

Occurrence of *Didymella* ascospores in western and southern Poland in 2004–2006

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Received: 21 March 2011 / Accepted: 9 July 2011 / Published online: 5 August 2011
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Abstract The concentration of airborne *Didymella* spores has been investigated at two monitoring sites situated along the west–south transect in Poland (Szczecin, Kraków), i.e. from a height of 100 to 219 m, respectively, above sea level. The aerobiological monitoring of fungal spores was performed by means of two Lanzoni volumetric spore traps. The high *Didymella* spore numbers were observed at both cities in June, July and August. Statistically significant correlations have been found mainly between the *Didymella* spore concentrations in the air and the minimum air temperature and relative air humidity. The spore count of *Didymella* is determined by the diversity of local flora and weather conditions, especially by the relative air humidity. The identification of factors that influence and shape spore concentrations may significantly improve the current methods of allergy prevention.

Keywords Aerobiological monitoring · *Didymella* spores · Meteorological parameters

1 Introduction

Fungal spores play a significant role in the plant pathology and human respiratory allergy. In industrialized countries, the prevalence of allergy is increasing. Aerobiological studies describe the aeroallergens' seasonal distribution in a given area. The combination of allergological and aerobiological studies is useful in adjusting allergy-alerting systems. Spore concentration in the atmosphere results from a complex interaction between biological and environmental factors. Quite pragmatically, one may speak of fungi producing 'dry spores' and those producing 'wet spores'.

Members of the Ascomycetes class are characterized by the production of sexual spores (ascospores) within a sac-like structure called an ascus. Within the ascus, karyogamy and meiosis occur followed by an additional mitotic division to produce eight haploid ascospores. In most Ascomycetes, active release of ascospores takes place. Release frequently depends upon the activity of turgid cells, which requires a supply of water. The increasing pressure causes the ascus tip to burst open, forcing the spores out explosively. Spores can be ejected for distance of 2–300 mm depending on species. Asexual reproduction is accomplished by the production of conidia.

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Most of the recognized fungal allergens are derived from conidial Ascomycetes. Dutch elm disease, chestnut blight and powdery mildews are among diseases caused by the members of Ascomycetes, which damaged plants (Levetin 1995). Ascospores vary morphologically, and often they are difficult to identify microscopically in air samples. In general, they are recognizable by the fact that they have no attachment points, and are sometimes enclosed in gelatinous sheaths or within a sac.

Ascospore concentrations increase during and after rainstorms (Bush 1989). Also Allit (1986) observed that ascospores occurred abundantly in the atmosphere with high levels of relative humidity. Spores of Ascomycetes found in the air are numerous, and many of them are known to produce positive reactions in the skin prick test (Kendrick 1990).

One of the fungi producing ‘wet spores’ is *Didymella* belonging to the Pleosporaceae family. Spores are one-septate and hyaline. Generally, spore shape is ellipsoid or biconic (Fig. 1). *Didymella* is a saprobic or weakly pathogenic fungus. Spores fitting the description of *Didymella* have been reported above the fields of wheat and barley as a possible reason for the infection of both crops. Because *Didymella* relies upon a crop as a substrate, spore numbers drop dramatically after the crops are harvested.

Allit (1986) found that hyaline, one-septate ascospores reached high levels following rainfall during July and August. The majority of these spores belong to various species of *Didymella*. The high spore



Fig. 1 *Didymella* ascospore (Szczecin)

concentrations in Poland occur during June, July and August (Stępańska and Wołek 2005).

The aim of the study was to analyse the *Didymella* seasons in Szczecin and Cracow (western and southern Poland) in 2004–2006 and to find a relationship between meteorological factors and the *Didymella* spore counts.

2 Materials and methods

The investigation of the *Didymella* spore concentrations in the atmosphere was carried out in two cities in Poland, Szczecin and Cracow in 2004–2006 (Fig. 2).

Both cities are surrounded by forests, farmlands and abandoned farmland areas, which provide suitable media for the spore production. The present, ‘baltic’ climate of Szczecin is influenced by the impact of the air masses from over the northern Atlantic and is characterized by mild winters and cool summers. Cracow is situated in a region where weather changes are frequent due to the friction of both humid air masses arriving from over the northern Atlantic Ocean and dry, continental masses of air incoming from the east. In Szczecin and Cracow, the mean annual precipitation is relatively



Fig. 2 Map of Poland showing the monitoring sites and their coordinates: Szczecin—149 m a. s. l.; 53°25′N, 14°35′E; Kraków—220 m a. s. l.; 50°03′N, 19°57′E. Arrows—prevailing wind directions; cereals—locality of croplands

Table 1 Meteorological parameters for examined cities

	Szczecin	Kraków
2004		
Air temperature annual mean [°C]	8.6	8.7
Relative humidity annual mean [%]	80.6	76.6
Wind velocities annual mean [m/s]	3.4	2.6
Wind directions	W, SW	W, SW
2005		
Air temperature annual mean [°C]	8.8	8.4
Relative humidity annual mean [%]	79.8	77.4
Wind velocities annual mean [m/s]	3.3	2.5
Wind directions	W, SW	W, SW
2006		
Air temperature annual mean [°C]	9.2	8.4
Relative humidity annual mean [%]	80.1	78.6
Wind velocities annual mean [m/s]	3.1	2.2
Wind directions	S, SW	W, SW

low, 500 mm and 750 mm, respectively. The dominant wind directions for both cities are west and south-west (Fig. 2). Vegetation seasons in the cities differ in length: 210–220 days in Szczecin and over 220 days in Cracow (Woś 1999) (Table 1; Fig. 2).

For this study, the volumetric method has been employed using Hirst spore trap (Hirst 1952) made by Lanzoni with a flow rate of 10 l min⁻¹. The VPPS Lanzoni 2000 trap is equipped with wind vane that orients the air inlet according to the wind direction. The traps were installed on the rooftops in the centres of the cities at the same height (20 m) above ground level. In Szczecin, both samplers (Lanzoni spore trap and weather station) were located approximately 30 km on the south from croplands and in Cracow, approximately 42 km on the west from croplands. The farmlands in the vicinity of Cracow are twice larger than those around Szczecin (Fig. 2).

Airborne spores were sampled continuously, 12 months a year in 2004, 2005 and 2006. The Melinex tape used for catching spores was replaced at the same day every week and cut into segments corresponding to 24 h periods. Then, the segments were stained using a solution of glycerine, distilled water and gelatin in a proportion of 6:6:1. Samples were stained with basic fuchsin (saturated aqueous solution) 0.25%. Daily records of the airborne fungal spores were obtained using the tangent field counting method. With this method, successive tangent fields

positioned along one line were examined. After having counted the fungal spores in one field, the slide was moved to the next tangential field. All spores were counted per a microscopical slide at 400× magnification and an appropriate conversion factor was applied. The factor (F) was calculated using following formula:

$$F = a/[(b)c]$$

where a is total area of a daily tape segment, b area of analysed traverse = width × length × 1, c volume of analysed air = 10 l/min = 14.4 m³/24 h

Results (average daily concentration) were expressed as the number of spores m⁻³ air/24 h.

The meteorological parameters taken into consideration to assess their effect on the airborne fungal spores were wind speed, relative humidity and air temperature. In Szczecin, the meteorological data covering 3 years of the studies were provided by the automatic weather station (Vaisala MAWS101 and MAWS201, Finland). The meteorological station was located in the immediate neighbourhood of the Lanzoni trap. In Cracow, the meteorological data were obtained from the weather station of the Department of Climatology, Institute of Geography and Spatial Management, Jagiellonian University, situated also in the immediate vicinity of the monitoring site.

The spore data were analysed to determine the start, the end and the duration of the season using the 90% method. The start 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 date when 95% of the seasonal cumulative spore count was reached (British Aerobiology Federation 1995).

The statistical relationship between spore concentration and meteorological factors was established using the Statistica program version 8.0 (StatSoft, Inc. 2008).

3 Results

In Szczecin as well as in Cracow, the *Didymella* spores were present in large numbers during the summer period with the high levels being reached in June, July and August. In both cities, the highest concentrations were noted in July. The lowest sum of

Table 2 Spearman's rank correlation coefficients between *Didymella* spore concentrations in Kraków and meteorological variables

Variables and years	<i>Didymella</i>
2004	
Maximum temperature	−0.340*
2005	
Minimum temperature	0.325**
2006	
Minimum temperature	0.305*

P-value * $P < 0.05$; ** $P < 0.01$

Table 3 Spearman's rank correlation coefficients between *Didymella* spore concentrations in Szczecin and meteorological variables

Variables and years	<i>Didymella</i>
2004	
Relative humidity	0.498*
2005	
Minimum temperature	0.325***
2006	
Relative humidity	0.380*
Wind speed	0.220*

P-value * $P < 0.05$; *** $P < 0.001$

spores occurred in 2006 (1,075 and 968 in Szczecin and Cracow, respectively) and the highest in 2004 (5,095 and 5,901).

The Spearman's rank correlations analysis was performed in order to determine which meteorological variables influenced spore concentrations. Meteorological data used in the analysis included minimum and maximum temperature, relative humidity and wind speed.

Results of the Spearman's rank correlations analysis suggest that temperature was the most influential factor for *Didymella* spore concentrations during the studied period. The positive, significant correlation between minimum temperature and *Didymella* spore count occurred in two analysed seasons (2005, 2006) in Cracow and in one season in Szczecin (2005) (Tables 2,3).

In Cracow only in one out of the three studied seasons (2004), a negative and statistically significant correlation between *Didymella* spore count and the maximum temperature was noted (Table 2).

The positive, significant correlation occurred in Szczecin between *Didymella* spore count and relative humidity during two seasons studied (2004 and 2006). The wind speed was correlated with the number of spores only in one season (2006) in Szczecin (Table 3).

In Cracow, the dynamics of the spore season was similar in each analysed period. The differences in length between single stages were not larger than a couple of days. Contrary to the seasons of 2004 and 2006, the 2005 season was markedly shorter. In 2004, the fourth and fifth stages (50–75%; 75–95%) were prolonged in comparison with 2005, 2006; in 2006, the same situation occurred in the second and third stage (5–25%; 25–50%) (Fig. 3a).

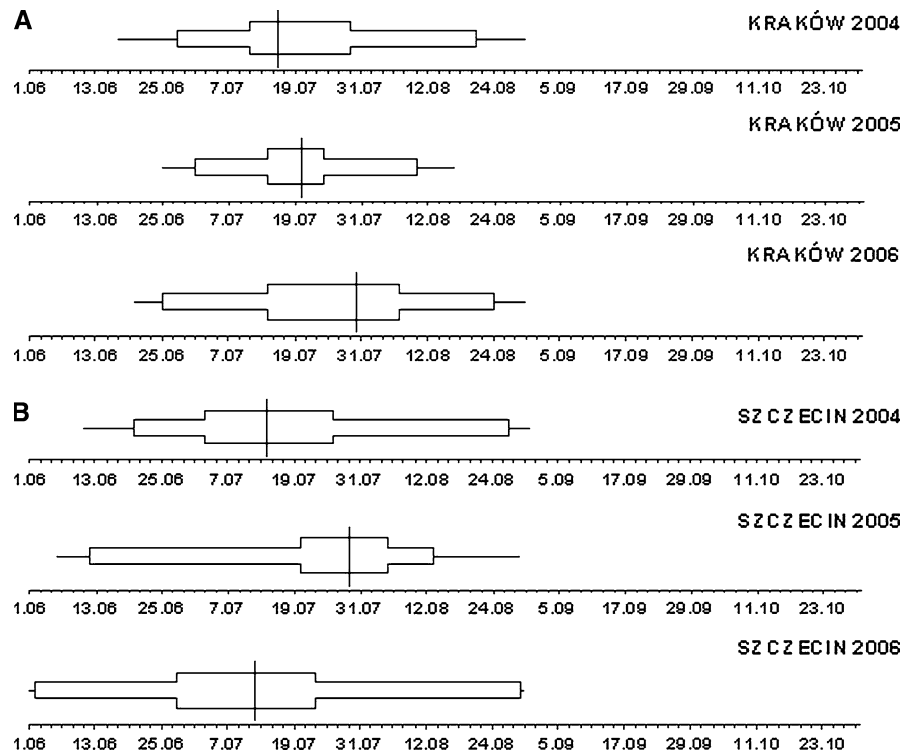
In Szczecin, the dynamics of the *Didymella* season was more differentiated than in Cracow. In 2004, the fifth (75–95%) stage was long; in 2005, the second and sixth stages (5–25%; 95–99%) were significantly longer and the fourth and fifth (50–75%; 75–95%) the shortest; in 2006, the second and fifth stages (5–25%; 75–95%) were prolonged and first and sixth (1–5%; 95–99%) the shortest (Fig. 3b).

4 Discussion

The occurrence of *Didymella* spores in the atmosphere throughout summer (June, July and August) is consistent with previous findings (Adams 1964; Allit 1986; Corden and Millington 1994; Frankland and Gregory 1973; Grinn-Gofroń and Mika 2008; Harries et al. 1985; Richardson 1996). The *Didymella* spores are important from an allergological point of view because they have been known to trigger allergic respiratory disease such as late summer asthma. Since the *Didymella* spore presence in the air is known to be influenced by meteorological parameters, some researchers undertook the studies to find which parameters have the strongest effect on spore concentration changes in a season (Corden and Millington 1994; Frankland and Gregory 1973; Packe and Ayres 1985; Stepalska and Wołek 2005; Wahl and Kersten 1991).

Earlier studies (Harries et al. 1985; Wahl and Kersten 1991) monitored *Didymella* spore concentrations over short periods. This paper deals with concentration of spores sampled in 2004–2006 and the relationship between spore concentrations and

Fig. 3 **a** Dynamics of *Didymella* seasons in Kraków, 2004–2006. Diagrams of consecutive stages of the fungal season: 1%, 5%, 25%, 50%, (vertical line), 75%, 95% and 99%. **b** Dynamics of *Didymella* seasons in Szczecin 2004–2006. Diagrams of consecutive stages of the fungal season: 1%, 5%, 25%, 50%, (vertical line), 75%, 95% and 99%



meteorological factors. Results of the analysis of Spearman's rank correlations showed that temperature correlated with spore concentration the strongest. The results agree with those reported by Corden and Millington (1994), Grinn-Gofroń and Mika (2008), Jones and Harrison (2004) and Stępałska and Wołek (2005). However, Wahl and Kersten (1991) found that the release and dispersal of *Didymella* spores was not greatly affected by air temperature. Similarly, Arseniuk et al. (1998) found a weak association between ascospore concentrations and temperature but this occurred probably due to the effect of temperature on ascospore growth rather than on release. In Cracow, minimum temperature was the most important factor in 2005 and 2006 and maximum temperature in 2004. Li and Kendrick (1995) found that in general, the concentrations of various fungal spores varied with mean, minimum and maximum temperature, mean wind speed, relative humidity and rainfall, but minimum temperature was the most important in the growing season. In Szczecin also minimum temperature influenced concentrations the strongest but only in 2005. In 2004 and 2006, relative humidity was the most influential variable correlating significantly and positively with

the concentration. In the earlier study performed in Cracow, the relative humidity appeared to be correlated with *Didymella* spore concentration in the pre-peak period but the percentage of explained variation was very low (Stępałska and Wołek 2005).

The association between the *Didymella* spore release and moist conditions has been well established (Corden and Millington 1994; Frankland and Gregory 1973; Harries et al. 1985). Wahl and Kersten (1991) suggested that *Didymella* spores might be dispersed by rain splashes but this was not supported by their own data, which showed that on a rainless night the spore release occurred at the same time as on rainy nights and it was 15% greater. The studies in Edinburgh showed clearly that rain was not essential for peak concentrations. A sharp peak occurred a few days after the last rain was recorded (Richardson 1996). Since *Didymella* spores are actively discharged in the early morning (Corden and Millington 1994; Frankland and Gregory 1973; Stępałska and Wołek 2009), Richardson (1996) concluded that the high humidity resulting from low temperature and dew formation in the early hours was the reason for high concentrations in the atmosphere, rather than rainfall. Significant and positive correlation of spore

concentrations with the relative humidity in Szczecin is consistent with the results given by Richardson (1996).

In Szczecin in 2006, wind speed correlated positively with the spore numbers. However, it is not supported by the earlier study performed in Cracow where wind speed and direction showed weak correlation with *Didymella* spore concentration, and in some cases, correlation was non-significant (Stępańska and Wołek 2005). McCartney (1991), reviewing spore take-off mechanisms and the threshold wind speed required for spore removal noted that information on the strength of spore attachment and values of threshold wind speed are not known for the majority of fungi. Dispersal patterns are varied and appear to be different for different species and for the same species at different locations and times. However, the mechanisms of dispersal are essentially the same for all spores. Understanding dispersal processes is by no means complete. Methods to deal with dispersal in complex flows such as near field boundaries, hedges or obstructions or over non-uniform terrain have still to be developed. More work is needed to realize the potential of random walk dispersal models. A greater understanding of spore release mechanisms is also needed for use with such models when they are available. Burt et al. (1997) analysing the concentrations of ascospores in banana and plantain plantations stated no relationship between wind speed and ascospore concentrations. Ascospore concentration was nearly significantly related (at the 90% level) to updraught and was significantly inversely related to turbulent intensity. In the covariance analysis of mean wind speed and log ascospore catches, the correlation was not statistically significant, even at the 10% level ($P > 0.1$). Lyon et al. (1984) found for Ascomycetes that radiation, minimum humidity, changes in humidity and minimum wind speed were related to airborne spore concentrations.

From the above discussion, it is seen that the same meteorological variables may have different effects on different parts of the life cycle (spore growth, source size and spore emission) and also that the effect of any meteorological factor on spore concentration may differ from year to year because of extremes in other factors (Jones and Harrison 2004).

The dynamics of *Didymella* spore seasons in Cracow and Szczecin described by the graphs

illustrates the consecutive phases in which the cumulative spore sums attain the designated levels of the annual totals. Throughout the years monitored, the *Didymella* spore season shows variations with respect to its dynamics that are larger in Szczecin. More differentiated dynamics (different length of consecutive phases) of the *Didymella* spore season in Szczecin than in Cracow could possibly be explained by variability in weather conditions in Szczecin caused by the vicinity of the Baltic Sea. Two phases (25–50% and 50–75%) of the *Didymella* spore season, when the cumulative spore sums obtain value between 25 and 75% of the annual total spore count, are usually defined as a peak concentration period (Corden and Millington 1994; Richardson 1996; Stępańska and Wołek 2005).

There are only few publications describing the *Didymella* spores in the air and the influence of meteorological factors on changes in their concentrations. We hope that our present studies will be stimulative for scientists to undertake further investigations on *Didymella* spores in view of their importance for medicine (allergies) and agriculture (crop parasites).

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