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The maturation rate of the generative stage of *Leptosphaeria maculans* and *Leptosphaeria biglobosa* in central and eastern Poland

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Abstract. Two pathogens from the genus *Leptosphaeria*: *Leptosphaeria maculans* and *Leptosphaeria biglobosa* are the cause of stem canker – a damaging disease of oilseed rape in Poland. The fungi belong to *Ascomycetes,* with both sexual and vegetative stages in their life cycle. The fruiting bodies of the teleomorph, called pseudothecia produce ascospores, which are the primary inoculum infecting young plants. The formation of pseudothecia takes place on infected stubble from a previous season's crop. Conducive weather conditions lead to ascospore release and development of disease symptoms. The main goal of the experiments was to evaluate the uniformity of pseudothecia maturation rate within and between the climatic regions as well as the relation between the development of pseudothecia and ascospore release of *L. maculans* and *L. biglobosa*. The study was done for three years (2005–2007) at 7 experiment sites located in three climatic regions of central and eastern Poland, with Puławy located centrally on a borderline between these regions. The maturation of pseudothecia was based on the morphology of asci and ascospores. The monitoring of ascospore density in the air was done using a seven-day volumetric spore trap. We have found that at early maturation stages the differences in pseudothecia maturation were small and did not exceed 2 weeks, but at advanced maturation stages the highest differences exceeded a month. The temperature profiles in studied locations were highly correlated, but the correlations between the rainfall data were sometimes very low or non existent. The differences in pseudothecial maturation were mainly associated with the quantity of precipitation both before and during the maturation process. Ascospore release greatly depended on the rate of pseudothecial maturation of *L. maculans* and *L. biglobosa.*

key words: *Leptosphaeria maculans, Leptosphaeria biglobosa,* stem canker, pseudothecial maturation, ascospore release, volumetric spore trap

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INTRODUCTION

Oilseed rape (*Brassica napus* L.) is currently one of the fastest expanding crops worldwide. According to the prognosis of OPEC, in 2008/2009 the world production of this crop will increase by ca. 9% and it shall soon reach 53 M tons (World Oil Outlook, 2007). European Union is at present the biggest producer of oilseed rape seeds and oil. In the season 2007/2008 it produced 18.2 Mt, which constituted 37.8% of the overall production of rapeseed. Such great increase of oilseed rape production and its common use in crop rotation brings the necessity to introduce new agronomic systems and to solve numerous problems connected with crop protection against different pests, including weeds, insects and plant pathogens. The most damaging pathogens of oilseed rape are fungi, with different importance of various species depending on the area of the world. From among 2.5 million farms in Poland about 278 thousands are situated in Lublin voivodship. The acreage and yield of oilseed rape in this region in 2006 were respectively 28.6 thousands hectares and 66 thousand tons (Dmochowska, 2007).

The increasing profitability of oilseed rape in agricultural production implies constant search of methods allowing to increase plant yield and quality. One of the most important conditions of high yield is the protection against fungal diseases. The most damaging disease of oilseed rape is stem canker of brassicas caused by the species *Leptosphaeria maculans* and *L. biglobosa.* Serious crop losses were reported in Europe (Fitt et al., 1997; Allard et al., 2002; West, 2001, 2004; Jędryczka, 2007), Canada (Gugel, Petrie, 1992) and Australia (Salisbury et al., 1995; Khangura, Barbetti, 2001).

Leptosphaeria is an ascomycete with both sexual and vegetative stages in its life cycle. The fungus produces fruiting bodies of the teleomorph (pseudothecia) which contain ascospores. In Poland, ascospores are the primary inoculum, which infects young rapeseed plants in the autumn (Kaczmarek et al., 2006). The formation of pseudothecia takes place on infected stubble from a previous season's crop (Petrie, 1994, 1995). Pseudothecium must reach a complete maturity to produce and release fully developed eight ascospores, each consisting of six cells. Released ascospores can be transferred by wind to several kilometres and infect plants far away from the source of disease. The maturation rate of pseudothecia and subsequent spore release depend on weather conditions, principally wetness (rain or dew) and temperature (Salam et al., 2003; Toscano-Underwood et al., 2003; Huang et al., 2005). Conducive weather conditions in summer and autumn lead to ascospore release and development of disease symptoms. Severity of the disease and symptoms of infection highly depend on date of infection and growth rate of the fungus inside plant tissues.

The aim of this study was to evaluate differences between pseudothecia maturation rates within and between the climatic regions of central and eastern Poland. We have also studied the relation between the development of pseudothecia and ascospore release of *L. maculans* and *L. biglobosa* at the same experiment site.

MATERIALS AND METHODS

Location of experiment sites

The area of study was composed of three climatic zones located in central and eastern Poland, as proposed by Wiszniewski and Chełchowski (1987): Region 1) Lublin and Zamość; Region 2) Mazovia Plain, and Region 3) Łódź-Masłowice Highland (Fig. 1). The samples of oilseed rape stubble originated from 7 experiment sites located in: Region 1) Kościerzyn (N 51°62'36.23", E 18°64'61.60"), Masłowice (N 51°22'19.31", E 18°56'57.88"), Radom (N 51°39'92.35", E 21°15'13.48"); Region 2) Siedlce (N 52°16'77.89", E 22°27'29.32"); Region 3) Bezek (N 51°19'60.37", E 23°16'48.65"), Leśniowice (N 50°98' 37.30", E 23°49'30.36"), with Puławy (N 51° 42'75.47", E 21° 95'40.41") located in the middle of the three regions, exactly on a borderline. The highest distance between Puławy and the experiment site (Masłowice) was 240 km westwards and Leśniowice located 115 km eastwards of Puławy. The highest distance to the north was the town of Siedlce, located 85 km from Puławy (Figure 1).

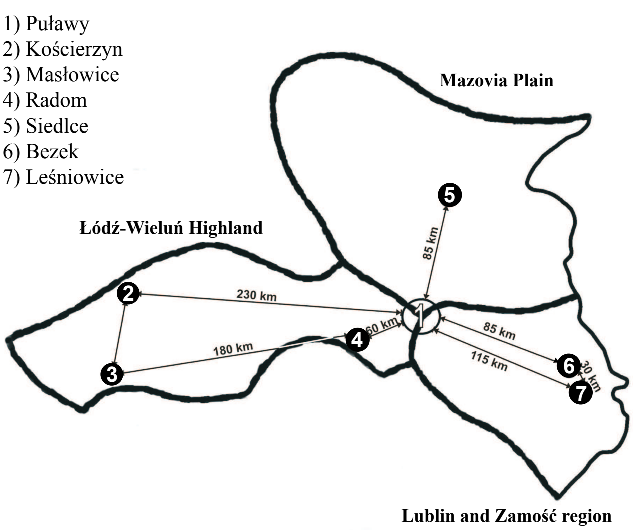

Fig. 1. Geographical location of experiment sites

Assessment of *Leptosphaeria maculans* and *Leptosphaeria biglobosa* pseudothecia maturation

The percent of *L. maculans* and *L. biglobosa* pseudothecia maturation was assessed on base of asci development stage and presence of ascospores. From five randomly chosen parts of infected oilseed rape stubble from the previous season's crop 10 fruiting bodies were isolated and microscope slides were prepared. Test samples were collected every seven days from 1 September until 30 November. The choice of this sampling period was selected on a base of previous results on pseudothecia maturation and ascospore release of *L. maculans* and *L. biglobosa* in the other regions of Poland. Pseudothecia maturation stages were classified to one of five categories (Toscano-Underwood et al., 2003):

- class A: pseudothecia without asci;
- class B: pseudothecia with asci, but without ascospores;
- class C: pseudothecia with asci, ascospores with 3–4 cells;
- class D: pseudothecia with asci, ascospores fully developed;
- class E: pseudothecia with empty asci.

Ascospore sampling

The *L. maculans* – *L. biglobosa* ascospore sampling was done at the same autumn period as the sampling of pseudothecia from oilseed rape stubble. The monitoring of ascospore concentration in the air was done using a Hirst type seven-day volumetric spore sampler (Burkard Manufacturing Co., Rickmansworth, UK). The trap was located at the grounds of the Institute of Soil Science and Plant Cultivation – National Research Institute in Puławy. The spore trap was surrounded by oilseed rape stubble infected with stem canker. The stubble was collected in the previous season at the Agricultural Experimental Station in Grabów and the Experimental Station in Osiny. The spore sampler was operated according to the instructions of Lacey and West (2006). The Melinex tape from the spore trap was cut into 7 pieces, each 48 mm long. Each piece was mounted onto a microscope slide, stained with 0.1% (w/v) trypan blue in lactophenol and examined with a light microscope under 400x magnification (Zeiss Axiostar, Germany). The numbers of spores were re-calculated to daily ascospore numbers per 1 m^3 of air.

Table 1. Rainfall data at seven experiment sites and the correlation coefficients of mean daily rainfall in Puławy and the other experiment sites in July–November, 2005–2007.

Year	Month	Experiment site						
		Puławy	Radom	Siedlce	Leśniowice	Bezek	Masłowice	Kościerzyn
		Mean daily rainfall (mm)						
2005	July	4.8	5.3	1.5	2.5	2.5	n.d.	n.d.
	August	4.4	2.2	2.1	9.7	9.7	n.d.	n.d.
	September	6.6	1.9	1.3	5.4	5.4	n.d.	n.d.
	October	0.7	0.5	0.8	0.9	0.9	n.d.	n.d.
	November	2.0	1.3	3.6	1.2	1.2	n.d.	n.d.
2006	July	4.7	2.3	2.2	5.2	5.2	4.1	5.8
	August	10.9	6.9	6.4	12.7	12.7	3.8	3.8
	September	3.3	4.9	2.6	1.7	1.7	4.5	2.1
	October	3.8	3.6	2.3	1.7	1.7	5.3	4.1
	November	2.9	2.3	8.6	2.2	2.2	3.6	4.3
2007	July	4.7	2.6	1.7	5.2	2.4	2.9	2.9
	August	12.0	2.6	1.2	12.7	1.6	1.9	1.9
	September	4.3	4.3	2.7	1.7	3.2	2.7	2.7
	October	3.5	0.9	1.9	1.7	2.6	3.6	3.6
	November	2.9	1.8	2.5	2.2	7.2	7.1	7.1
		Number of rainy days						
2005	July	14	13	17	10	10	n.d.	n.d.
	August	9	12	16	9	9	n.d.	n.d.
	September	5	10	12	4	4	n.d.	n.d.
	October	5	11	15	7	7	n.d.	n.d.
	November	14	16	20	14	14	n.d.	n.d.
2006	July	4	5	6	5	5	4	12
	August	21	20	31	19	19	22	27
	September	3	7	19	4	4	5	25
	October	9	11	20	8	8	8	29
	November	12	14	22	13	13	14	29
2007	July	5	21	20	6	26	28	31
	August	20	11	12	19	24	23	27
	September	4	15	13	4	27	26	26
	October	10	14	12	8	29	31	31
	November	13	18	16	13	23	27	27
		Correlation coefficients between rainfall in Puławy and the other experiment sites						
2005	July	1.000	0.290	0.260	0.040	0.040	n.d.	n.d.
	August	1.000	0.740	0.430	0.790	0.790	n.d.	n.d.
	September	1.000	-1.000	0.400	0.500	0.500	n.d.	n.d.
	October	1.000	0.400	0.400	0.800	0.800	n.d.	n.d.
	November	1.000	0.090	0.090	-0.870	-0.870	n.d.	n.d.
2006	July	1.000	0.400	0.500	-0.400	-0.400	-0.400	-0.400
	August	1.000	0.060	0.050	0.550	0.550	0.280	-0.440
	September	1.000	0.400	0.500	0.500	0.500	-0.400	-0.400
	October	1.000	0.400	-0.400	0.740	0.740	0.890	0.700
	November	1.000	0.590	0.280	0.720	0.720	0.710	0.390
2007	July	1.000	-0.400	-0.500	0.400	0.280	-0.740	-0.740
	August	1.000	0.440	-0.060	0.550	0.550	-0.010	-0.010
	September	1.000	0.500	-0.500	0.400	0.280	0.500	0.500
	October	1.000	-0.300	0.500	0.740	0.740	-0.140	-0.140
	November	1.000	0.360	0.180	0.720	0.720	-0.070	-0.070

n.d. – no data

All correlation coefficients are significant at $P < 0.05$

Meteorological analysis

The rainfall (mm) and temperature (°C) data were gathered from 1 July until 30 November, except experiment sites at Kościerzyn and Masłowice in 2005, where failure of a meteorological station deprived us from the rainfall data. The mean distance between experiment point and meteorological station was 13 kilometres. Spearmann's correlation coefficient was used to calculate relationships between the temperature and between the rainfall at all experiment sites, with $P<0.05$. To check normality of used data Shapiro-Wilk test was used. All calculations were performed with the use of STATISTICA version 7.0 (Hill, Lewicki, 2007)

RESULTS

The fully mature pseudothecia of *L. maculans* and *L. biglobosa* were observed at all experiment sites over the whole monitoring period. At the earliest they occurred in 2007, when pseudothecia belonging to class D were found already at the first sampling on 5 September (Fig. 2c). In 2006 the same phenomenon was observed one week later (Fig. 2b). The latest production of fully mature pseudothecia was found in the autumn 2005, when class D pseudothecia were not observed before the third week of study, *i.e.* the second half of September (Fig. 2a). In most cases a rapid increase of maturation took place, but in general it coincided with the beginning of maturation; the earlier it was observed, the earlier increase of class D pseudothecia was found. The maturation pattern was similar for the most of experiment sites, although each location experienced the maturation rate specific for the place. In 2005 and 2006 the data found for Puławy greatly differed from the results found in the remaining experiment sites (Fig. 2ab), whereas in 2007 this phenomenon was reversed and class D pseudothecia in Puławy were found early and in big numbers (Fig. 2c). This coincided with the mean daily rainfall and the number of rainy days; in 2005 this parameter was 3.7 mm of rain per day and there were 47 rainy days in total from July to November. In 2006 these values were 5.1 mm and 49 days respectively, but in 2007 the rainfall parameters were the highest, with 5.5 mm of mean rainfall per day and as many as 52 rainy days (Table 1). The mean data for the first two months of the study were comparable – the lowest in 2005 (4.6 mm, 23 days), higher in 2006 (7.8 mm, 25 days) and the highest in 2007 (8.4 mm and 25 days).

The experiment site in Puławy, although located centrally to the remaining sampling sites (Fig. 1) showed different rate of *L. maculans* and *L. biglobosa* pseudothecia maturation process. In the first two years of this study most class D pseudothecia developed later (Fig. 3ab) and in 2007 they matured one to four weeks later at most experiment sites and thresholds. Moreover, the sampling place located in Leśniowice, namely *ca*. 115 km south-eastwards from Puławy (Fig. 1) was the place of the most similar data in 2005 and 2006, and greatly different from 2007. In all

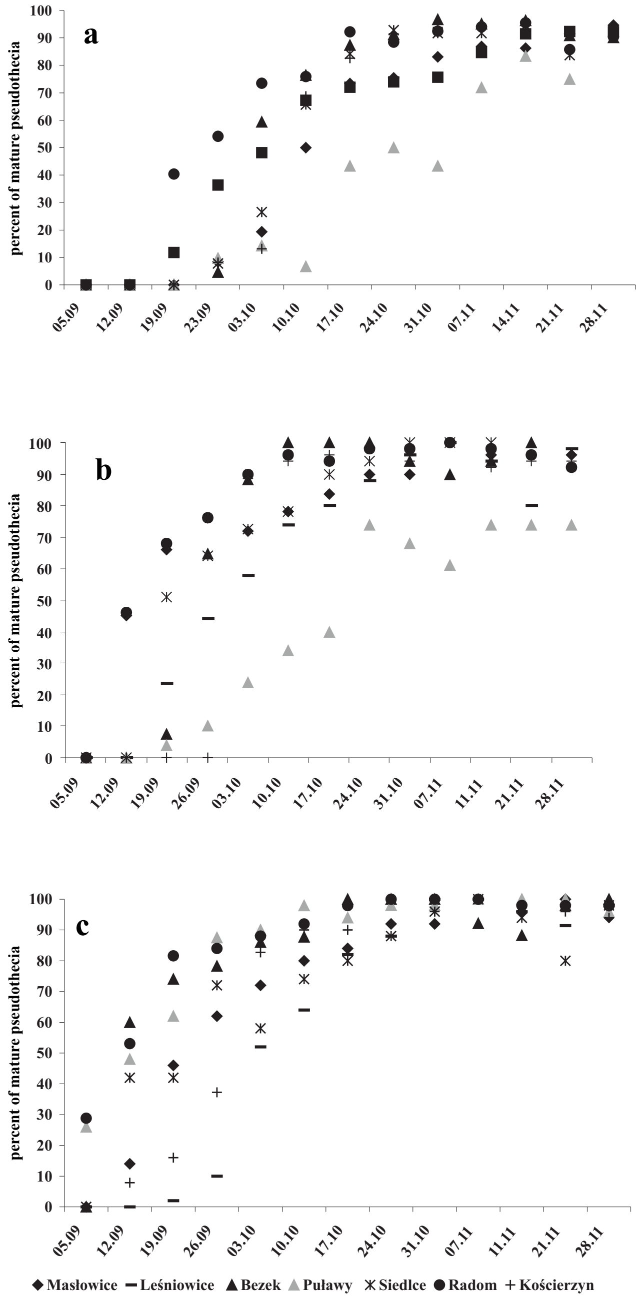

Fig. 2. The percent of mature pseudothecia of *L. maculans* and *L. biglobosa* on the stubble of oilseed rape at seven experiment sites in central and east Poland in: a) 2005, b) 2006, c) 2007.

cases the correlation coefficients between the temperature profiles of Puławy and of the other experiment sites were very high, they ranged from 0.728 to 0.984 (Table 2). It is however possible that subtle differences of daily rainfall and temperatures could affect the maturation process of generative stage fruiting bodies.

The differences between autumn periods were most clearly visible for the *L. maculans* and *L. biglobosa* as-

Table 2. The correlation coefficients of temperature profiles between Puławy and six experiment sites located in central and east Poland (July–November, 2005–2007).

Year	Month	Experiment site					
		Leśniowice	Radom	Siedlce	Masłowice	Kościerzyn	Bezek
2005	July	0.864	0.743	0.784	0.868	0.868	0.864
	August	0.920	0.845	0.814	0.784	0.784	0.920
	September	0.967	0.894	0.931	0.905	0.905	0.967
	October	0.982	0.811	0.877	0.915	0.915	0.982
	November	0.966	0.893	0.886	0.825	0.825	0.966
2006	July	0.948	0.795	0.775	0.780	0.870	0.948
	August	0.957	0.841	0.728	0.756	0.881	0.957
	September	0.924	0.831	0.887	0.875	0.809	0.924
	October	0.984	0.748	0.757	0.829	0.934	0.984
	November	0.938	0.732	0.731	0.759	0.747	0.938
2007	July	0.957	0.841	0.870	0.756	0.881	0.957
	August	0.841	0.881	0.881	0.957	0.957	0.957
	September	0.984	0.748	0.809	0.829	0.934	0.984
	October	0.748	0.934	0.934	0.984	0.984	0.984
	November	0.773	0.747	0.747	0.938	0.938	0.938

All correlation coefficients are significant at $P < 0.05$

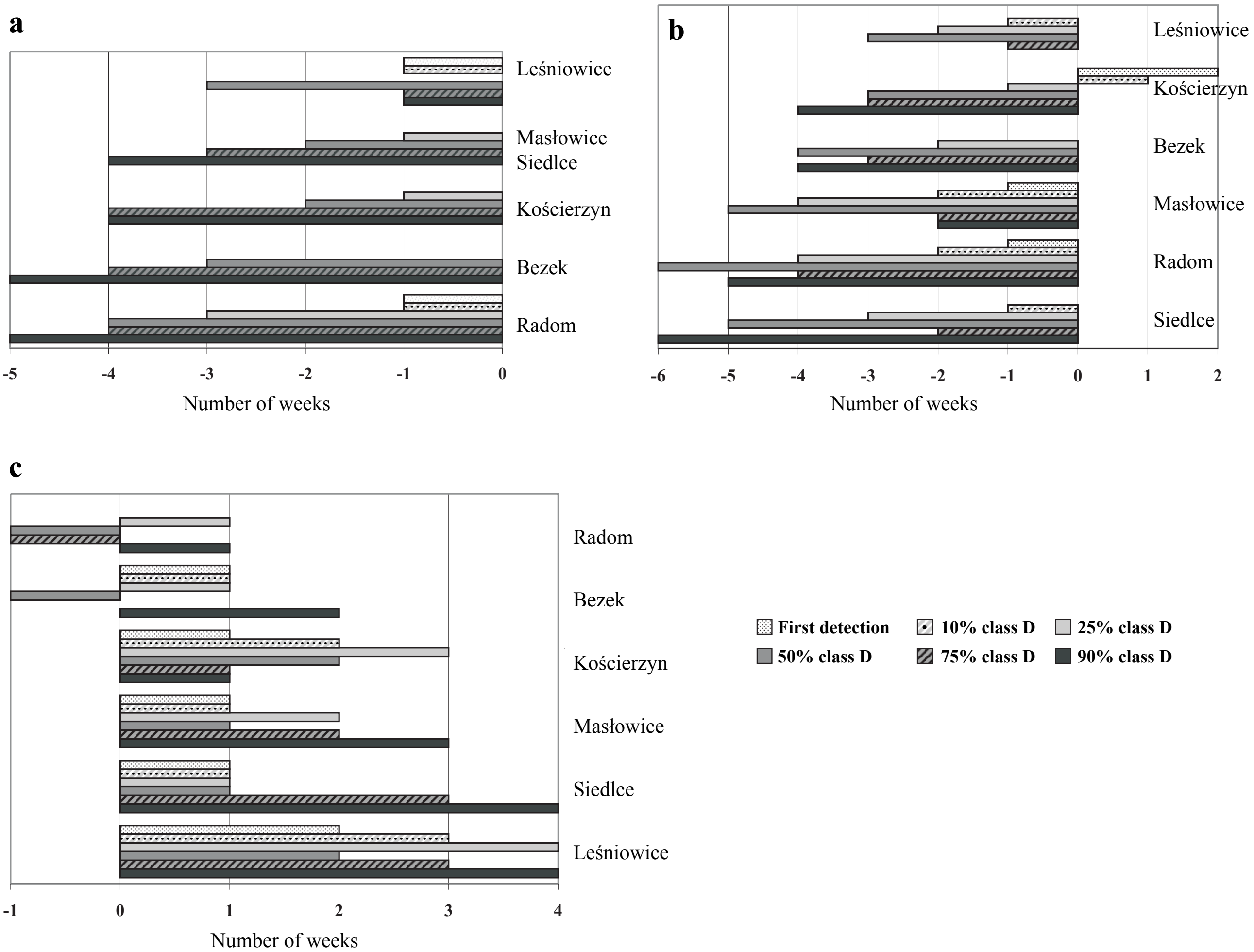

Fig. 3. The difference in maturation rate of class D pseudothecia between Puławy and the remaining experiment sites of study in autumn: a) 2005, b) 2006, c) 2007.

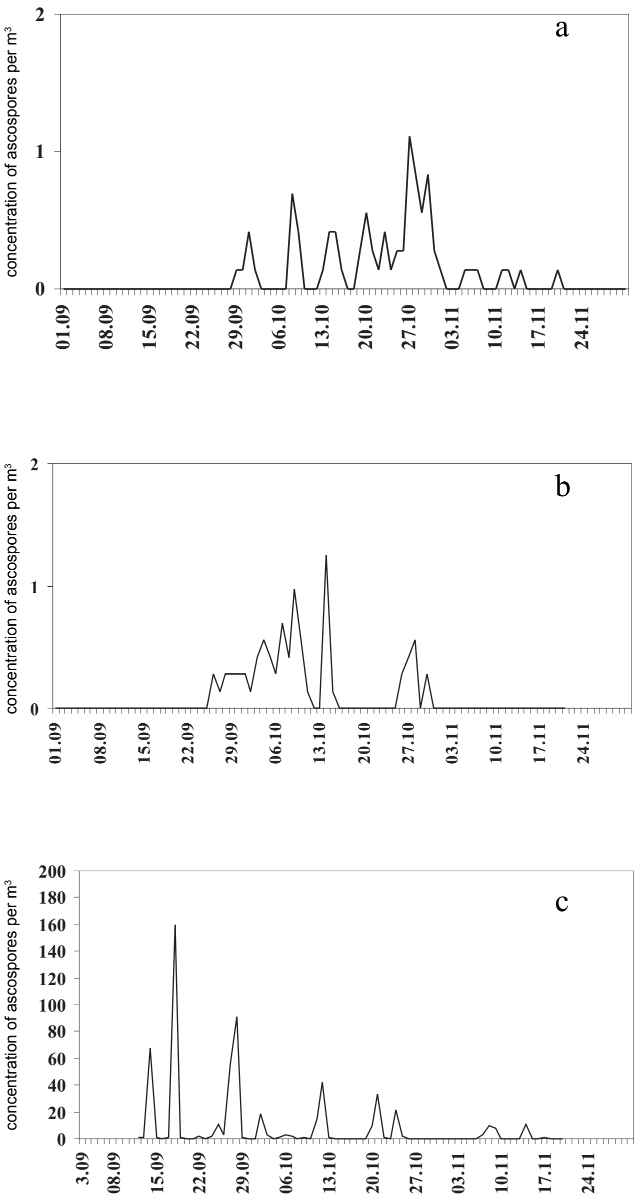

Fig. 4. The concentration of *L. maculans* and *L. biglobosa* ascospores in Puławy in: a) 2005, b) 2006, c) 2007

cospore density numbers. In 2005 and 2006 the daily concentrations of ascospores in the air was below 1, and in 2007 the peak ascospore day was *ca.* 160 ascospores per 1 m^3 (Fig. 4a-c).

In contrast to small differences of the temperatures and great similarities in temperature profiles (Table 2), great differences were observed in summary precipitation noted at different locations (Table 1). The lowest number of rainy days in the studied period was 44 days, found in Leśniowice and Bezek in 2005. The highest summary number of rainy days was noted in Kościerzyn, both in 2006 (122 days) and 2007 (142 days). The data cannot be compared to the same experiment site in 2005; the failure of the weather station did not allow us to calculate the results. Leśniowice and Puławy had the lowest numbers of rainy days.

DISCUSSION

Detailed data about *L. maculans* and *L. biglobosa* life cycles in different climatic regions allow to improve stem canker management through optimization of fungicide treatments. The ascospores of the studied species form primary inoculum infecting young plants of numerous Brassica crops, including oilseed rape. The formation of pseudothecia takes place on infected stubble from a previous season's crop (Petrie, 1995). In our hemisphere ascospores are mainly produced and dispersed in the autumn (Huang et al., 2005; Kaczmarek et al., 2006).

Suitable weather conditions in summer and autumn cause fast maturation of the fruiting bodies of *L. maculans* and *L. biglobosa*, leading to ascospore release and subsequent infections of host plants. Intensity of pseudothecial maturation of *L. maculans* and *L. biglobosa* depend on weather conditions, e.g. higher temperature within 5–20°C increases pseudothecia maturation (Toscano-Underwood et al., 2003). In susceptible plants the infections cause leaf spots. The fungus spreads from a leaf via a petiole to a stem (Hammond et al., 1985). The intensity of symptoms and severity of the disease depend on the date of infection and fungal growth rate in tissues of the host-plant, related to air temperature (Huang et al., 2003).

The experiments performed in the central and eastern part of Poland showed that pseudothecia maturation rate strongly depended on a cropping season. High and statistically significant correlation coefficients were obtained between temperature profiles originating from different monitoring sites. Even the lowest archived values were statistically significant. It is therefore postulated that – within an optimal temperature range – rainfall is the main factor responsible for pseudothecia maturation and subsequent ascospore showers. In all three years of this study the weather conditions were suitable to complete the maturation process of *L. maculans* and *L. biglobosa*. In most situations the pseudothecia of class D were observed in September. A rapid increase of fully matured pseudothecia was observed soon after their first detection.

The release of ascospores from pseudothecia is humidity and rainfall dependent (Huang et al., 2007; Oliveira et al., 2009). In this study, the amount of rainfall was doubled in 2006 and tripled in 2007, as compared to 2005. High rainfall coincided with the high number of *L. maculans* and *L. biglobosa* ascospores. This phenomenon was mainly observed in 2007 when much higher rainfall triggered abundant maturation of class D pseudothecia, followed by high concentrations of the ascospores, greatly outnumbering the negligible amounts of fungal spores in air samples, observed in 2005 and 2006. The results of this study confirm the finding of Toscano-Underwood et al. (2003), that an influence of wetness is more crucial than temperature.

Nevertheless, simple dependency of pseudothecia maturation and ascospore release on rainfall was not always found. In some cases the weather conditions were similar between years or locations, but the pseudothecia and spore numbers were different. The complicated nature of the *Leptosphaeria* spp. fungi life cycles and conditions for their growth and development, leading to plant infections were also demonstrated by Thürwächter et al. (1999). The authors studied the relations between ascospore discharge, oilseed rape plant infestation and pathogenicity of fungal strains trying to relate the impact of *L. maculans* and the healthiness, as well as the yield of oilseed rape. The relations between these characters were not straightforward, but they could be mathematically modelled (Salam et al., 2007) and subsequently – used to support decisions on chemical protection of agricultural crops against stem canker of oilseed rape (Jędryczka et al., 2008).

Based on the results of this study it can be concluded, that weather data observed at different experiment sites and years had a strong, positive effect on *L. maculans* and *L. biglobosa* maturation process. We have proved that each vegetative season differed in pseudothecia maturation rate and intensity as well as timing of ascospore release. As a consequence, a fixed term of fungicide treatment against stem canker of crucifers is not justified.

CONCLUSIONS

1. There are big differences in pseudothecia maturation speed of *L. maculans* and *L. biglobosa* in different vegetative seasons and locations in Poland.
2. There is a high relationship between pseudothecia maturation rate of *L. maculans* and *L. biglobosa* and meteorological conditions, especially rainfall.
3. The weather data observed at different experiment sites and years had a strong, positive effect on *L. maculans* and *L. biglobosa* maturation process.
4. A fixed period of fungicide treatment against stem canker of brassicas is not effective, when using chemical sprays on oilseed rape.

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