

An analysis of the exposure time to very high concentrations of *Cladosporium* conidia in the air of an urban site

Magdalena Sadyś 

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Abstract *Cladosporium* spp. is a ubiquitous mould present both in indoor and outdoor environments and affecting a wide array of substrates. It is considered to be the second most important fungal aeroallergen in countries of temperate climates, after *Alternaria* species. In order to establish the time of exposure to very high concentrations of *Cladosporium* conidia by sensitised individuals, a 5-year study was undertaken in the UK in a densely populated area using a volumetric air sampler. Weather data, comprised air temperature, precipitation, relative humidity and wind speed were collected simultaneously. *Cladosporium* showed a mono-modal pattern with the majority of conidia observed between 09:30 and 19:00 as indicated by descriptive statistics and multivariate regression tree analysis. On the other hand, circular statistics showed that the maximum hourly concentrations were found within 1.5-h window before 09:30. The highest conidia concentrations were observed when dry conditions occurred (40–55%). The maximum temperature associated with peak concentrations was oscillating within the range of 20–25 °C. All findings

were confirmed using Kruskal–Wallis, Friedman and sign tests.

Keywords Bioaerosol · Asthma · Circadian rhythm · Air quality · Fungal ecology · Circular statistics

1 Introduction

Many aerobiological studies demonstrated that air is never free from biological components (Ianovici et al. 2008), and it has been estimated that during the summer months, we can breathe in $>50,000$ spores m^{-3} of air day^{-1} (Pashley et al. 2012). An inhalation contact with spores produced by some of the fungi does not necessarily lead to fungal infection, but can instead elicit allergic response resulting in the development of rhinitis or asthma (Denning et al. 2014). The chronic exposure to the very high levels of allergens in both indoor and outdoor environments causes a sensitisation towards moulds (Alves et al. 2016). To date, more than 112 fungal taxa have been identified as a source of allergens, and four genera have been widely studied, i.e. *Alternaria*, *Cladosporium*, *Aspergillus* and *Penicillium* (Twaroch et al. 2015). The contribution of *Cladosporium* spp. (hereafter *Cladosporium*) spores is site-specific, and it may account for up to 80–85% of all spores present in the air (D’Amato and Spieksma 1995; Sindt et al. 2016). In addition, Fukutomi and Taniguchi (2015) reported that the primary source of

M. Sadyś (✉)
Institute of Science and the Environment, University of
Worcester, Henwick Grove, Worcester WR2 6AJ, UK
e-mail: magdalena.sadys@rothamsted.ac.uk

M. Sadyś
Rothamsted Research, West Common,
AL5 2JQ Harpenden, UK

Cladosporium constitutes the outdoor environment over the indoors.

Cladosporium spores are highly allergenic, and also, they can cause pulmonary and cutaneous infections through the mycelial growth (Horner et al. 1995; Breitenbach and Simon-Nobbe 2002). *C. herbarum* and *C. cladosporioides* have been studied the most intensively out of all *Cladosporium* species; *Cladosporium cladosporioides* is known for production of the secondary metabolite called as either Cladosporin or Asperentin which inhibits the growth of other fungi and thus, it is commonly used as antibiotic (Fromme et al. 2016). Among other mycotoxins produced by *Cladosporium* species can be found Altersolanol J, Calphostin A-C, Calphostin D, Emodin, Epicladosporic acid, Fagicladosporic acid, Herbarin A, B (Bräse et al. 2009; Weidenböcker 2001). The most important isolated allergens are Cla h 1 and Cla h 2 (Horner et al. 1995; Breitenbach and Simon-Nobbe 2002). A study of Bouziane et al. (2005) showed that mycelium and spores produced by *C. cladosporioides* have different allergenic properties with the latter containing a greater amount of allergens. Although infection can only be caused by viable fungal spores, they do not need to be viable to trigger an allergic reaction (Denning et al. 2014). According to Zureik et al. (2002), overall sensitisation to fungal spores in the UK and Republic of Ireland was found to be the highest in comparison with Northern, Central and Southern Europe. Sensitisation to *Cladosporium herbarum* affected 6.8% of patients with diagnosed asthma (Zureik et al. 2002). Lower results of sensitisation to *Cladosporium* spores were reported by the European Community Respiratory Health Survey (ECRHS), which indicated 2.3% of adults aged between 20 and 44 years to be allergic to the above-mentioned fungal spores (Bousquet et al. 2007). It is notable that more than 11,300 adults were examined, and overall it was found that 35.6% of subjects were atopic (Bousquet et al. 2007). The clinical threshold, which has been estimated for *Cladosporium* spore concentration in the air, in the UK (Frankland and Davies 1965) and Croatia (Pernel et al. 2004) is equal to 3000 spores m^{-3} , while it is 2800 spores m^{-3} in Poland (Rapiejko et al. 2004) and 4000 spores m^{-3} in Finland (Ranta and Pessi 2006).

Studying diurnal distribution of allergenic fungal spores, such as those of *Cladosporium*, can determine when increased spore concentrations in the air may

occur. As described by Denning et al. (2014), avoidance of fungi was the cornerstone step in the management of fungal allergy in asthma. Thus, this study aimed to examine temporal variations in *Cladosporium* conidia concentrations measured at densely populated area in relation to time, as well as in relation to changing weather conditions. This has been accomplished by (1) measurement of conidia concentration throughout a 5-year survey using a volumetric air sampler, (2) simultaneous collection of weather data, (3) microscopy analysis of the spores and (4) statistical evaluation of spore and weather data using advanced tools, such as circular statistics and multivariate regression tree (MRT) analysis.

2 Materials and methods

2.1 Data collection

Air samples were collected continuously for a 5-year period (January 2006–December 2010) using a 7-day volumetric spore trap (Hirst 1952). This device enables measurement of hourly and daily mean fungal spore and pollen grain concentration in the air. The trap was placed on the roof of the University of Worcester's building in Worcester, UK (52°11'N, 2°14'W) approximately 10 m above the ground level. *Cladosporium* conidia were impacted on the surface of a collection tape, which was previously coated with an adhesive (*i.e.* a mixture of petroleum jelly and paraffin wax) and tightly fastened to a rotating drum inside the air sampler. The speed with which drum was rotating was 2 mm h^{-1} . Thus, one full round was equal to a 7-day period. Once a cycle was completed, then drum was changed and taken to the laboratory for further processing. The collection tape was cut into seven 48 mm long pieces, stained using lacto phenol Cotton Blue and secured with coverslips and nail varnish, which prevented damage to the samples by the loss of moisture.

Cladosporium herbarum is the most common species identified outdoors in temperate and cold climates, while *C. cladosporioides* is found mostly indoors. Colonies of *Cladosporium herbarum* are powdery–velvety in structure, and their colour may vary from olivaceous to brown. Conidiophores have smooth walls and terminal and intercalary protuberances. Single or multi-celled conidia are formed at the

end of the conidiophores in either simple or occasionally branched chains. Thus, depending on the location, spores may have a shield or round shape at the ends, visible black scars of attachment points. Occasionally, conidia may have up to 3 transverse walls, while spores towards the end of chain are smaller and aseptate (Bensch et al. 2012). The shape of the spores varies from cylindrical, through ellipsoidal and ovoid up to sub-spherical. They are usually small in size (40–60 $\mu\text{m} \times 3\text{--}22 \mu\text{m}$) and from olivaceous to brown in colour. The surface of the wall may be either smooth or rough (verrucose or echinulate), (Ellis 1971). Conidia germinate on moist surfaces resulting in black lesions (König et al. 2009).

Fungal spores were identified up to the genus level using a light microscope (Nikon Eclipse E400) under 400 $\times$ magnification and enumerated lengthwise from a single traverse located in the central part of the microscope slide. Mediavilla Molina et al. (1998), followed by Sabariego et al. (2000) and Rodríguez-Rajo et al. (2005) identified microscopically, classified and enumerated conidia of two types of *Cladosporium*, i.e. *C. herbarum*-type and *C. cladosporioides*-type. These were first works towards the distinction between different *Cladosporium* species using the light microscopy, which was further examined at the molecular level by Crous et al. (2007) and Bensch et al. (2012).

Under each examined microscope slide, a printed grid corresponding to an hourly division was placed. Finally, the concentration of conidia [number of spores m^{-3}] was calculated by multiplying the number of conidia by a specific for the microscope correction factor (Lacey and West 2006). Weather data were obtained from the weather station co-located with the air sampler at the University of Worcester. All meteorological parameters (air temperature, rainfall, relative humidity, wind speed) were recorded from 00:00 to 23:59 according to UTC local time. A thorough analysis of the entire data set based upon daily records can be found in Sadyś et al. (2016).

2.2 Statistical evaluation

Due to the atmospheric processes, particles that once were released into the air stream and deposited, they can become airborne once more (Jäger 2003). Thus, Nilsson and Persson (1981) introduced a definition of a spore/pollen season and proposed several counting

methods. This way, also particles rarely observed within a year's time will not contribute towards an artificial extension of the actual fungal spore/pollen season length. In this study, fungal spore seasons were estimated using the "90% method", which was comprised of 5% reduction at the beginning and at the end of the accumulative sum of daily mean spore concentration.

Data obtained this way were then a subject of a preliminary screening, which comprised of data distribution examination using 3 normality tests (Kolmogorov–Smirnov test, Kolmogorov–Smirnov test with Lilliefors correction and Shapiro–Wilk test), and detection of the linearity or lack thereof between all examined variables by visual analysis of the scatter plots. The non-parametric tests were applied since the preliminary screening revealed the non-normal distribution of the spore data.

The annual differences in the hourly mean and daily mean distribution of *Cladosporium* conidia were investigated with the aid of the Kruskal–Wallis test. The sign test was used to examine whether there was any statistically significant difference in conidia distribution on dry and rainy days. The impact of hourly changes in maximum air temperature, precipitation, relative humidity and wind speed on conidia temporal distribution was analysed using the Friedman test. All selected meteorological parameters were classified into 4 levels, upon preliminary data screening, and examined against spore counts.

Subsequently, circular statistical tests were used in order to estimate the average time, when the maximum conidia concentration in the air of Worcester was found (regardless the impact of weather). This approach required a transformation of all hours into a corresponding angle (expressed in degrees) and selection of hours with the maximum concentration only. In case, if the same value occurred two or more times within a single day, then all those hours were chosen. Further trigonometric calculations were based on the formula described in Zar (1984). In the end, mean angle expressed as a function of sine and cosine was converted back to the time format (h min). In addition to that, an angular dispersion was also considered.

The circular statistics was complemented by the multivariate regression tree (MRT) analysis, which was carried out to identify the threshold hour when high/low conidia concentrations were observed

(De'ath and Fabricius 2000). For this reason, all spore seasons were pooled together. Two thousand multiple cross-validations were applied to stabilise the cross-validated relative error (CVRE). The final MRT model was chosen based on the lowest obtained CVRE. All statistical analyses were performed with the aid of Statistica Statsoft (2012), Genstat (v. 17), R (v. 2.9.2) and Microsoft Excel (2016).

3 Results

3.1 Changes in conidia concentration in time

After application of the “90% method” in order to indicate the *Cladosporium* spore seasons, selected number of days for the analyses was equal to $n = 729$. The length of *Cladosporium* spore seasons varied annually, and it was found within the range of 111–186 days (Fig. 1). The intensity of the seasons in terms of a number of trapped conidia was inversely proportional to the season length. The highest maximum daily mean concentrations were observed in 2006 and 2010, when *Cladosporium* concentration

was equal to $46,831 \text{ spores m}^{-3}$ and $36,783 \text{ spores m}^{-3}$, respectively. The midpoint of the seasons tended to occur in the second half of July (Fig. 1). Inter-annual differences using both daily mean and hourly mean values were confirmed by the Kruskal–Wallis test ($p < 0.01$).

Five-year mean temporal variations in *Cladosporium* spore distribution are shown in Fig. 2, while Fig. 3 presents each year individually. For *Cladosporium* spores, the most representative years were 2006 and 2010 (Fig. 3). In these particular years, the maximum concentrations of *Cladosporium* spores were observed at 15:00 and 13:00, respectively, exceeding $10,000 \text{ spores m}^{-3}$ within an hour (Fig. 3). The lowest concentrations were found at 09:00 and 07:00, respectively, decreasing to slightly below $3000 \text{ spores m}^{-3}$. However, an analysis of the 5-year mean distribution showed that the peak concentrations occurred at 13:00, while the lowest concentrations were found at 06:00 (Fig. 2). Further analysis of total 5-year sums of hourly spore concentrations indicated contributions equal to 6.3 and 2.1%, respectively.

Fig. 1 Variations in *Cladosporium* conidia seasons between examined years observed at Worcester, UK. Season dynamics are comprised of the following stages: 1, 5, 25, 50% (upright line), 75, 95 and 99%

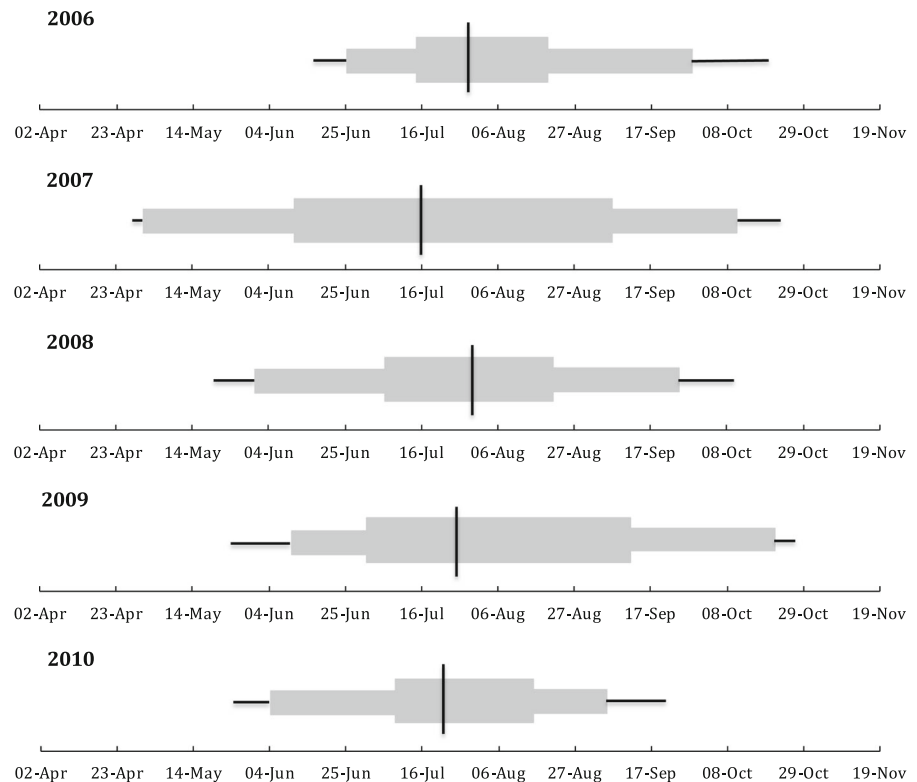
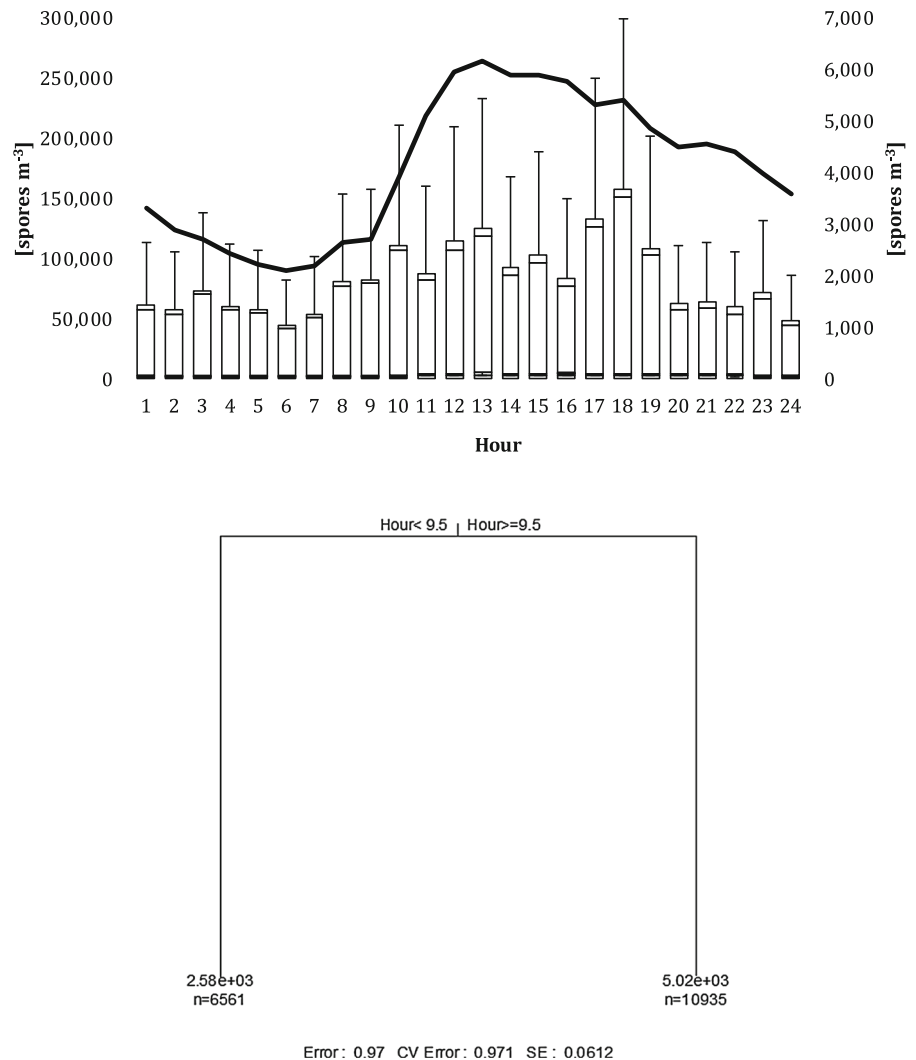


Fig. 2 Overall variations in *Cladosporium* conidia hourly distribution observed at Worcester, UK and computed upon 5-year spore seasons pooled together ($n = 17,496$). The negative error bars were equal to the result of subtraction between the first quartile and the minimum value, while the positive error bars were equal to the result of subtraction between the maximum value and the third quartile. The continuous line on the secondary axis represents the five-year mean



The multivariate regression tree model divided all concentrations into two categories, of low (mean cluster concentration was <2580 spores m^{-3}) and high (mean cluster concentration was >5020 spores m^{-3}). The 09:30 h was found to be the border value when the discrepancy between clusters occurred (Fig. 3). In addition to that, results computed using circular statistics showed that the peak concentrations were observed on average between 08:00 and 09:30 (Table 1). The difference in angular deviations between years was equal to 2° (Table 1).

3.2 Weather impact on conidia abundance

A 5-year mean hourly distribution of *Cladosporium* spores measured in Worcester for all days, days with

lack of rainfall (“dry days”, $n = 406$) and days with the presence of a minimum of 0.20 mm of rainfall (“rainy days”, $n = 323$) is shown in Fig. 3. A general trend in the distribution pattern of examined fungal spores was repeated, although at some time periods, a significant reduction in fungal spore concentration was found (Fig. 3). It was observed that 12-h rainfall had a positive impact on *Cladosporium*, as its concentration increased significantly. This effect was seen after 07:00. The maximum spore concentration was also registered at 12:00, an hour earlier in a comparison with “all days” and “dry days” distribution patterns, approaching 6400 spores m^{-3} . The sign test showed that the distribution of *Cladosporium* spores remained unaffected, even after pooling together five years of observations (Table 2).

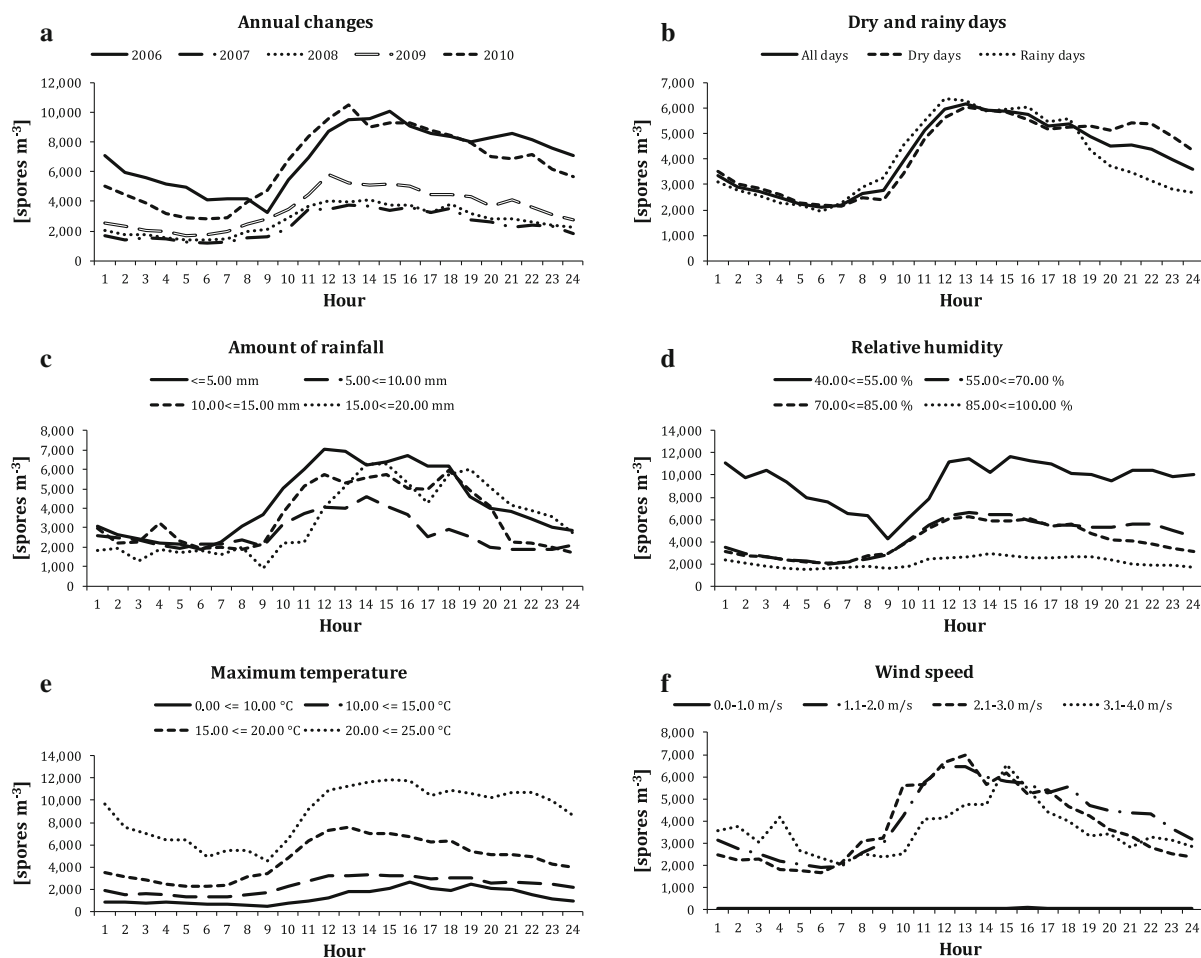


Fig. 3 Mean hourly distributions of *Cladosporium* conidia depending on: **a** the spore season/year, **b** the presence or absence of rain, **c** amount of rainfall, **d** relative humidity, **e** maximum temperature and **f** wind speed registered within 24 h at Worcester, UK

Table 1 Variations in the peak occurrence calculated using circular statistics

Factor	2006	2007	2008	2009	2010	2006–2010
Mean angle of the peak	142°	120°	126°	121°	120°	123°
	09:28 h	08:00 h	08:24 h	08:04 h	08:00 h	08:12 h
Angular deviation	79°	77°	78°	79°	77°	78°
	5 h 16 m	5 h 8 m	5 h 12 m	5 h 16 m	5 h 8 m	5 h 12 m

The volume of rainfall that fell within 24 h was further examined, as observations made by the author during fungal spore counting suggested that a small amount of precipitation induced the simultaneous presence of *Cladosporium* spores in greater quantities, in the air in Worcester. Figure 3 shows the impact of the amount of rainfall (n), classified into four categories: $0 < n \leq 5$ mm ($n = 240$), $5 < n \leq 10$ mm ($n = 49$), $10 < n \leq 15$ mm ($n = 18$) and $15 < n \leq 20$ mm

($n = 16$) on the diurnal distribution of *Cladosporium* spores. Low volumes of rainfall were beneficial for dispersal of *Cladosporium* spores ($0 < n \leq 5$ mm), which achieved the maximum trapped concentration (>7000 spores m^{-3}) at 12:00 (Fig. 3). The Friedman test confirmed that there was a statistically significant difference in the diurnal distribution of *Cladosporium* spores depending on the amount of rainfall that occurred during a 24-h period (Table 3). This

Table 2 Results of sign test for the comparison of changes in diurnal pattern of *Cladosporium* conidia

Factor	Year	2006	2007	2008	2009	2010	2006–2010
± rainfall	<i>z</i>	−0.20	1.43	1.02	0.61	0.20	0.27
	<i>p</i>	ns	ns	ns	ns	ns	ns

ns not significant

Level of statistical significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

association was observed for each of the examined years as well as for all 5-year sampling period (Table 3).

The lowest relative humidity (n), which occurred within the study period, was 40%. To enable an easy comparison with the impact of precipitation on diurnal pattern of selected fungal spores, relative humidity was divided into four groups: $40 < n \leq 55\%$ ($n = 22$), $55 < n \leq 70\%$ ($n = 263$), $70 < n \leq 85\%$ ($n = 386$), and $85 < n \leq 100\%$ ($n = 58$). *Cladosporium* showed that drier conditions favoured spore dispersal, i.e. where relative humidity varied between 40 and 55% (Fig. 3). The maximum concentration of *Cladosporium* was found at 15:00, when spore concentration $>11,000$ spores m^{-3} was registered (Fig. 3). The Friedman test confirmed that there was a statistically significant difference in the diurnal pattern of *Cladosporium* spores associated with relative humidity during 24-h period (Table 3). These relationships were observed in each sample year as well as for an averaged 5-year period (Table 3).

Four classes for the maximum air temperature were identified: $0 < n \leq 10$ °C ($n = 38$), $10 < n \leq 15$ °C ($n = 298$), $15 < n \leq 20$ °C ($n = 353$) and $20 < n \leq 25$ °C ($n = 40$), for this investigation (Fig. 3). The maximum temperatures between 20 and 25 °C were favourable for dispersal of *Cladosporium* spores,

although maximum spore concentrations were recorded at different times (Fig. 3). *Cladosporium* was found in the greatest concentrations approximating 12,000 spores m^{-3} at 15:00. A statistically significant difference between maximum temperature and diurnal distributions of *Cladosporium* for all individual years and a 5-year average was indicated by the Friedman test (Table 3).

The four classes, which were distinguished for the wind speed, were following: $0 < n \leq 1$ $m\ s^{-1}$ ($n = 437$), $1 < n \leq 2$ $m\ s^{-1}$ ($n = 235$), $2 < n \leq 3$ $m\ s^{-1}$ ($n = 51$) and $3 < n \leq 4$ $m\ s^{-1}$ ($n = 6$), for this investigation (Fig. 3). The wind speed between 1.1 $m\ s^{-1}$ and 3 $m\ s^{-1}$ was favourable for dispersal of *Cladosporium* spores (Fig. 3). *Cladosporium* was found in the greatest concentrations approaching 70,000 spores m^{-3} at 13:00. The Friedman test confirmed that there was a statistically significant difference in the diurnal pattern of *Cladosporium* spores associated with wind speed during 24-h period (Table 3).

4 Discussion

The first reports on the diurnal periodicity of *Cladosporium* conidia described a “daytime double

Table 3 Results of Friedman test for the comparison of changes in diurnal pattern of *Cladosporium* conidia

ns Not significant

Level of statistical significance: * $p \leq 0.05$,

** $p \leq 0.01$,

*** $p \leq 0.001$

Factor	Year	2006	2007	2008	2009	2010	2006–2010
Rainfall (mm)	χ^2	58.25	36.55	41.90	36.15	38.75	59.14
	<i>p</i>	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
Relative humidity (%)	χ^2	68.00	13.55	29.75	30.05	67.25	60.32
	<i>p</i>	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
Max. temperature (°C)	χ^2	70.85	59.00	72.00	55.55	66.05	279.11
	<i>p</i>	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
Wind speed ($m\ s^{-1}$)	χ^2	45.35	57.75	57.15	64.48	62.74	201.50
	<i>p</i>	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*

maxima” pattern with a greater peak in the forenoon, and second smaller occurring either in the afternoon or early evening hours (Calderón et al. 1997; Gregory and Stedman 1958; Lacey 1996; Rich and Waggoner 1962). This phenomenon was then explained that the increase in conidia concentrations was mainly driven by changes in the turbulence of the air caused by a rise in the air temperature and drop in relative humidity (Rich and Waggoner 1962). The production of *Cladosporium* conidia requires 8 continuous hours of darkness and their further maturation another 3–4 h; thus, none of the spores could be discharged at the night-time hours (Rich and Waggoner 1962). Results presented in this study show a daytime single maximum pattern, which has previously been reported by Fernández et al. (1998). The contribution of this single peak to the total daily spore concentration (occurring at 12:00–14:00) was established for 20%, while the current survey showed a contribution equal to 18%. A single peak pattern was distinct for the years 2006 and 2010, where the maximum concentration of *Cladosporium* spores was found at 15:00 and 13:00, respectively, with spore counts exceeding 10,000 spores m^{-3} . The minimum concentration, below a clinical threshold equal to 3000 spores m^{-3} (Frankland and Davies 1965), was observed in the morning, between 07:00 and 09:00. Previously, Adams (1964) reported that in Cardiff, UK, *Cladosporium* was seen at the greatest concentration between 12:00 and 15:00, and the lowest concentration from 01:00 to 07:00. However, in these studies, spore peaks were approaching 45,000 spores m^{-3} (Adams 1964). Furthermore, Rich and Waggoner (1962) stated that the earliest hour at which spores were ready to become airborne was 09:00 during the summer time. The MRT analysis showed 09:30 as a border hour and thus corresponding well to the previous findings. In contrast, the concentration of *Cladosporium* spores measured in the wheat field was found to be the greatest between 07:00 and 08:00 (Pady et al. 1969). Occasionally, peaks were also observed at 03:00 (Pady et al. 1969).

This study also showed that the amount of recorded rainfall and wind speed had the greatest influence on *Cladosporium*, which resulted in very high concentrations ($<80,000$ spores m^{-3}). The number of *Cladosporium* spores per cubic metre of air significantly increased during “rainy days”, especially between 07:00 and 19:00. Stępańska and Wołek (2009) did not find any statistically significant

difference between “dry” and “rainy” days, although a small positive influence of rainfall was seen between hours 14:00–19:00 and 00:00–08:00. Further examination of the amount of rainfall revealed that up to a maximum of 5 mm within 24-h period was needed for the highest *Cladosporium* spore release into the atmosphere. The diurnal pattern remained unchanged, as a peak was found at 12:00. Similar observations were made by Peternel et al. (2004) who observed a rise in *Cladosporium* spore concentration preceded by a rain of up to 5 mm in Zagreb, Croatia while Fernández et al. (1998) reported the same positive impact of rain at higher rates of 8–11 mm in León, Spain. Rich and Waggoner (1962) suggested that wind-driven drops of turbulent storms caused vibration facilitating detachment of spores and their release into the air streams. The importance of vibration on *Cladosporium* conidia discharge was then further examined by Byung Uk (2010), who found that vibration rate equal to 20 Hz resulted in sixfold greater spore discharge compared to the naturally released spore rate without any disturbance. Troutt and Levett (2001) reported that 30 mm of rainfall within one hour (at 02:00) was sufficient enough to reduce *Cladosporium* spore concentration from 3000 spores m^{-3} to 0 spores m^{-3} .

Relative humidity had a significant impact on the trapped *Cladosporium* spore concentration. The maximum concentration was registered three hours after a peak in relative humidity. The spore count increased by 36% in comparison with the propitious amount of rainfall. Changes in relative humidity affected the hourly variations of *Cladosporium* spores, and this was in agreement with the results obtained by the Friedman test. Similar observations were made by Fernández et al. (1998) who reported a decrease in *Cladosporium* spore concentrations during the months of June and July caused by low amount of rainfall and simultaneous decline in relative humidity recorded during this period. Lacey (1996) classified liberation mechanism of *Cladosporium* conidia as passive, yet requiring water. A mist pickup by *Cladosporium* was first reported by Davies (1959) who found that conidia can be detached from conidiophores by moving water droplets in a haze or cloud. The number of released spores, measured at the same wind velocity, in the air with water droplets was sixfold to eightfold higher compared with the air without them (Davies 1959). The impact of relative humidity was also studied by

Reponen et al. (1996). They found that when relative humidity increased from 30% to 100%, at the same time, the size and aerodynamic diameter of *Cladosporium cladosporioides* increased for 27%. This has further clinical implications since the spore saturation facilitates 20–30% increase in a successful deposition in human respiratory tract (Reponen et al. 1996).

Wind speed plays an important role in the dissemination of various pathogens, including conidia of *Cladosporium* species (Lacey 1996). Pasanen et al. (1991) studied the release of *Cladosporium* spores from a culture by changing air velocity ($0.5\text{--}1.5\text{ m s}^{-1}$) and range of relative humidity (from 12–18% to 71–73%). The minimum wind speed facilitating spore discharge was 1 m s^{-1} ; this result corresponds well with the outcomes received herein. Similar findings were described by Rich and Waggoner (1962) who collected the greatest amount of *Cladosporium cucumerinum* conidia, when the wind velocity was within the range of $2.2\text{--}2.7\text{ m s}^{-1}$, as well as by Kurkela (1997) who found the peak concentrations at 14:00–15:00 when wind velocity was varying between 2 m s^{-1} and 3 m s^{-1} .

The effect of the maximum air temperature in Worcester was less influential than other examined variables. In contrast, Troutt and Levetin (2001) indicated that the maximum air temperature was the most important weather parameter that affected hourly variations of *Cladosporium* spores in the air of Tulsa, USA. The application of multiple regression analysis explained the hourly variation in *Cladosporium* spores by maximum air temperature in 21%, but no range of the temperature was given by the authors (Troutt and Levetin 2001). Gravesen (1979) reported that the optimal temperature range for *Cladosporium* species fell within the range of 18–28 °C. Thus, the temperature scope identified herein, *i.e.* 20–25 °C, was very well corresponding although it was narrowed down to 6 °C range instead. In contrast, Shaheen (1992) reported that psychrophilic fungi, such as *Cladosporium*, thrived at the temperature <20 °C. However, it should be noticed that this study was conducted in Jordan, which is characterised by a semi-arid climate. The impact of the air temperature was also highlighted by Grinn-Gofroń and Strzelczak (2013) who studied rapid changes in weather associated with summer storms and the presence of airborne fungal spores. They found that just before the thunderstorm concentrations of *Cladosporium* conidia drastically elevated

simultaneously with a rapid rise in the air temperature and ozone concentration. The predicting model they built using the artificial neural networking enabled them a 2-hour prediction of such spore events in advance (Grinn-Gofroń and Strzelczak 2013).

5 Conclusions

The majority of *Cladosporium* spores were present in the air of studied area between the end of June and beginning of September. Results showed that until 09:30, concentrations of *Cladosporium* conidia in the air did not exceed a clinical threshold related to the asthma exacerbation. The exposure to these aeroallergens should be reduced to the minimum in the afternoon hours, between 13:00 and 19:00. No statistically significant difference was found between dry and rainy days; those the findings would apply to all days. The presence of high spore counts in the air was mainly apparent during warm (20–25 °C), dry (40–55%) and breezy days ($1.1\text{--}3.0\text{ m s}^{-1}$).

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References

- Adams, K. F. (1964). Year to year variations in the fungus spore content of the atmosphere. *Acta Allergologica*, 19, 11–50.
- Alves, C., Duarte, M., Ferreira, M., Alves, A., Almeida, A., & Cunha, Â. (2016). Air quality in a school with dampness and mould problems. *Air Quality, Atmosphere and Health*, 9, 107–115.
- Bensch, K., Braun, U., Groenewald, J. Z., & Crous, P. W. (2012). The genus *Cladosporium*. *Studies in Mycology*, 72, 1–401.
- Bousquet, P.-J., Hooper, R., Kogevinas, M., Jarvis, D., & Burney, P. (2007). Number of allergens to be tested to assess allergenic sensitization in epidemiologic studies: results of the European Community Respiratory Health Survey I. *Clinical and Experimental Allergy*, 37, 780–787.
- Bouziane, H., Latgé, J. P., Fitting, C., Mecheri, S., Lelong, M., & David, B. (2005). Comparison of the allergenic potency of spores and mycelium of *Cladosporium*. *Allergologia et Immunopathologia*, 33, 125–130.
- Bräse, S., Encinas, A., Keck, J., & Nising, C. F. (2009). Chemistry and biology of mycotoxins and related fungal metabolites. *Chemical Reviews*, 109, 3903–3990.

- Breitenbach, M., & Simon-Nobbe, B. (2002). The allergens of *Cladosporium herbarum* and *Alternaria alternata*. In M. Breitenbach, R. Crameri, & S. B. Lehrer (Eds.), *Fungal allergy and pathogenicity* (pp. 48–72). Basel: Krager.
- Byung Uk, L. (2010). Effect of vibration on dispersal of *Cladosporium cladosporioides* bioaerosols. *Journal of Microbiology and Biotechnology*, 20(5), 904–907.
- Calderón, C., Lacey, J., McCartney, A., & Rosas, I. (1997). Influence of urban climate distribution of airborne Deuteromycete spore concentrations in Mexico City. *International Journal of Biometeorology*, 40, 71–80.
- Crous, P. W., Braun, U., Schubert, K., & Groenewald, J. Z. (2007). Delimiting *Cladosporium* from morphologically similar genera. *Studies in Mycology*, 58, 33–56.
- D'Amato, G., & Spiekma, F. T. M. (1995). Aerobiologic and clinical aspects of mould allergy in Europe. *Allergy*, 50, 870–877.
- Davies, R. R. (1959). Detachment of conidia by cloud droplets. *Nature*, 4676, 1695.
- De'ath, G., & Fabricius, K. E. (2000). Classification and regression trees: A powerful and yet simple technique for ecological data analysis. *Ecology*, 81, 3178–3192.
- Denning, D. W., Pashley, C., Hartl, D., Wardlaw, A., Godet, C., Del Giacco, S., et al. (2014). Fungal allergy in asthma—state of the art and research needs. *Clin Transl Allergy*, 4, 14.
- Ellis, M. B. (1971). *Dematiaceous Hyphomycetes*. London: The Eastern Press Ltd.
- Fernández, D., Valencia, R. M., Molnár, T., Vega, A., & Sagüés, E. (1998). Daily and seasonal variations of *Alternaria* and *Cladosporium* airborne spores in Leon (North-West, Spain). *Aerobiologia*, 14, 215–220.
- Frankland, A. W., & Davies, R. R. (1965). Allergie aux spores de moisissures en Angleterre. *Poumon Coeur*, 21, 11–23.
- Fromme, H., Gareis, M., Völkel, W., & Gottschalk, C. (2016). Overall internal exposure to mycotoxins and their occurrence in occupational and residential settings – An overview. *International Journal of Hygiene and Environmental Health*, 219, 143–165.
- Fukutomi, Y., & Taniguchi, M. (2015). Sensitization to fungal allergens: Resolved and unresolved issues. *Allergol Int*, 64, 321–331.
- Gravesen, S. (1979). Fungi as a cause of allergic diseases. *Allergy*, 34, 135–154.
- Gregory, P. H., & Stedman, O. J. (1958). Spore dispersal in *Ophiobolus graminis* and other fungi of cereal foot rots. *Trans Brit Mycol Soc*, 41, 449.
- Grinn-Gofroń, A., & Strzelczak, A. (2013). Changes in concentrations of *Alternaria* and *Cladosporium* spores during summer storms. *International Journal of Biometeorology*, 57, 759–768.
- Hirst, J. (1952). An automatic volumetric spore trap. *Ann Appl Biol*, 39, 257–265.
- Horner, W. E., Helbling, A., Salvaggio, J. E., & Lehrer, S. B. (1995). Fungal allergens. *Clinical Microbiology Reviews*, 8, 161–179.
- Ianovici, N., Popescu, A., & Nemeş, C. (2008). Aeromycological monitoring of *Cladosporium* spores in Timișoara. *Annals of West University of Timișoara*, 11, 1–8.
- Jäger, S. (2003). Plant taxonomy and nomenclature. *Postep Derm Alergol*, 20, 218–226.
- König, H., Uden, G., & Fröhlich, J. (Eds.). (2009). *Biology of microorganisms on grapes, in must and in wine*. Heidelberg: Springer.
- Kurkela, T. (1997). The number of *Cladosporium* conidia in the air in different weather conditions. *Grana*, 36, 54–61.
- Lacey, J. (1996). Spore dispersal – its role in ecology and disease: the British contribution to fungal aerobiology. *Mycological Research*, 100, 641–660.
- Lacey, M. E., & West, J. (2006). *The air spora. A manual for catching and identifying airborne biological particles*. Dordrecht: Springer.
- Mediavilla Molina, A., Angulo Romero, J., Infante García-Pantaleón, F., Comtois, P., & Domínguez Vilches, E. (1998). Preliminary statistical modeling of the presence of two conidial types of *Cladosporium* in the atmosphere of Córdoba, Spain. *Aerobiologia*, 14, 229–234.
- Nilsson, S., & Persson, S. (1981). Tree pollen spectra in the Stockholm region (Sweden), 1973–1980. *Grana*, 20, 179–182.
- Pady, S. M., Kramer, C. L., & Clary, R. (1969). Periodicity in spore release in *Cladosporium*. *Mycologia*, 61, 87–98.
- Pasanen, A.-L., Pasanen, P., Jantunen, M. J., & Kalliokoski, P. (1991). Significance of air humidity and air velocity for fungal spore release into the air. *Atmospheric Environment*, 25A, 459–462.
- Pashley, C. H., Fairs, A., Free, R. C., & Wardlaw, A. J. (2012). DNA analysis of outdoor air reveals a high degree of fungal diversity, temporal variability, and genera not seen by spore morphology. *Fungal Biol*, 116, 214–224.
- Peternel, R., Culig, J., & Hrga, I. (2004). Atmospheric concentrations of *Cladosporium* spp. and *Alternaria* spp. spores in Zagreb (Croatia) and effects of some meteorological factors. *AAEM*, 11, 303–307.
- Ranta, H., & Pessi, A.-M. (2006). Pollen Bulletin Summary 2005. *Finnish Pollen Bulletin*, 30, 1–12.
- Rapiejko, P., Lipiec, A., Wojadas, A., & Jurkiewicz, D. (2004). Threshold pollen concentration necessary to evoke allergic symptoms. *Internat. Rev. Allergol Clin Immunol*, 10, 91–94.
- Reponen, T., Willeke, K., Ulevicius, V., Reponen, A., & Grinshpun, S. A. (1996). Effect of relative humidity on the aerodynamic diameter and respiratory deposition of fungal spores. *Atmospheric Environment*, 30, 3967–3974.
- Rich, S., & Waggoner, P. E. (1962). Atmospheric concentration of *Cladosporium* spores. *Science*, 137, 962–965.
- Rodríguez-Rajo, F. J., Iglesias, I., & Jato, V. (2005). Variation assessment of airborne *Alternaria* and *Cladosporium* spores at different bioclimatical conditions. *Mycological Research*, 109, 497–507.
- Sabariego, S., Díaz de la Guardia, C., & Alba, F. (2000). The effect of meteorological factors on the daily variation of airborne fungal spores in Granada (southern Spain). *International Journal of Biometeorology*, 44, 1–5.
- Sadyś, M., Adams-Groom, B., Herbert, R. J., & Kennedy, R. (2016). Comparisons of fungal spore distributions using air sampling at Worcester, England (2006–2010). *Aerobiologia*, 32, 619–634.
- Shaheen, I. (1992). Aeromycology of Amman area, Jordan. *Grana*, 31, 223–228.

- Sindt, C., Besancenot, J.-P., & Thibaudon, M. (2016). Airborne *Cladosporium* fungal spores and climate change in France. *Aerobiologia*, 32, 53–68.
- Stępańska, D., & Wołek, J. (2009). Intradiurnal periodicity of fungal spores concentrations (*Alternaria*, *Botrytis*, *Cladosporium*, *Didymella*, *Ganoderma*) in Cracow, Poland. *Aerobiologia*, 25, 333–340.
- Troutt, C., & Levetin, E. (2001). Correlation of spring spore concentrations and meteorological conditions in Tulsa, Oklahoma. *International Journal of Biometeorology*, 45, 64–74.
- Twaroch, T. E., Curin, M., Valenta, R., & Swoboda, I. (2015). Mold allergens in respiratory allergy: From structure to therapy. *Allergy Asthma Immunol Res*, 7, 205–220.
- Weidenbörner, M. (2001). *Encyclopedia of food mycotoxins*. Heidelberg: Springer.
- Zar, J. H. (1984). *Biostatistical Analysis*. New Jersey: Prentice Hall.
- Zureik, M., Neukirch, C., Leynaert, B., Liard, R., Bousquet, J., & Neukirch, F. (2002). Sensitisation to airborne moulds and severity of asthma: cross sectional study from European Community respiratory health survey. *BMJ*, 235, 1–7.