This was not yet considered to date.

Keywords: aeromycology, agrometeorology, ascospore, phoma stem canker, pseudothecium

Phoma stem canker of brassicas is of economic importance in Europe, North America and Australia (West et al., 2001; Dilmaghani et al., 2010). It is the most damaging disease of winter oilseed rape in the UK and disease losses have been estimated at over 40 million GBP in the harvest years 2000–2002 (Fitt et al., 2006; Stonard et al., 2009). The disease is caused by a complex of fungal pathogens, comprising at least two species, *Leptosphaeria maculans* (Sowerby) P. Karst and *L. biglobosa* Shoemaker & H. Brun (Shoemaker & Brun, 2001). The pathogens are able to infect and cause disease in species within the genus *Brassica* such as cabbage, turnip, broccoli and cauliflower (Gugel & Petrie, 1992).

In addition to large losses in agricultural production, these fungi can also cause allergic reactions in humans. It is believed that in Poland, with its very high concentrations of spores, the

fungus can be harmful in regions with intensive oilseed rape cultivation such as Kujavia. According to Emsl Analytical, Inc. (2011), fungi belonging to the *Leptosphaeria* genus may cause type I and II allergic responses. This was confirmed in studies in New Zealand, where 5% of people exposed to spores of this genus had allergic symptoms (Hasnain, 1993). The life cycles of *L. maculans* and *L. biglobosa* are similar (Kaczmarek & Jedryczka, 2011). For both species, the primary mode of dissemination is by wind-dispersed ascospores released from pseudothecia formed on infected stubble in the autumn (Hall, 1992). Ascospores deposited on leaves of newly sown oilseed rape plants germinate and invade the leaf via stomata or wounds (Hammond et al., 1985; Huang et al., 2003). After initial penetration, *L. maculans* and *L. biglobosa* colonise the palisade layer biotrophically, then become necrotrophic

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behind the hyphal front, resulting in large lesions with abundant pycnidia. Ascospores are released from the infected stubble for most of the growing season, causing lesions on newly emerging leaves. Following initial infection of the leaf, *L. maculans* colonises the leaf beyond the visible leaf lesion, advancing through the intercellular spaces of the mesophyll cells, eventually reaching the petiole. The pathogen continues to grow down the petiole, mainly in the xylem vessels of the vascular bundle or in between the cells of the xylem parenchyma and the cortex, eventually reaching the stem (Hammond et al., 1985). At the stem base, the nutritional mode of the fungus changes but once at the stem base; invasion and death of stem cortex cells take place, resulting in cankers (West et al., 2001). After harvest, the pathogens rapidly colonise crop debris and complete their life cycles by sexual out-crossing, followed by the production of pseudothecia and ascospores (Petrie et al., 1995).

Crop disease control consists mostly of the use of resistant cultivars, stubble management, fungicide application and a high level of agrotechnology (Kutcher et al., 2011). Among management practices, the most important were crop rotation and tillage (Guo et al., 2008) and, if possible, greater spatial distribution of fields (Marcroft et al., 2004). As ascospores and splash-dispersed pycnidiospores (asexual spores) constitute inocula in phoma disease epidemics (West et al., 2001; Ghanbarnia et al., 2009), information on spore concentration and seasonal discharge pattern is crucial both for understanding the epidemiology of phoma stem canker and to design appropriate strategies for disease management. Subsequent steps of pathogen development greatly depend on meteorological parameters. It is well known that the process of pseudothecial maturation and ascospore release is highly weather dependent, particularly on rainfall and temperature (Salam et al., 2003, 2007; Toscano-Underwood et al., 2003; El Hadrami et al., 2009).

The ascospores of *Leptosphaeria* belong to the group of 'wet spores', which means that their release from pseudothecia is triggered by rainfall (Grinn-Gofron & Mika, 2008). Subsequently, the spores land on wet leaves and initiate infection. The effect of the weather on patterns of ascospore release differs between countries (Huang et al., 2005). There is a great need to study the influence of meteorological factors on pathogen development to improve forecasting the appearance of inoculum in a particular season and region. Moreover, regions also differ in the proportion of the inoculum produced by *L. maculans* and *L. biglobosa* (Fitt et al., 2006); this phenomenon was evident in Poland (Jedryczka, 2007; Kaczmarek et al., 2009, 2011; Dawidziuk

et al., 2010a). In the western part of Poland, the predominant species was *L. maculans*. The climate of this zone is mild compared to the central and eastern part of the country, which have much colder, continental climate. This coincides with the predominance of *L. biglobosa* (Jedryczka, 2007). In view of the fact that the climate in eastern and central Poland is much colder and crop development significantly lags plant growth in the west, there was a need to undertake studies in eastern and central Poland on the impact of weather on the release of spores from the genus *Leptosphaeria*.

The aim of this work was to determine the meteorological conditions required for triggering the release of *Leptosphaeria maculans* and *L. biglobosa* ascospores in central and eastern Poland. To achieve this, statistical calculations such as multiple regression and correlations, were employed on a set of spore and weather data. The materials were obtained at four experiment sites located in different climatic regions of east Poland, over four consecutive autumn seasons.

Material and methods

Evaluation of ascospore concentration in air samples

The analysis of the spore count was performed based on data collected at four experimental sites located in central and eastern Poland (Figure 1) for four consecutive autumns (1 September–30 November) in the years 2005–2008. Over the four years of the study, 1584 samples were collected. Each experimental site was located in a different climatic region based on the climate zones of Poland described by Wiszniewski and Chechlowski (1987). The experimental sites were Drogosze in the Warmia-Mazuria region (north-eastern Poland), Glebokie in the Kujawy-Pomerania region (central Poland), Pulawy in the Lublin region (eastern Poland) and Krasne at the Carpathian Foothills (south-eastern Poland).

Air sampling was done by using volumetric samplers (Burkard Manufacturing Co. Ltd, Rickmansworth, UK, and Lanzoni s.r.l., Bologna, Italy). Spore traps were surrounded by infected oilseed rape stubble collected at the end of the previous season and kept outdoors exposing it to the typical weather conditions of the region. After each week, tapes were cut half longitudinally and transversely in seven equal parts representing seven days (24 h-periods) of each week. Ascospore concentration was expressed as daily average spore concentration per cubic metre of air, according to the formula published by Lacey and West (2006). The spore data were analysed to determine the start, end and duration of the season using the 95% method. The start

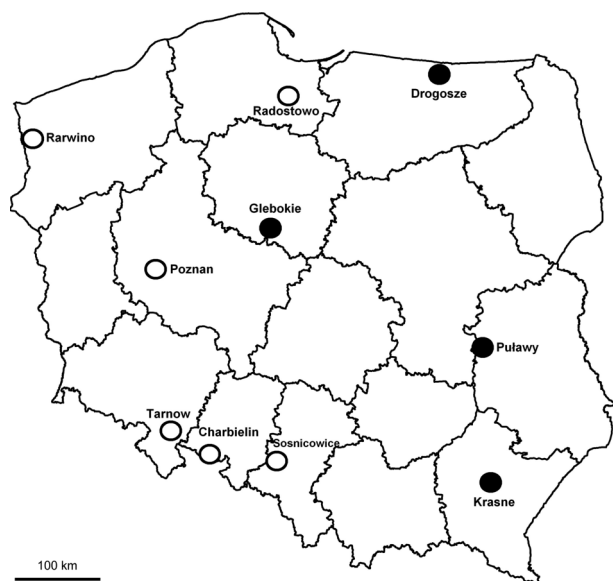


Figure 1. Location of the experimental sites considered in this study (for coordinates, etc. cf. Table I). White dots: reference sites; black dots: experimental sites.

of the season was defined as the date when 5% of the seasonal cumulative spore count was trapped and the end of the season as the day of the year when 95% of the seasonal cumulative spore count was reached. The studies took into account the maximum concentration of *Leptosphaeria maculans* and *L. biglobosa* ascospores in air samples.

Meteorological data

Meteorological data were provided by the weather stations from the Central Station for Variety Testing COBORU in Slupia Wielka and Trax Elektronik Company in Cracow. The parameters considered in the assessment of the effect of meteorological conditions on the airborne fungal spores were the daily level of precipitation and the mean daily air temperature at 2 m height of the years 1998–2008 (Table II).

Statistical analyses

To perform a detailed analysis of the impact of weather conditions on ascospore release, data from the entire country were examined. This paper describes the results obtained for eastern and central Poland. The statistical relationship between spore concentration and meteorological factors was established using the programs Statistica 7.0 (StatSoft, Inc., Tulsa, USA), SAS Systems for Windows 6.12. (SAS Institute, Inc., Cary, USA) and the Excel application of the Microsoft Office 2003 package (Microsoft, Inc., Redmond, USA). The null hypothesis that a sample came from a normally distributed

population was analysed with the Shapiro–Wilk test. Significant variables for spore release were analysed using stepwise regression. Regressions were performed for 54 different variables of meteorological measurements from four experimental sites for the years 2005–2008. The data chosen for the statistical calculations contained the numbers of days with rainfall events and cumulative rainfall, decade (10 days) by decade, starting from 1 July till the end of November and all variants of decades joined from two to 15. The latter variable contained all days in the period from 1 July–30 November. The same scheme of selection of variables concerned the mean temperature and solar radiation (Table II). Mean squared error of prediction (MSEP) procedure was used as a criterion of the models' predictive quality (Aubertot et al., 2006). The values of this criterion range from zero to one, where zero means no differences between the predicted and observed data and one if the model is totally unrealistic. Due to normal distribution of the data, confirmed by the Shapiro–Wilk test, observations and simulations were compared using the Pearson correlation method ($p = 0.05$).

Results

Evaluation of ascospore concentration in air samples

The date of the beginning and the end of the season differed between sample sites in several seasons. The earliest ascospore release was observed in Drogosze and Glebokie in the autumn of 2007. The observation was made on 6 September, 249 days after

Table I. Monitoring sites and years of data collection of ascospores of *Leptosphaeria maculans* and *L. biglobosa*.

Monitoring site (region)	Geographical coordinates	Years of study
Poznan (Wielkopolska) ^a	52° 22' 52" N, 16° 53' 54" E	1998–2008
Rarwino (West Pomerania) ^a	53° 55' 35" N, 14° 50' 11" E	2004–2008
Radostowo (Pomerania) ^a	53° 59' 27" N, 18° 43' 50" E	2004–2008
Sosnicowice (Silesia) ^a	50° 16' 19" N, 18° 31' 37" E	2004–2008
Tarnow (Lower Silesia) ^a	50° 34' 42" N, 16° 47' 26" E	2004–2008
Charbielin (Opole) ^a	50° 20' 37" N, 17° 25' 49" E	2004–2008
Pulawy (Lublin) ^b	51° 25' 39" N, 21° 57' 14" E	2005–2008
Krasne (Carpathian Foothills) ^b	50° 01' 46" N, 22° 01' 05" E	2005–2008
Drogosze (Warmia-Mazuria) ^b	54° 12' 34" N, 21° 14' 11" E	2005–2008
Glebokie (Kujawy-Pomerania) ^b	52° 38' 40" N, 18° 26' 16" E	2005–2008

^aExperimental sites used for mathematical modelling.

^bExperimental sites studied with the model.

Table II. Meteorological data recorded in the surveyed regions (data according to Dmochowska, 2007, 2008, 2009, 2010).

Measurement	Year	Region			
		Kujawy-Pomerania	Warmia-Mazuria	Lublin	Carpathian Foothills
Average temperature (°C)	2005	8.6	7.6	7.8	8.3
	2006	8.9	8.1	8.0	8.7
	2007	9.5	8.6	8.8	9.4
	2008	9.5	8.6	8.9	9.6
Average air temperature (°C) during the period of fungal development (July–October)	2005	15.8	14.7	15.1	15.3
	2006	16.9	16.0	16.1	16.5
	2007	14.6	14.1	14.4	14.9
	2008	14.9	14.6	14.6	15.3
Total annual precipitation (mm)	2005	459	518	559	774
	2006	503	632	521	612
	2007	666	757	662	692
	2008	512	666	649	736
Total precipitation (mm) during the period of fungal development (July–October)	2005	111	254	180	302
	2006	249	259	225	169
	2007	311	272	317	344
	2008	265	344	285	332
Solar radiation (hours)	2005	2460	1810	1890	2107
	2006	2502	1722	1862	2284
	2007	2306	1690	1807	1909
	2008	2241	1670	1764	2083
Area of the region in km ²		1 797 169	2 417 346	2 512 250	1 784 573
Area of oilseed and turnip rape in km ² (percentage area of the region)		119 400 (6.6%)	60 800 (2.5%)	29 300 (1.2%)	13 000 (0.7%)

the beginning of the year. The latest start of the ascospore season was observed in Drogosze in 2005 (day 273, 30 September). This phenomenon did not coincide with the dates of the end of the season; the earliest end of the spore season was noted on day 305 both in Krasne in 2006 (1 November) and in Glebokie in 2008 (31 October). In contrast, the last day of the ascospore season was observed on day 335 (1 December); this situation was reported in Pulawy, in 2006. Large differences were also observed between seasons. The greatest differences were observed in Drogosze; the earliest start of the season was noted in 2007 on day 249 (6 September) but in 2005, it was as late as day 273 (30 September). The difference between these seasons was 24 days (Table III).

The number of days with ascospores detected in air samples also differed between experimental sites. Ascospores were detected for only 16 days at Krasne (2007), located in the very east of Poland, but as long as 63 days at Glebokie (2006), located in Kujavia, the centre of Poland. Large variation in the number of days with airborne ascospores was also found between seasons. The highest variation was found in Glebokie, where it ranged from 29 days in 2008 to 63 days in 2006 (Table III).

The earliest day of the maximum concentration of ascospores in the air was observed in Pulawy

in 2007 on day 262 (19 September). The latest detection of the highest peak of the ascospores was in Glebokie in 2005 at day 311 (7 November). Such a great difference (49 days) observed at a single location, clearly indicates the necessity of developing decision support systems to monitor inoculum of *Leptosphaeria maculans* and *L. biglobosa* in air samples. The extreme differences in the number of ascospores collected in the air ranged from 9 spores/m³ observed in Krasne in 2007 to 1404 spores/m³ in Glebokie in the same year (Table III).

Weather variables influencing ascospore release

Weather variables with the most significant effect on the ascospore release of *Leptosphaeria maculans* and *L. biglobosa* were the number of days with rainfall in the first ten days of July and the cumulative rainfall from 1 to 30 July. Those two variables explained approximately 77% ($R^2 = 0.77$) of the variability connected to the day of the first ascospore release. MSEP used to check prediction quality of the regression for the three variables chosen by stepwise regression showed that the error of prediction was 0.045 only (Table IV). There was a high correlation ($r = 0.88$) between simulation and observation (Figure 2). These calculations were

Table III. Characterisation of the spore season of *Leptosphaeria maculans*/L. *biglobosa* in central and eastern Poland over four years of study.

Year	Experiment site			
	Pulawy	Krasne	Drogosze	Glebokie
<i>Start of the season (Julian days)</i>				
2005	273 (30 Sep)	260 (17 Sep)	273 (30 Sep)	272 (29 Sep)
2006	270 (27 Sep)	257 (14 Sep)	257 (14 Sep)	259 (16 Sep)
2007	256 (13 Sep)	256 (13 Sep)	249 (6 Sep)	249 (6 Sep)
2008	255 (12 Sep)	—	262 (19 Sep)	255 (12 Sep)
Mean	263.5	257.7	260.3	258.8
Confidence interval	263.5 ± 7.9	257.7 ± 1.7	260.3 ± 8.5	258.8 ± 8.3
Median	255.5	256.0	254.6	252.0
<i>End of the season (Julian days)</i>				
2005	317 (13 Nov)	331 (27 Nov)	321 (17 Nov)	329 (25 Nov)
2006	335 (1 Dec)	305 (1 Nov)	334 (30 Nov)	331 (27 Nov)
2007	322 (18 Nov)	328 (24 Nov)	326 (22 Nov)	330 (26 Nov)
2008	329 (25 Nov)	—	333 (29 Nov)	305 (1 Nov)
Mean	325.8	321.3	328.5	323.8
Confidence interval	325.7 ± 6.7	321.3 ± 11.4	328.5 ± 5.2	323.7 ± 10.6
Median	323.9	321.3	327.3	329.5
<i>Number of days spores were detected during the season</i>				
2005	29	27	31	33
2006	22	24	57	63
2007	44	16	34	61
2008	30	—	27	29
Mean	31.3	22.3	37.3	46.5
Confidence interval	31.2 ± 7.8	22.3 ± 4.5	37.2 ± 11.4	46.5 ± 15.3
Median	29.5	24.0	32.5	47.0
<i>Day of maximum spore concentration</i>				
2005	301 (28 Oct)	276 (3 Oct)	297 (24 Oct)	311 (7 Nov)
2006	288 (15 Oct)	278 (5 Oct)	272 (29 Sep)	272 (29 Sep)
2007	262 (19 Sep)	294 (21 Oct)	266 (23 Sep)	292 (19 Oct)
2008	291 (18 Oct)	—	302 (29 Oct)	289 (16 Oct)
Mean	285.5	282.7	284.2	291.0
Confidence interval	285.5 ± 14.1	282.7 ± 7.9	284.2 ± 15.2	291.0 ± 13.6
Median	289.5	278.0	284.5	290.5
<i>Summarised seasonal number of spores per m³ air</i>				
2005	9.7	11.9	13.6	26.6
2006	9.0	10.0	139.7	1038.5
2007	560.0	8.9	36.8	1403.6
2008	182.7	—	27.2	218.3
Mean	190.3	10.2	54.3	671.7
Confidence interval	190.34 ± 220.4	10.25 ± 1.2	54.32 ± 48.9	671.75 ± 556.9
Median	96.2	9.9	32.0	628.4
<i>Maximum spore concentration per m³ air</i>				
2005	1.1	2.2	1.9	5.3
2006	1.2	1.5	19.4	183.4
2007	159.7	2.5	6.2	293.3
2008	53.7	—	7.9	44.6
Mean	53.9	2.1	8.9	131.6
Confidence interval	53.9 ± 63.4	2.1 ± 0.4	8.9 ± 6.3	131.3 ± 112.1
Median	27.5	2.22	7.1	114.0

based on three variables and include the cumulative rainfall between 1 and 30 September. However, the impact of this variable is extremely low (0.052), and we suggest not including it in further investigations (Table IV).

The stepwise regression calculated for the day of the maximum ascospore concentration in the air pointed to three variables: cumulative rainfall

from 1 to 10 July, cumulative rainfall from 21 to 30 September and the number of days with rainfall from 1 to 10 August. These three variables explained 86 of the variability ($R^2 = 0.86$). MSEF was low (0.06; Table IV). Based on these three variables, the Pearson's correlation procedure indicated a high correlation ($r = 0.81$) between simulation and observation (Figure 3).

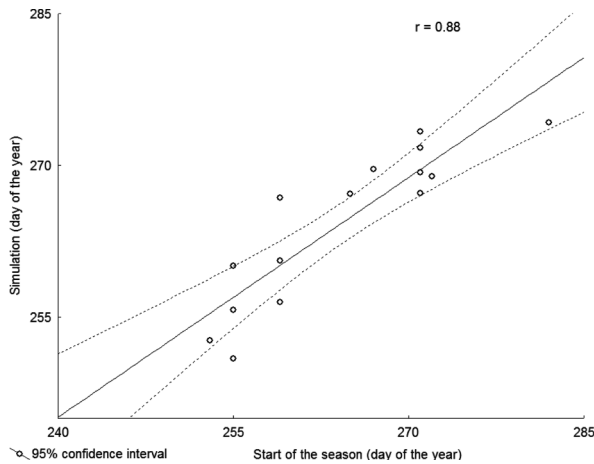


Figure 2. Comparison between simulation and observed start of ascospore release of the *Leptosphaeria maculans*/*L. biglobosa* species complex.

Discussion

This work indicates that the time of the first ascospore release, the date of maximum spore concentration in the air and the quantity of spores is highly dependent on weather conditions and varies among regions and seasons. In this study, the first detection of the ascospores of *Leptosphaeria maculans* and/or *L. biglobosa* was observed on average in mid-September. These data are consistent with the results obtained in west Poland (Kaczmarek & Jedryczka, 2008a, 2008b) as well as in the Czech Republic (Jedryczka et al., 2010). In the UK, experiments performed in years 1998–2003 showed that the beginning of ascospore release occurred later in the autumn, usually at the end of September (Huang et al., 2005). The end of ascospore release was noted on average in mid-November. However, the dates of start and end of the season varied greatly among years and regions and in many cases, they fell significantly outside the estimated confidence intervals. Among experimental sites and seasons, the average number of days when spores were observed ranged from 22 to 47. Moreover, large differences among seasons were observed at the same experimental site. On average, the day of maximum ascospore concentration of *L. maculans* and *L. biglobosa* ranged from 8 October (day 283) to 17 October (day 291). Again, these dates varied greatly and in many cases were outside of the confidence interval.

Based on the mathematical models applied, it was clear that weather conditions influence the growth and development of the fruiting bodies and the sporulation of the pathogens (Aubertot et al., 2006; Salam et al., 2007). At present, most models are based on average daily temperature and precipitation. In France, a simple model forecasting ascospore

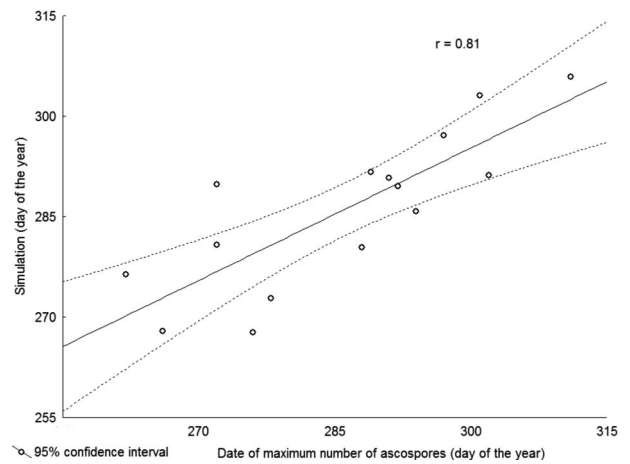


Figure 3. Comparison between simulation and the actual day with the maximum number of ascospores.

release indicated that the process takes place after the occurrence of 16 to 19 rainy days following harvest (Peres et al., 1999). In Australia, a system based on temperature and precipitation indicated that the risk of spore release occurs 43 days after harvest, if the temperature is lower than 22 °C and precipitation is equal or higher than 4 mm (Salam et al., 2003). In England, mathematical models indicated that rainfall in August and September was closely associated with stem canker of brassicas (Gladders & Symonds, 1995). Gladders and Symonds (1995) also reported that of the numerous parameters connected with temperature and solar radiation, the three most important variables were always connected with precipitation, either calculated as cumulative rainfall over a certain period of time or the number of wet days.

Work under controlled conditions and in the field showed that in England 130 to 169 °C-days (thermal time) were needed to proceed from infection to lesions appearing on leaves (Biddulph et al., 1999). Similar work in Poland indicated that from time of infection to visible symptoms on leaves required 278 to 387 °C-days (Huang et al., 2005). In the UK, from the appearance of leaf lesions to stem cankers on oilseed rape plants, 1120 to 1240 °C-days were recorded. At present, several models predicting the incidence of stem canker have been proposed based on the combined effects of moisture factors and temperature (Salam et al., 2007). They also confirmed the important effect of rainfall in pathogen development and subsequent disease symptoms. These models recognise ascospores as primary inocula, however, an important role in pathogen dispersal in Canada has also been attributed to pycnidiospores (Ghanbarnia et al., 2009).

It was found that the number of days where spores were present in air and the highest concentration of spores depended on the acreage of oilseed rape in the region. Within Kujavia in central Poland, oilseed rape occupied 6.6% of the province (Dawidziuk et al., 2008, 2010b). At other locations, the acreage of oilseed rape was less than 3%. In Kujavia, the highest temperature and greatest solar radiation were recorded, but there was no direct relationship between these variables and ascospore release or concentration. No direct relationship between the average annual or seasonal temperature or rainfall on the development of *Leptosphaeria maculans* and *L. biglobosa* was found. The frequency and amount of rainfall over short periods (several days) were more important than annual or seasonal rainfall. The first ascospore event depended mainly on the number of days with rainfall from 1 to 10 July and the cumulative rainfall in July. The start of ascospore release by *L. maculans* and *L. biglobosa* usually took place in mid-September. It is therefore quite puzzling why the third variable, chosen as important for this maturation process, was the cumulative rainfall over the whole September. In this case, however, the partial R^2 was very low, which means that this parameter played a minor role in the release of the first ascospores of *Leptosphaeria*.

The MSEP, which is the criterion to compare the observed and simulated values, calculated for the three top variables, was minute (0.045), which

Table IV. Variables chosen by stepwise regression as the most important for start of the season and the day with the maximum ascospore concentration.

Variable	Partial R^2	Model R^2	F	p -level
<i>Start of the season^a</i>				
Number of days with rainfall 1–10 July	0.465	0.465	13.202	<0.001
Cumulative rainfall 1–30 July	0.307	0.772	20.596	<0.001
Cumulative rainfall 1–30 September	0.052	0.824	6.593	0.018
MSEP		0.045		
<i>Day with the maximum number of spores</i>				
Cumulative rainfall 1–30 September	0.560	0.560	4.575	0.002
Cumulative rainfall 1–10 July	0.254	0.714	6.273	0.005
Number of days with rainfall 1–10 August	0.145	0.859	4.671	0.009
MSEP		0.060		

^aAccording to the 95% method.

confirms very high predictive quality of the selected variables. These results are consistent with the knowledge on the development of *Leptosphaeria maculans* and *L. biglobosa* (Huang et al., 2003). Results presented in this paper clearly show that the quantity of rainfall in July greatly affected time required for pseudothecial maturation and rainfall in September triggered ascospore release from fruiting bodies. For the highest airborne ascospore concentration, the most important variables detected in this experiment were cumulative rainfall in the beginning of July and at the end of September and the number of wet days in the beginning of August. The MSEP value for the predictive quality of these three variables was 0.06. The results confirmed that wet straw provided better conditions for the development of pathogen fruiting bodies than dry infected oilseed rape stubble.

At present, all models based on weather data start their calculations after the harvest. The rationale is based on the hypothesis that the development of pseudothecia begin after a certain number of highly wet and moderately warm days following harvest. This paper illustrates the importance of the period between 1 and 10 July for the first time. In Poland, the first ten days of July always preceded the harvest. Hence, it can be concluded that the maturing, but still moist and living stems are in an ideal condition for the pathogen to quickly overgrow stem tissues and form pycnidia what, in turn, directly influences the earlier and more abundant formation of pseudothecia with the ascospores of *Leptosphaeria* species. The number of days with rainfall had a greater influence on day of detection of the first ascospores than the cumulative rainfall, which means that it was more desirable for the fungus to receive regular rain. The cumulative rainfall in the first ten days of July was more important than the number of wet days in the estimation of the date of the maximum ascospore concentration. This means that the release of ascospores requires higher straw moisture than the establishment and the development of the pathogen on oilseed rape stubble.

However, to determine the day with maximum spore release, the cumulative rainfall at the end of September was much more useful. This result is in full agreement with the empirical observations showing that the day with the maximum ascospore release was usually in October or even November (66.7% of the days), although sometimes at the very end of September (28.2%). In 5.1% of the cases, it was earlier, usually in the second half of September (detailed data not presented here). Comparison of the data obtained in western, central and eastern Poland does not support the hypothesis for differences in time of the maximum concentration of the *Leptosphaeria* ascospores in air samples. On the one hand, the percentage of site-years when the

maximum ascospore concentration was observed in October or November, was 10.8% more frequent in the east, but the average number of days from the beginning of the year until mass ascospore release was almost identical on the other hand, with 288 days in west Poland and 286 in central and east Poland, based on 24 and 15 site-years of data from the respective areas.

The quantity of rainfall in the beginning of August and at the end of September influenced ascospore shower dynamics and the development of fungal mycelium inside oilseed rape plants. This data led to the production of new fruiting bodies and the formation of new ascospores. This study provided data necessary to develop a precise model to forecast the first and the maximum release of *Leptosphaeria maculans* and *L. biglobosa* ascospores in central and eastern Poland. The accuracy of the elaborated model was sufficient to provide advice to farmers in order to improve the timing of the fungicide application used for the control of blackleg on oilseed rape.

Conclusion

The beginning of ascospore release and the day of maximum airborne ascospore concentration of *Leptosphaeria maculans* and *L. biglobosa* depend on rainfall in the period preceding the collection of oilseed rape plants in the previous season. Using stepwise regression, we have demonstrated the importance of cumulative precipitation in the last ten days of September; the abundance of this rainfall directly influenced the time of the peak ascospore release, subsequently leading to more severe stem canker epidemics.

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