Aerobiologia

Effects of wind speed and direction on monthly fluctuations of Cladosporium conidia concentration in the air

--Manuscript Draft--

Manuscript Number:	AERO-D-17-00008R1
Full Title:	Effects of wind speed and direction on monthly fluctuations of Cladosporium conidia concentration in the air
Article Type:	Original paper
Keywords:	fungal spores; atmosphere; HYSPLIT; circular statistics; dynamic; airborne transmission
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Funding Information:	University of Worcester (Ph.D. scholarship) — Dr. Magdalena Sadyś
Abstract:	This study determined the relationship between airborne concentration of Cladosporium spp. spores and wind speed and direction using real data (local wind measured by weather station) and modelled data (air mass flow computed with the aid of Hybrid Single Particle Lagrangian Trajectory model). Air samples containing fungal conidia were taken at an urban site (Worcester, UK) for a period of five consecutive years using a spore trap of the Hirst design. A threshold of ≥ 6,000 s m-3 (double the clinical value) was applied in order to select high spore concentration days, when airborne transport of conidia at a regional scale was more likely to occur. Collected data were then examined using geospatial (GIS) and statistical tools, including circular statistics. Obtained results showed that the greatest numbers of spore concentrations were detected in July and August, when Cladosporium herbarum, C. cladosporioides and C. macrocarpum sporulate. The circular correlation test was found to be more sensitive than Spearman's rank test. The dominance of either local wind or the air mass on Cladosporium spore distributions varied between examined months. Source areas of this pathogen had an origin within the UK territory. Very high daily mean concentrations of Cladosporium spores were observed when daily mean local wind speed was vs ≤ 2.5 m s-1 indicating warm days with a light breeze.
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Title: Effects of wind speed and direction on monthly fluctuations of Cladosporium conidia concentration in the air

Dear Carmen Galán, Ph.D.
Editor-in-Chief
Aerobiologia

I would like to thank you and my anonymous reviewer for the manuscript evaluation. I found all the comments very supportive and encouraging.

I now feel that after applying changes suggested by all reviewers this manuscript has improved and hence, it will be matching the standards of the Aerobiologia journal.

Furthermore, I would like to confirm that this work is original and has not been published elsewhere or has currently been under consideration for publication elsewhere.

I have attached below my replies to the specific reviewer’s comments as well as revised manuscript for your kind perusal.

Yours sincerely,
Dr Magdalena Sadys

Reviewer 1:

Comment 1
I accept the manuscript to be published. The manuscript needs a minor revision. The comments to improve the work are the following.

Reply
I would like to thank the reviewer for the manuscript evaluation and an indication of areas requiring the improvement.

Comment 2
Keywords
Consider the change transport by dynamic.

Reply
As suggested by the reviewer I changed “transport” by “dynamic”, and also I replaced “aerobiology” by “airborne transmission”.

Comment 3
Abstract
Consider the change dependency by relation.

Reply
As suggested by the reviewer I changed “dependency” for “relationship” as it was more appropriate taking the grammar into account.

Comment 4
1 Introduction
Line 95. Introduce "," before "they constituted…"

Reply
As suggested by the reviewer I added comma before “they constituted”.

Comment 5
I think that the introduction is quite short. It would be appropriate to introduce a small review the urban dynamics of particles into the atmosphere on a small scale for a city. Moreover, it would convenient a small review over allergy. On the other hand, it would be accurate a small review on GIS tools for fungal spores.

Reply
Following the reviewer's suggestions the introduction has been extended. Now it counts 915 words. I also extended the bibliography for further 9 references.

Comment 5
4 Discussion

Line 264. Add n to "a unimodal…"

Reply
As suggested by the reviewer correction has been applied.

Line 267. Add point before "Cladosporium…"

Reply
As suggested by the reviewer correction has been applied.

Line 335. Add grains to pollen

Reply
As suggested by the reviewer correction has been applied.

Comment 6
Figure 1b. It would be advisable to edit the years with colours

Reply
As suggested by the reviewer Fig. 1 has been edited. The years are now clearer, and the same applied to the months.

Comment 7
Figure 4. What is the criteria to establish as limit 2.5 m s-1?

Reply
The meaning of applied threshold of 2.5 m s-1 has been previously explained in the results section. I have now given the same in the legend of the Fig. 4 to clarify this.

Comment 8
Table 1 and 3. Indicate the meaning of the bold colour.

Reply
I removed bold font. I have previously used it to strengthen the visibility of results which have achieved a statistical significance but this is unnecessary since I have also used an asterisk to indicate it.

General comments:
I consider that the manuscript presents a novel topic applied to fungal spores.

Reply
I would like to thank the reviewer for his/her positive opinion on the manuscript.

Effects of wind speed and direction on monthly fluctuations of *Cladosporium* conidia concentration in the air

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Abstract

This study determined the relationship between airborne concentration of *Cladosporium* spp. spores and wind speed and direction using real data (local wind measured by weather station) and modelled data (air mass flow computed with the aid of Hybrid Single Particle Lagrangian Trajectory model). Air samples containing fungal conidia were taken at an urban site (Worcester, UK) for a period of five consecutive years using a spore trap of the Hirst design. A threshold of $\geq$ 6,000 s m^{-3} (double the clinical value) was applied in order to select high spore concentration days, when airborne transport of conidia at a regional scale was more likely to occur. Collected data were then examined using geospatial (GIS) and statistical tools, including circular statistics. Obtained results showed that the greatest numbers of spore concentrations were detected in July and August, when *Cladosporium herbarum*, *C. cladosporioides* and *C. macrocarpum* sporulate. The circular correlation test was found to be more sensitive than Spearman's rank test. The dominance of either local wind or the air mass on *Cladosporium* spore distributions varied between examined months. Source areas of this pathogen had an origin within the UK territory. Very high daily mean concentrations of *Cladosporium* spores were observed when daily mean local wind speed was $v_s \leq 2.5$ m s^{-1} indicating warm days with a light breeze.

Keywords: fungal spores; atmosphere; HYSPLIT; circular statistics; dynamic; airborne transmission;

1. Introduction

Cladosporium spp. conidia have become of special interest to scientists since 1932 when Cobe first reported their allergenic properties (Hyde et al. 1956). Bouziane et al. (2005) and Bouziane et al. (2006) found that conidia of *Cladosporium cladosporioides* contained a larger concentration of allergens than mycelia. Furthermore, Green et al. (2003) showed that increased allergen production varying from 5% to 40% was observed during germination by *Cladosporium herbarum*. A cross-sectional study of Zureik et al. (2002) examined sensitization rates in 1,132 patients living in six regions, *i.e.*, northern Europe, central Europe, southern Europe, United Kingdom/Republic of Ireland, Portland (US) and Australia/New Zealand using allergen extracts from two fungi (*Alternaria* spp., *Cladosporium* spp.), five pollen types (grass, birch, ragweed, olive, pellitory of the wall), cat and house dust mites. Their survey showed that the sensitization rates to fungi increased along with the severity of asthma. In Europe, the sensitization to *Cladosporium* spp. (hereafter *Cladosporium*) was found within the range of 0.7-9.9% with an upward trend towards the North; in the case of British and Irish population this was equal to 6.8% (Zureik et al. 2002). Another study conducted in 16 European countries confirmed the highest sensitization rate to *Cladosporium herbarum* to occur in Ireland, UK and other northern countries, and an average sensitization rate to this type of spores equal to 5.8% (Heinzerling et al. 2005).

The outcomes of these clinical surveys are in agreement with the observations previously made by Lacey (1981) who reviewed a number of aerobiological reports and established the dominance of *Cladosporium* spores in the air of areas characterized by cooler humid continental climates with warm summers; in Madrid (Spain) overall contribution of *Cladosporium* spores to the total air spora was estimated for 41%, while in Worcester (UK) this can reach up to 75% (Sadyś et al. 2015a; Díez Herrero et al. 2006). Other sites, such as Krasne (Poland) reported contributions up to 92% (Kasprzyk and Worek 2006). Harvey (1970) estimated spore production in six species of *Cladosporium* to be within a range from 7.3×10^2 to 2.61×10^4 s mg^{-1} dry weight of mycelium. Spore production by individual species turned out to be independent of spore frequency in the air (Harvey 1970). Frankland and Davies (1965) established a threshold value of spore concentration above which susceptible individuals exhibit symptoms of sensitization, *i.e.* 3,000 s m^{-3} in the United Kingdom. Different threshold values for *Cladosporium* were estimated in Finland (4,000 s m^{-3}) and Poland (2,800 s m^{-3}), (Ranta and Pessi 2006; Rapiejko et al. 2004).

Cladosporium spores were found to be present in the upper atmosphere layer at 3.3 km above the ground and together with spores of *Alternaria* spp. and *Aspergillus* spp., they constituted 75% of total collected fungal spores (Fulton 1966). However, the vertical stratification in *Cladosporium* spore concentration measured at 300 m and at 1,650 m above ground level revealed to be similar (Hirst et al. 1967). Subsequently, Hirst (1973) concluded that *Cladosporium* spores may be suspended in the atmosphere for a period longer than a week, based on the analysis of air samples collected during several flights, and indicated their potential for a long-distance transport. A case study from Taiwan has confirmed this when *Cladosporium* was found as a major biological component of the dust blown from China (Wu et al. 2004). A qualitative and quantitative study of free tropospheric air in the North America has also detected *Cladosporium* conidia among identified fungal taxa that originated from inoculum sources located in Asia (Smith et al. 2012). The transport of bioaerosols in the atmosphere can be studied with the aid of atmospheric models, such as CALifornia PUFF Model (CALPUFF 1990), HYbrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT; Draxler and Rolph 2014; Rolph 2014) and System for Integrated modeLling of Atmospheric composition (SILAM; Sofiev and Siljamo 2004; Sofiev et al. 2006). However, a limited number of surveys investigated regional and long-distance airborne transmission of fungal spores, e.g. rust spores (Isard et al. 2005), *Leptosphaeria biglobosa* (Grinn-Gofroń et al. 2015), *Alternaria* spp. (Fernández-Rodríguez et al. 2015; Sadyś et al. 2015b; Skjøth et al. 2012). To date only one article was focused exclusively on *Cladosporium* (Sadyś et al. 2015c) and many more are needed since this is one of the most important fungal aeroallergens. Grinn-Gofroń (2009) reviewed a large number of reports which examined the dependence of *Cladosporium* on the meteorological variables. She found positive statistically significant correlations with maximum, minimum, and mean temperature, sunshine hours while negative statistically significant relationships with dew point temperature and air pressure. Contrary results were found for rainfall, relative humidity and wind speed by other researchers (Grinn-Gofroń 2008; Herrero and Zaldivar 1997; Hjelmroos 1993; Katial et al. 1997; Kurkela 1997; Mediavilla Molina et al. 1998; Mitakakis et al. 1997; Oliveira et al. 2009a; Stępalska and Wołek 2005; Troutt and Levetin 2001). However, this review did not include a wind direction analysis as this is a rarely studied parameter (Recio et al. 2012; Sánchez Reyes et al. 2009).

The aim of this study was to analyze the impact of local wind and air mass flow over an urban area (Worcester, UK) in connection with the monthly concentration pattern in *Cladosporium* conidia in the air of a chosen location. This has been accomplished by (1) collecting air samples throughout a 5-year period (2006-2010) using air sampler of the Hirst design, (2) microscopy analysis, (3) atmospheric modelling using the Hybrid Single Particle Lagrangian Integrated Trajectory model, (4) circular statistics and (5) geospatial evaluations of collected data.

2. Materials and methods

2.1. Bioaerosol specimen

The volumetric air sampler (Burkard Manufacturing Co. Ltd., Rickmansworth, UK) was operating continuously from 2006 to 2010. The spore trap (Hirst 1952) was installed permanently at a height of 10 m above ground level, on the rooftop of the University of Worcester building (52° 11’ 48” N, 2° 14’ 31” W). Measurements were taken following the guidance given by Lacey and West (2006). Drums with trapping surface were changed every Thursday at 09:00 UTC, and then processed at the laboratory as described by Lacey and West (2006).

The shape of the *Cladosporium* spores varies from cylindrical, through ellipsoidal and ovoid up to sub-spherical. They are usually small in size (40-60 µm × 3-22 µm) from olivaceous to brown in colour, frequently observed in branched chains. Hence, depending on the location, spores may have a shield or round shape at the ends, visible black scars of attachment points. Spores towards the end of chain are smaller and aseptate (Bensch et al. 2012). The surface of the wall may be either smooth or rough (verruculose or echinulate), (Ellis 1971).

Spores of *Cladosporium* species were identified up to the genus level under ×400 magnification and counted from one central lengthwise stripe, with an hourly division. Obtained spore counts were then multiplied by a correction factor, specific for the microscope used (Nikon Eclipse E400) to acquire the spore concentration expressed in *n* spores per cubic meter of air (Lacey and West 2006).

Throughout a 5-year period the clinical threshold of $\geq 3{,}000$ s m^{-3} established for *Cladosporium* conidia (Frankland and Davies 1965) was recorded on 330 days and varied from 47 to 88 days in a single year (Sadyś et al. 2016). Such atmospheric concentrations achieved by *Cladosporium* can be produced by a local source. Thus, in order to examine the impact of local wind and air mass transport on *Cladosporium* concentrations, and possible transport of conidia at a regional scale, days when clinical threshold was two-folded were selected for this study (*n*=131). As this study focused on monthly fluctuations in bioaerosol distribution, data for a single day with above-mentioned concentration, which occurred on the

30th of April 2007, was discarded as it would not constitute a representative value for the entire month.

2.2. Meteorological data and atmospheric modelling

The meteorological data were obtained using the Weather Link Vantage Pro2 weather station which was placed next to the air sampler. Out of a number of recorded parameters this study focused on the impact of the local wind direction extracted for days when very high concentrations (≥ 6,000 s m^{-3}) of *Cladosporium* spore were found. During the first 4 years, the wind direction data were recorded with 5 min intervals. In 2010 year the number of records was reduced to 96 per 24 h period. Finally, hourly mean values were computed in order to allow a comparison between years of sampling as well as a comparison of fungal spore counts with clusters of backward trajectories.

The HYSPLIT model was employed in order to calculate the clusters of back trajectories graphically presenting transport of the air masses during the examined period of time (Draxler and Rolph 2014; Rolph 2014). The Global Data Analysis System (GDAS), which has been made available by the National Oceanic and Atmospheric Administration (NOAA) Air Resources Laboratory (ARL), formed the foundation of the back trajectories. The temporal resolution was chosen to equal to 1 h while the total of 24 trajectories was generated for the period of 24 h. Due to the design of trajectory models, such as HYSPLIT, it is therefore recommended to use a receptor height from 200 m to 1,000 m to simulate the overall transport in the planetary boundary layer (Fernández-Rodríguez et al. 2015; Hernández-Ceballos et al. 2014), which includes convection and dispersion near the source. In this study, the back trajectories were computed at the height of 500 m above ground. Further analysis of the air masses transport was performed using the Geographic Information System (GIS) techniques (ArcMap v. 10.0).

2.3. Statistical analyses

Directions of local wind and air mass were investigated using the circular statistics. The linear-circular correlation analysis between spore occurrence in the atmosphere and wind direction was possible thanks to "cassociation" module available in GenStat (v. 17) software. This methodology was described in more detail by Sadyś et al. (2015c) and Maya-Manzano et al. (*in press*). In addition to that, Spearman's rank test was applied. The level of statistical association was classified following Mukaka (2012). Hours, when calm was recorded in local wind data, were excluded in order to perform a correlation analysis. From June to September the contribution of calm hours did not exceed 2% of a total number of records. Upon the primary results that would show the greater influence either of local wind or air mass direction, a further velocity analysis was performed.

3. Results

3.1. Distribution of *Cladosporium* spores

Concentrations of *Cladosporium* spores varied significantly between months (Fig. 1a, Fig. 1b). Overall the greatest number of spores was trapped in July and these constituted 31.79% of the total 5-year spore catch. August (22.97%) and June (15.87%) were the second and third months in order when large numbers of conidia were observed in the air of Worcester (Fig. 1a). However, this pattern was not repeated each year, as in 2007 the greatest concentration of *Cladosporium* spores was collected in June, followed then by July and September (Fig. 1b). In 2008, once again spore counts recorded in September outnumbered counts observed in June the same year (Fig. 1b). An interesting situation also occurred in 2009, when the second largest monthly sum of daily mean spore concentration was found in October, not in August (Fig. 1b). A total number of spores collected between December and April contributed less than 4% of the total number of spores recorded within five years of investigation (Fig. 1a). The number of high spore concentration days was also a subject of change. A number of days, when daily mean spore concentration was ≥ 6,000 s m^{-3}, turned out to be within a range from 8 in 2007 to 47 in 2006. The year 2010 showed a lot of similarities to 2006, as 44 high spore concentration days were found and exactly the same order in monthly contribution occurred (Fig. 1b).

3.2. Influence of wind direction on spore counts

The Spearman's rank test (Table 1) showed that the relationship between hourly mean spore concentration and local wind direction was inversely proportional, and it reached the level of statistical significance ($p \leq 0.05$) only in July and September. The highest correlation coefficient of $r_s = -0.47$ was found in September (Table 1). With regard to air mass, the relationship with spore concentration also revealed to be inversely proportional (Table 1). The highest correlation coefficient value arose in September ($r_s = -0.43$). No statistically significant association was found between *Cladosporium* presence and air mass direction in June, August and October (Table 1).

The analysis of spore dependence on wind direction examined using linear-circular correlation is also presented in Table 1. Both associations with local wind and air mass directions with *Cladosporium* spores were statistically significant in each investigated month with an exception of August (Table 1). The vector of these relationships was found to be proportional, yet weak to moderate (Table 1). In October, a slightly larger impact on spore occurrence revealed local wind above the air masses, while this has changed in favour of the air mass in remaining months.

3.3. Air mass analysis

The analysis of the back trajectories revealed that the durations air masses spent over the non-UK areas were only a minor fraction of the time within the 24 h before they reached Worcester (Table S1, Fig. 2). In the annual summaries for high spore concentration days, this fraction of the time was found to be ≤ 16%. The influence of possible sources of *Cladosporium* spores from Ireland was estimated at 4% or less (Table S1).

Figure 3 presented the distribution of the air mass for each studied month when the daily mean concentration of *Cladosporium* spores was equal to or above 6,000 s m^{-3}. Overall, the air masses were coming from the SSE to the WNW directions, while none or very little contribution was detected from N-E bearings (Figs 2, 3). Obtained results were in agreement with an analysis of clustered trajectories points, which showed that majority of the air masses originated from the southern directions (SW-SE) when increased levels of *Cladosporium* spores were trapped at Worcester station (Table 2, Fig. 3). Throughout the period of study, the mean angle remained within a range of 135°-293° (Table 2). A lack of uniformity in the sampled data was confirmed jointly by von Mises and Rayleigh tests (Table 2). The values of kappa also greatly varied, with an agreement from 0.60 (October) to 2.14 (September), (Table 2). The relationship between local wind direction and air mass directions (Table 3) varied from low (August) to high (October) level of association.

3.4. Local wind analysis

The overall distribution of local wind direction was examined using daily mean values recorded within five years of study. Results of this analysis are presented in Fig. 3. No influence of the northern direction was observed from the end of spring and throughout summer. Its contribution started to be apparent with the advent of autumnal months (Fig. 3). A similar pattern was found for NE direction. Wind blowing from the eastern bearings (E-SE) was mainly recorded in spring (June) and autumn (September-October) while its contribution decreased to a minimum of 1% input in August (Fig. 3). The dominance of southern directions (S-SW) was pervasive and reached a maximum of 35% in July (Fig. 3).

Western wind constituted the second fraction with regard to its impact on the overall distribution of the local wind measured in Worcester. The greatest input was noted in August when it scored 22% while its importance diminished with the beginning of autumn (Fig. 3). Similar results were true for NW wind direction.

A more detailed analysis of local wind direction during high spore concentration days is given in Table 2. Both the Chi-square von Mises and the Rayleigh tests revealed that the null hypothesis must be rejected, and hence local wind direction did not have a uniform circular distribution. The correlation between observed and expected from a von Mises distribution, expressed as kappa, varied monthly from 0.64 (June) to 1.44 (September). Figure 3 showed wind histograms for each individually examined month, produced upon spore concentration threshold equal to or above 6,000 s m^{-3}. Both the size of analyzed samples varied, as well as the monthly fluctuations in local wind direction (Fig. 3). High spore counts of *Cladosporium* conidia were recorded, when wind direction was observed within the span of ESE to the WNW directions (121°-294°), (Table 2, Fig. 3).

Upon these results, it was decided to perform a further analysis of the local wind speed recorded during high spore count days, individually for each month (Fig. 4). Obtained histograms showed that regardless the time of the year and the spore concentration levels, 95% of observations were made when daily mean wind speed was equal to or lower than 2.5 m s^{-1} (Fig. 4). A Spearman's rank test did not find this relationship to be statistically significant (r_s=0.019, p=0.847).

4. Discussion

This study indicated an unimodal distribution in *Cladosporium* spore frequency, with a single peak occurring mostly in July. Morrow Brown and Jackson (1978a) reported that at 8 locations across England, *i.e.*: Derby, Birmingham, Ashby, Church Broughton, Hartington, Crich, Attenborough, Sutton Bonington, *Cladosporium* spores similarly showed a single peak between the end of July and mid-August although the spore counts did not vary greatly between sampling sites. In contrast, a concentration of *Cladosporium* spores in Spain (Madrid, Malaga, Valladolid) and Turkey (Sivrihisar) was reported to follow a bimodal distribution with first peak occurring either by the end of spring or at the beginning of summer (May-June) and second peak observed in autumn (September-October), (Díez Herrero et al. 2006; Sánchez Reyes et al. 2009; Recio et al. 2012; Erkara et al. 2009). The magnitude of spring-summer and autumn peaks differed between locations, and the latter peaks in Madrid were more important than in Malaga or Valladolid. With regard to the monthly sum of daily mean spore concentrations, October catch was a factor of 2 higher than June (Díez Herrero et al. 2006). Such high contribution of autumn months was explained by more susceptible environmental conditions for the fungal growth while too high daily maximum temperature and lack of precipitation over the summer prevented numerous spore production and dissemination (Díez Herrero et al. 2006; Sánchez Reyes et al. 2009; Erkara et al. 2009). However, none of the authors reported which *Cladosporium* species were responsible for the spring-summer and autumn peaks.

A study of Harvey (1967) determined that spores produced by six *Cladosporium* species dominated in the air of Cardiff (Wales), *i.e.*: *C. herbarum, C. cladosporioides, C. sphaerospermum, C. macrocarpum, C. elatum* and *C. resinae.* They jointly constituted more than 97% of the total *Cladosporium* spore catch (Harvey 1967). Similar results were reported by Calvo Torras et al. (1981) who isolated from the air in Barcelona (Spain) the same species of *Cladosporium* with the exception of *C. resinae.* Also, the frequency of *C. macrocarpum* was found to be greater than that of *C. sphaerospermum* (Calvo Torras et al. 1981). In the UK conidia of *Cladosporium herbarum* are present in the air mainly between June and October, and usually, they peak twice during the vegetation season, *i.e.*: (1) in June-July, (2) in August-October. The presence of *Cladosporium cladosporioides* overlaps with *C. herbarum,* as spores are found from July to September with peak protruding mostly in August. Increased concentrations of *Cladosporium sphaerospermum* are observed largely in colder, autumnal months (September-November). Finally, *Cladosporium macrocarpum* sporulates simultaneously with *C. herbarum*, thus its presence is difficult to detect (Harvey 1967). Moreover, Oliveira et al. (2009b) examined spore levels in urban and rural areas of Portugal and showed that throughout a 3-year survey the highest levels of *Cladosporium* spores in a rural area were found in autumn (September-October). This trend was not the same for an urban area where spores peaked primarily during summer time (July-August). The latter results were, however, similar to those recorded at Worcester sampling station. This study also showed that overall percentage directions of the local wind remained constant throughout the examined period of time (Fig. 3). Despite this, Spearman’s rank test indicated statistically significant correlations between very high spore concentration days and local wind direction only in July and September (Table 1). These results were not confirmed by circular statistics, as the dominance of local winds was found only in October (Table 1). Considering the overall local wind direction distribution, it seems that this could be explained by the greatest contribution of wind blowing from E-SE directions (26%) in comparison with other examined months (Fig. 3). With regard to the impact of air mass on fungal spore levels, Spearman's rank test showed statistically significant associations for the same months as with local wind direction (Table 1). Lack of any sort of correlation between *Cladosporium* and both local wind and air mass directions was observed in August (Table 1). Despite that during this month, the air mass spent over the non-UK areas the lowest amount of their time (Table S1). Also, the relationship between local wind and air mass direction showed to be the weakest in August (r_s=0.41) out of five examined months (Table 3). Taking into account the overall pattern in local wind direction, August was notable for a significant decrease in the contribution of E-SE wind direction (Fig. 3). Hence, within a span of 91°-180° there must be a considerable source area of *Cladosporium* spores. Studies that investigate the impact of wind direction on bioaerosol concentration are very scarce (Sadyś et al. 2015c). An exception is a study of Sánchez Reyes et al. (2009) who examined the impact of wind direction on the presence and concentration levels of *Cladosporium* spores in the air of Valladolid (Spain). Another exception is a survey made by Recio et al. (2012) who performed the same analysis in Malaga (Spain). Sánchez Reyes et al. (2009) found that although NE direction was dominant throughout two years of sampling (37.4% and 31.4%, respectively), Spearman's rank test indicated statistically significant correlation with SE wind direction only. Sánchez Reyes et al. (2009) reported that along this direction an extensive grassland area was found that most likely constituted an inoculum source of *Cladosporium* spores (Sánchez Reyes et al. 2009). Contrary findings were reported by Recio et al. (2012) who found statistically significant relationships between dominant wind directions (SW and NE) and an increase in fungal spore concentration. Moreover, wind blowing from the sea (SE) was correlated negatively with the presence of *Cladosporium* spores in the air of Malaga (Recio et al. 2012).

Morrow Brown and Jackson (1978b) investigated the difference in the contribution of local wind direction to the overall fungal spore (including *Cladosporium*) and pollen grains (grass, nettle) concentration recorded at three coastal sites (Point Lynas, Withernsea, Cromer) and one inland (Derby). In general, the lowest pollen and spore concentration were found in Point Lynas (West coast) where the wind from the sea dominated over the wind from the land. In contrast, the highest counts of biological particles were detected in Derby located in the center of the East Midlands of England. Similar high concentration of *Cladosporium* conidia was observed in Cromer (East coast), where wind blowing from the land contributed more significantly than the wind originating from the North Sea, thus over passing potential source areas of the fungus. Likewise, Rodríguez-Rajo et al. (2005) reported a rise in *Cladosporium cladosporioides* type proportionally to the increase of the Continental Index, and inversely proportionally to the effect of the sea. Out of three sampling stations, the coastal site (Vigo) exhibited a strong positive correlation (r_s=0.45-0.52), simultaneously with a mountainous site in Trives (r_s=0.29-0.58) between *Cladosporium* concentration and wind calm at the significance level of $p \leq 0.001$ (Rodríguez-Rajo et al. 2005). The inland site located in Ourense demonstrated the greatest contribution in spore concentration when NE-S wind direction occurred (r_s=0.32, $p \leq 0.001$).

Finally, although the correlation between local wind and high concentration of *Cladosporium* spores was not found to be statistically significant, yet 95% of observations were made when daily average wind speed was $v_s \leq 2.5$ m s^{-1}. This finding is in agreement with previously reported a value of $v_s \leq 3$ m s^{-1} in relation to the overall dispersal of biological particles in the atmosphere (Reynolds et al. 2007), as well as v_s varying between 2 and 3.5 m s^{-1} established in particular for *Cladosporium* spores (Kurkela 1997).

Conclusion

The major findings of this aerobiological survey were following: (1) the greatest numbers of spore concentrations were recorded in July and August when *Cladosporium herbarum*, *C. cladosporioides* and *C. macrocarpum* sporulate; (2) sources of *Cladosporium* conidia must have an origin within the UK territory; (3) local wind had a greater impact on *Cladosporium* conidia occurrence in the air of Worcester than the air masses; (4) taking into account the strength of statistical significance of detected dependencies of *Cladosporium* on local wind, it must be stressed that the origin of conidia had a rather regional than local character; (5) the most contributing sources of the fungus were located in the SE to SW directions; (6) very high daily mean concentrations of *Cladosporium* spores, *i.e.* between 6,000 and 32,000 spores per cubic meter of air, were observed when daily mean local wind speed was $v_s \leq 2.5$ m s^{-1} indicating warm days with light breeze.

Acknowledgements

This project was funded by the University of Worcester and conducted within the framework of the doctoral studies. The author would like to thank Dr. Andrew M. Reynolds (Rothamsted Research) for a critical evaluation of the manuscript. Subsequently, thanks go to Dr. Carsten Ambelas Skjøth (University of Worcester) for producing Fig. 2 used in this study. Finally, the author would like to acknowledge the NOAA ARL for the provision of the HYSPLIT model used in this publication as well as access to input data (GDAS archive) for running the HYSPLIT model.

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Figure captions

Fig. 1a Five year sums of daily mean concentration of *Cladosporium* spores, recorded monthly, measured in Worcester, UK (2006-2010) and expressed in percentage. Contribution of less than 5% was not shown.

Fig. 1b Monthly sums of daily mean concentration of *Cladosporium* spores measured in Worcester, UK (2006-2010).

Fig. 2 Foot print area computed upon frequency distribution of the air mass trajectories recorded during very high *Cladosporium* spore count days ($\geq 6{,}000$ s m^{-3}).

Fig. 3 Histograms showing a distribution pattern of the air masses, local wind direction both expressed in percent and *Cladosporium* spore concentrations recorded during high spore count days between June and October in Worcester, UK.

Fig. 4 Histograms showing (a) a daily mean concentration of *Cladosporium* recorded between June and October in Worcester, UK, (b) daily mean local wind speed with an indication of 5% error bars, (c) threshold line – 95% of observations were found when daily mean wind speed was ≤ 2.5 m s^{-1}. In all examined cases concentration of *Cladosporium* was equal to or higher than 6,000 spores per cubic meter of air. The number of examined cases varied between months.

Table captions

Table 1 Results of Spearman's rank test (r_s) and linear-circular correlation (r_c) between *Cladosporium* spore concentration and local wind (a) and air mass (b) directions

Table 2 Results of descriptive circular statistics for local wind and air mass direction, when high *Cladosporium* spore count occurred (n=130)

Table 3 Results of Spearman's rank test (r_s) between local wind and air mass directions

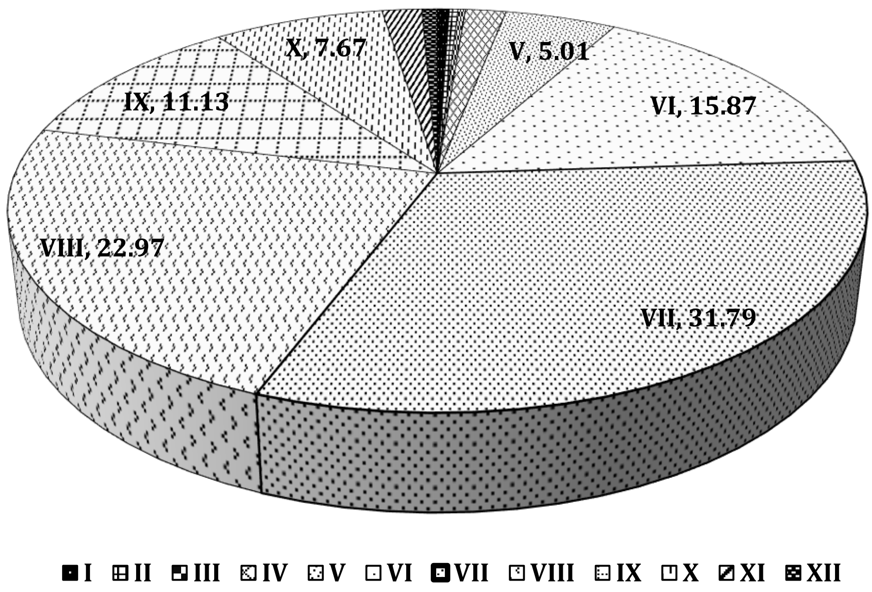


Fig. 1a Five year sums of daily mean concentration of *Cladosporium* spores, recorded monthly, measured in Worcester, UK (2006-2010) and expressed in percentage. Contribution of less than 5% was not shown.

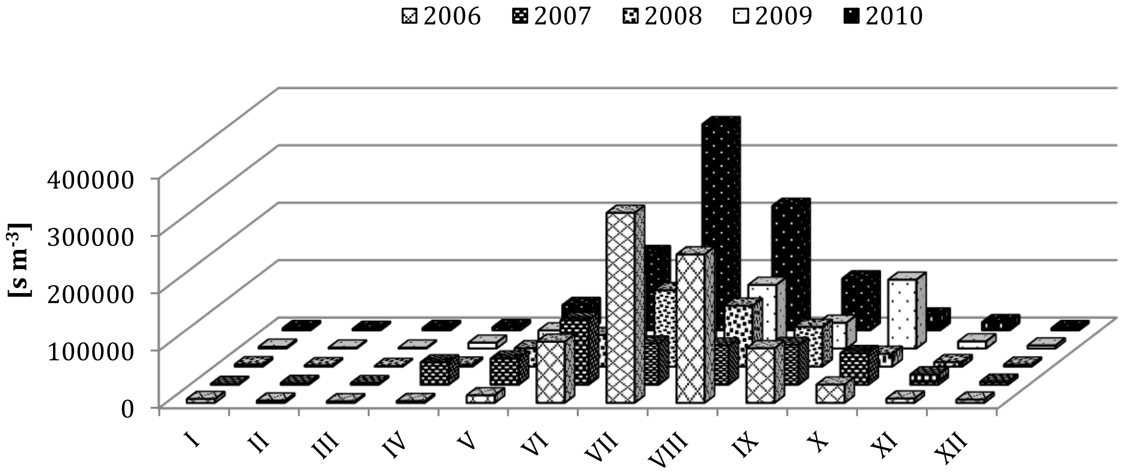


Fig. 1b Monthly sums of daily mean concentration of *Cladosporium* spores measured in Worcester, UK (2006-2010).

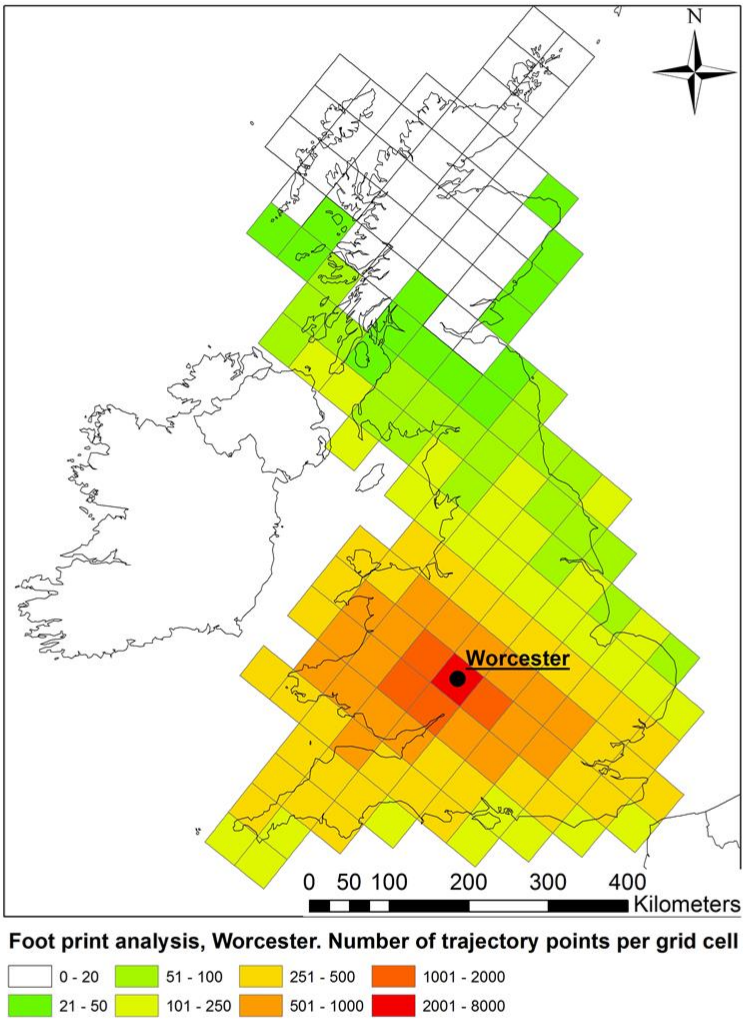
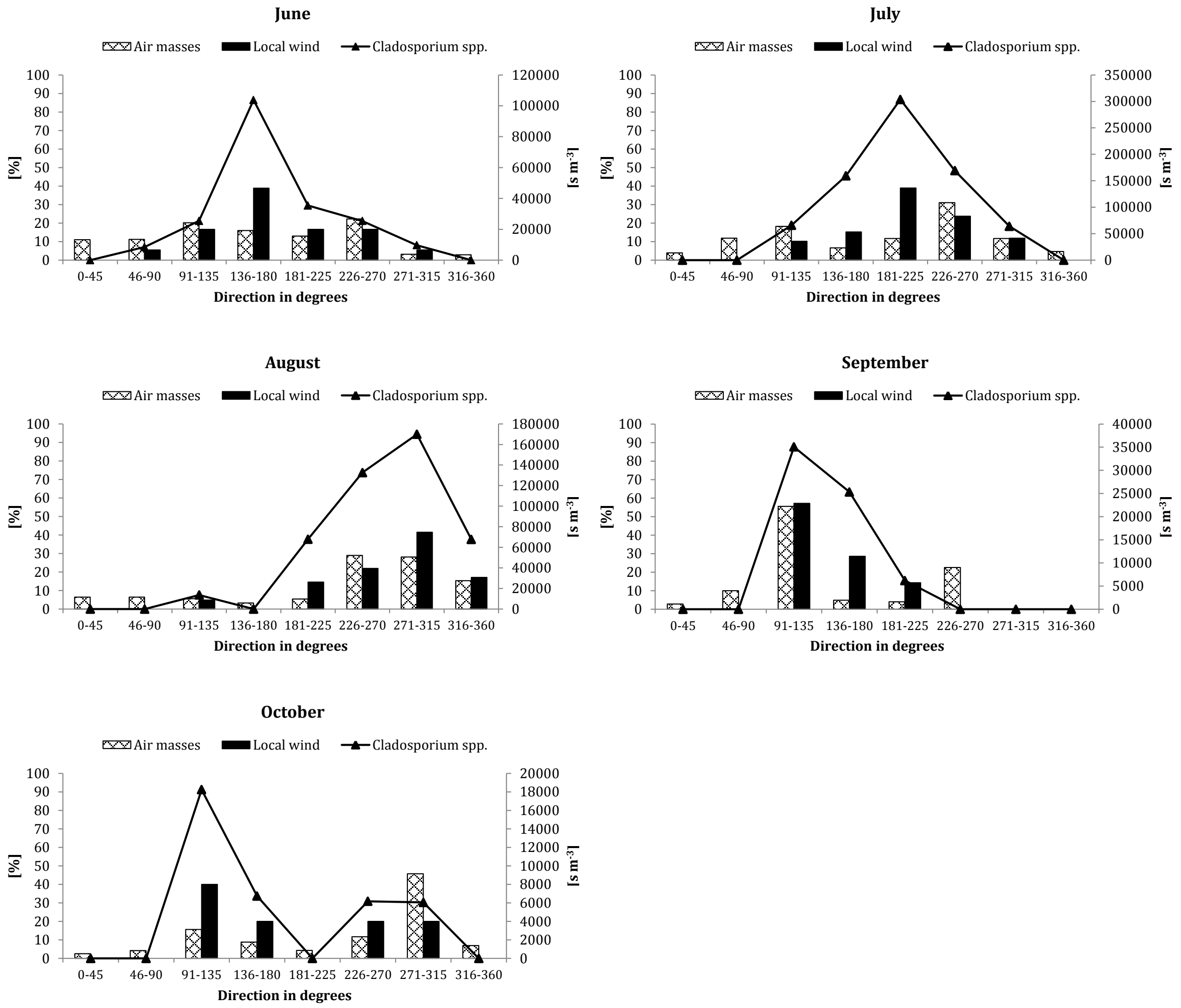


Fig. 2 Foot print area computed upon frequency distribution of the air mass trajectories recorded during very high *Cladosporium* spore count days ($\geq$ 6,000 s m^{-3}).



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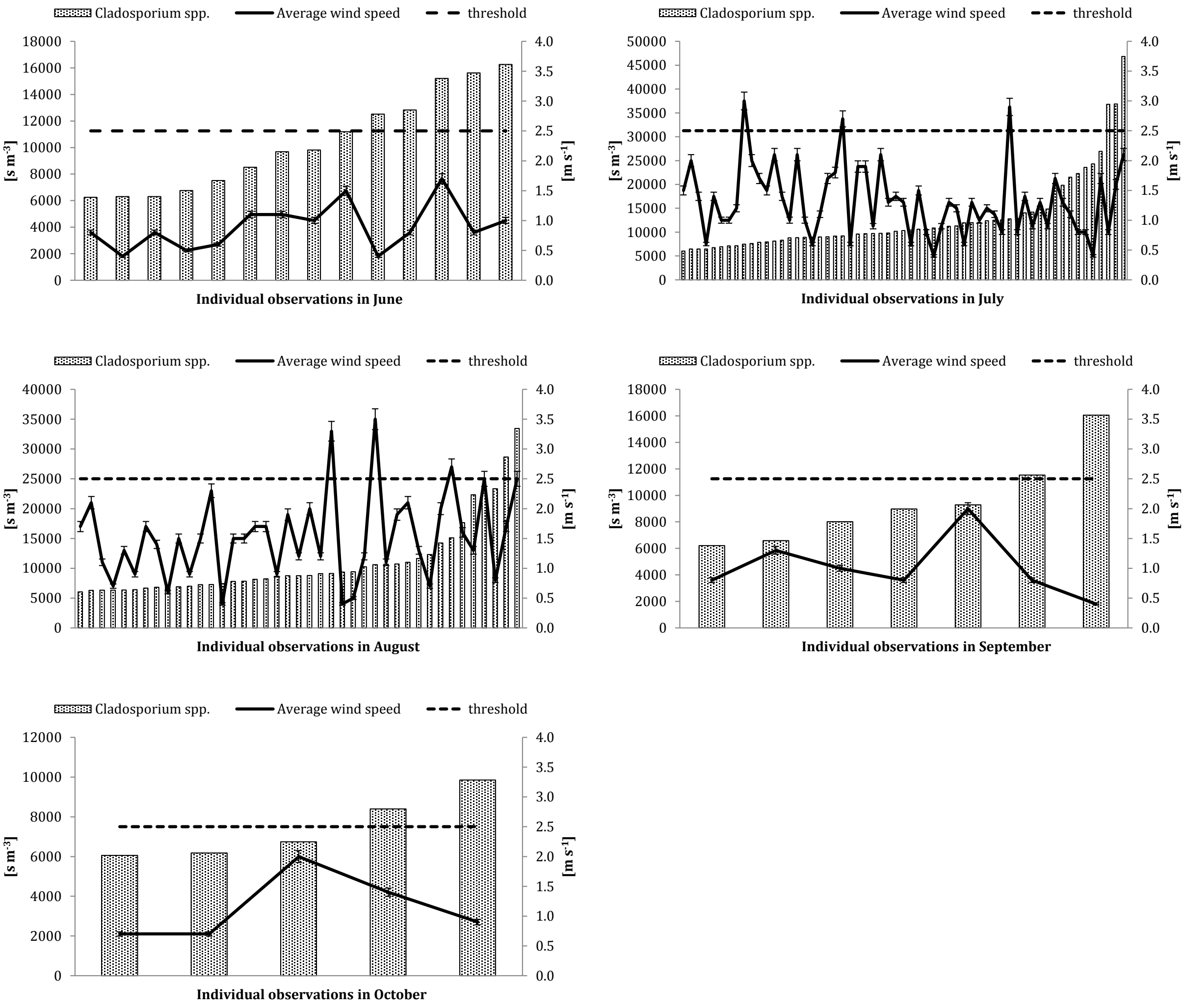

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Table 1 Results of Spearman’s rank test (r_s) and linear-circular correlation (r_c) between *Cladosporium* spore concentration and local wind (*a*) and air mass (b) directions.

Year	June		July		August		September		October	
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
r_s	-0.06	-0.04	-0.09*	-0.12*	-0.02	-0.00	-0.47*	-0.43*	-0.18	-0.16
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
r_c	0.15*	0.25*	0.08*	0.16*	0.05	0.07	0.20*	0.28*	0.41*	0.25*

* Statistical significance at $p \leq 0.05$

Table 2 Results of descriptive circular statistics for local wind and air mass direction, when high *Cladosporium* spore count occurred (*n*=130)

Class		Month				
		June	July	August	September	October
Air masses [a]	Mean direction [°]	161.62°	212.98°	292.63°	135.25°	232.82°
	Circular standard deviation [°]	74.39°	78.93°	62.83°	46.43°	90.59°
	Mean resultant length	0.43	0.39	0.55	0.72	0.29
	Skewness	-0.19	0.75	-0.18	-2.21	0.49
	Kappa estimate	0.96	0.84	1.32	2.14	0.60
	Prob. test of randomness	1.00	0.00	1.00	1.00	1.00
	Prob. Rayleigh test of uniformity	0.00	0.00	0.00	0.00	0.00
	Chi-square von Mises*	696.53	448.42	157.34	1105.26	1189.49
	Prob. Chi-square von Mises	0.00	0.00	0.00	0.00	0.00
Local wind [b]	Mean direction [°]	164.96°	212.46°	293.63°	121.13°	279.58°
	Circular standard deviation [°]	88.51°	73.97°	68.05°	59.68°	75.45°
	Mean resultant length	0.30	0.44	0.49	0.58	0.34
	Skewness	-0.07	-0.01	0.49	-0.12	0.42
	Kappa estimate	0.64	0.97	1.14	1.44	0.93
	Prob. test of randomness	1.00	0.00	1.00	1.00	1.00
	Prob. Rayleigh test of uniformity	0.00	0.00	0.00	0.00	0.00
	Chi-square von Mises*	704.86	91.89	205.84	1103.82	1281.86
	Prob. Chi-square von Mises	0.00	0.00	0.00	0.00	0.00

[a] Measured at 500 m above ground level.

[b] Measured at 10 m above ground level.

* All results with 5 degrees of freedom

Table 3 Results of Spearman's rank test (r_s) between local wind and air mass directions.

Year	June	July	August	September	October
r_s	0.57*	0.57*	0.41*	0.63*	0.71*

* Statistical significance at $p \leq 0.001$

Effects of wind speed and direction on monthly fluctuations of *Cladosporium* conidia concentration in the air

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Abstract

This study determined the ~~dependency~~ relationship ~~of~~ between airborne concentration of *Cladosporium* spp. spores ~~dispersal on~~ and wind speed and ~~wind~~ direction using real data (local wind measured by weather station) and modelled data (air mass flow computed with the aid of Hybrid Single Particle Lagrangian Trajectory model). Air samples containing fungal conidia were taken at an urban site (Worcester, UK) for a period of five consecutive years using a~~n~~ ~~air sampler~~spore trap of the Hirst design. A threshold of ≥ 6,000 s m^{-3} (double the clinical value) was applied in order to select high spore concentration days, when airborne transport of conidia at a regional scale was more likely to occur. Collected data were then examined using geospatial (GIS) and statistical tools, including circular statistics. Obtained results showed that the greatest numbers of spore concentrations were detected in July and August, when *Cladosporium herbarum*, *C. cladosporioides* and *C. macrocarpum* sporulate. The circular correlation test was found to be more sensitive than Spearman's rank test. The dominance of either local wind or the air mass on *Cladosporium* spore distributions varied between examined months. Source areas of this pathogen had an origin within the UK territory. Very high daily mean concentrations of *Cladosporium* spores were observed when daily mean local wind speed was $v_s \leq 2.5$ m s^{-1} indicating warm days with a light breeze.

Keywords: fungal spores; atmosphere; HYSPLIT; circular statistics; ~~transport~~dynamic; airborne transmission~~aerobiology~~;

1. Introduction

Cladosporium spp. ~~(hereafter *Cladosporium*)~~ conidia have become of special interest to scientists since 1932 when Cobe first reported their allergenic properties ~~were reported for the first time in 1932 by Cobe~~ (Hyde et al. 1956). Bouziane et al. (2005) and Bouziane et al. (2006) found that conidia of *Cladosporium cladosporioides* contained a larger concentration of allergens than mycelia. Furthermore, Green et al. (2003) showed that increased allergen production varying from 5% to 40% was observed during germination by *Cladosporium herbarum*. A cross-sectional study of Zureik et al. (2002) examined sensitization rates in 1,132 patients living in six regions, *i.e.*, northern Europe, central Europe, southern Europe, United Kingdom/Republic of Ireland, Portland (US) and Australia/New Zealand using allergen extracts from two fungi (*Alternaria* spp., *Cladosporium* spp.), five pollen types (grass, birch, ragweed, olive, pellitory of the wall), cat and house dust mites. Their survey showed that the sensitization rates to fungi increased along with the severity of asthma. In Europe, the sensitization to *Cladosporium* spp. (hereafter *Cladosporium*) was found within the range of 0.7-9.9% with an upward trend towards the North; in the case of British and Irish population this was equal to 6.8% (Zureik et al. 2002). Another study conducted in 16 European countries confirmed the highest sensitization rate to *Cladosporium herbarum* to occur in Ireland, UK and other northern countries, and an average sensitization rate to this type of spores equal to 5.8% (Heinzerling et al. 2005).

The outcomes of these clinical surveys are in agreement with the observations previously made by Lacey (1981) who reviewed a number of aerobiological reports and established the dominance of *Cladosporium* spores in the air of areas characterized by cooler humid continental climates with warm summers; in Madrid (Spain) overall contribution of *Cladosporium* spores to the total air spora was estimated for 41%, while in Worcester (UK) this can reach up to 75% (Sadyś et al. 2015a; Díez Herrero et al. 2006). Other sites, such as Krasne (Poland) reported contributions up to 92% (Kasprzyk and Worek 2006). ~~Based on analysis of a number of aerobiological reports Lacey (1981) concluded that *Cladosporium* spores dominated in cooler humid continental climates with warm summers; in Madrid (Spain) overall contribution of *Cladosporium* spores to the total air spora was estimated for 41%, while in Worcester (UK) this can reach up to 75% (Sadyś et al. 2015a; Díez Herrero et al. 2006). Other sites, such as Krasne (Poland) reported contributions up to 92% (Kasprzyk and Worek 2006).~~ Harvey (1970) estimated spore production in six species of *Cladosporium* to be within a range from 7.3 × 10^2 to 2.61 × 10^4 s mg^{-1} dry weight of mycelium. Spore production by individual species turned out to be independent of spore frequency in the air

(Harvey 1970). Frankland and Davies (1965) established a threshold value of spore concentration above which susceptible individuals exhibit symptoms of sensitization, *i.e.* 3,000 s m^{-3} in the United Kingdom. Different threshold values for *Cladosporium* were estimated in Finland (4,000 s m^{-3}) and Poland (2,800 s m^{-3}), (Ranta and Pessi 2006; Rapiejko et al. 2004). ~~Bouziane et al. (2005) and Bouziane et al. (2006) found that conidia of *Cladosporium cladosporioides* contained a larger concentration of allergens than mycelia. Furthermore, Green et al. (2003) showed that increased allergen production varying from 5% to 40% was observed during germination by *Cladosporium herbarum*.~~

~~Grinn-Gofroń (2009) reviewed a large number of reports which examined the dependence of *Cladosporium* on the meteorological variables. She found positive statistically significant correlations with maximum, minimum, and mean temperature, sunshine hours while negative statistically significant relationships with dew point temperature and air pressure. Contrary results were found for rainfall, relative humidity and wind speed by other researchers (Grinn-Gofroń 2008; Herrero and Zaldivar 1997; Hjelmroos 1993; Katial et al. 1997; Kurkela 1997; Mediavilla Molina et al. 1998; Mitakakis et al. 1997; Oliveira et al. 2009a; Stępalska and Wołek 2005; Troutt and Levetin 2001). However, this review did not include a wind direction analysis as this is a rarely studied parameter (Grinn-Gofroń 2009).~~

~~Moreover,~~ *Cladosporium* spores were found to be present in the upper atmosphere layer at 3.3 km above the ground and together with spores of *Alternaria* spp. and *Aspergillus* spp., they constituted 75% of total collected fungal spores (Fulton 1966). ~~Also~~However, the vertical stratification in *Cladosporium* spore concentration measured at 300 m and at 1,650 m above ground level revealed to be similar (Hirst et al. 1967). Subsequently, Hirst (1973) concluded that *Cladosporium* spores may be suspended in the atmosphere for a period longer than a week, based on the analysis of air samples collected during several flights, and indicated~~. This would suggest that *Cladosporium* spores have a great~~ their potential for a long-~~–~~distance transport. A case study~~ case~~ from Taiwan has~~, in fact, already shown~~ confirmed this when~~ that~~ *Cladosporium* was found as~~constituted~~ a major biological component of the dust blown from China (Wu et al. 2004). A qualitative and quantitative study of free tropospheric air in the North America has also detected *Cladosporium* conidia among identified fungal taxa that originated from inoculum sources located in Asia (Smith et al. 2012). The transport of bioaerosols in the atmosphere can be studied with the aid of atmospheric models, such as CALifornia PUFF Model (CALPUFF 1990), HYbrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT; Draxler and Rolph 2014; Rolph 2014) and System for Integrated modeLling of Atmospheric composition (SILAM; Sofiev and

Siljamo 2004; Sofiev et al. 2006). However, a limited number of surveys investigated regional and long-distance airborne transmission of fungal spores, e.g. rust spores (Isard et al. 2005), *Leptosphaeria biglobosa* (Grinn-Gofroń et al. 2015), *Alternaria* spp. (Fernández-Rodríguez et al. 2015; Sadyś et al. 2015b; Skjøth et al. 2012). To date only one article was focused exclusively on *Cladosporium* (Sadyś et al. 2015c) and many more are needed since this is one of the most important fungal aeroallergens. Grinn-Gofroń (2009) reviewed a large number of reports which examined the dependence of *Cladosporium* on the meteorological variables. She found positive statistically significant correlations with maximum, minimum, and mean temperature, sunshine hours while negative statistically significant relationships with dew point temperature and air pressure. Contrary results were found for rainfall, relative humidity and wind speed by other researchers (Grinn-Gofroń 2008; Herrero and Zaldivar 1997; Hjelmroos 1993; Katial et al. 1997; Kurkela 1997; Mediavilla Molina et al. 1998; Mitakakis et al. 1997; Oliveira et al. 2009a; Stępalska and Wołek 2005; Troutt and Levetin 2001). However, this review did not include a wind direction analysis as this is a rarely studied parameter (Recio et al. 2012; Sánchez Reyes et al. 2009).

The aim of this study was to analyze the impact of local wind and air mass flow over an urban area (Worcester, UK) in connection with the monthly concentration pattern in *Cladosporium* conidia in the air of a chosen location. This has been accomplished by (1) collecting air samples throughout a 5-year period (2006-2010) ~~with the aid of the~~using air sampler of the Hirst design, (2) microscopy analysis, (3) atmospheric modelling using the Hybrid Single Particle Lagrangian Integrated Trajectory ~~Model~~model, (4) ~~advanced statistical~~circular statistics and (5) geospatial evaluations of collected data.

2. Materials and methods

2.1. Bioaerosol specimen

The volumetric air sampler (Burkard Manufacturing Co. Ltd., Rickmansworth, UK) was operating continuously from 2006 to 2010. The spore trap (Hirst 1952) was installed permanently at a height of 10 m above ground level, on the rooftop of the University of Worcester building (52° 11’ 48” N, 2° 14’ 31” W). Measurements were taken following the guidance given by Lacey and West (2006). Drums with trapping surface were changed every Thursday at 09:00 UTC, and then processed at the laboratory as described by Lacey and West (2006).

The shape of the *Cladosporium* spores varies from cylindrical, through ellipsoidal and ovoid up to sub-spherical. They are usually small in size (40-60 μm × 3-22 μm) from olivaceous to brown in colour, frequently observed in branched chains. Hence, depending on the location, spores may have a shield or round shape at the ends, visible black scars of attachment points. Spores towards the end of chain are smaller and aseptate (Bensch et al. 2012). The surface of the wall may be either smooth or rough (verruculose or echinulate), (Ellis 1971).

Spores of *Cladosporium* species were identified up to the genus level under ×400 magnification and counted from one central lengthwise stripe, with an hourly division. Obtained spore counts were then multiplied by a correction factor, specific for the microscope used (Nikon Eclipse E400) to acquire the spore concentration expressed in *n* spores per cubic meter of air (Lacey and West 2006).

Throughout a 5-year period the clinical threshold of ≥ 3,000 s m^{-3} established for *Cladosporium* conidia (Frankland and Davies 1965) was recorded on 330 days and varied from 47 to 88 days in a single year (Sadyś et al. 2016). Such atmospheric concentrations achieved by *Cladosporium* can be produced by a local source. Thus, in order to examine the impact of local wind and air mass transport on *Cladosporium* concentrations, and possible transport of conidia at a regional scale, days when clinical threshold was two-folded were selected for this study (*n*=131). As this study focused on monthly fluctuations in bioaerosol distribution, data for a single day with above-mentioned concentration, which occurred on the 30th of April 2007, was discarded as it would not constitute a representative value for the entire month.

2.2. Meteorological data and atmospheric modelling

The meteorological data were obtained using the Weather Link Vantage Pro2 weather station which was placed next to the air sampler. Out of a number of recorded parameters this study focused on the impact of the local wind direction extracted for days when very high concentrations (≥ 6,000 s m^{-3}) of *Cladosporium* spore were found. During the first 4 years, the wind direction data were recorded with 5 min intervals. In 2010 year the number of records was reduced to 96 per 24 h period. Finally, hourly mean values were computed in order to allow a comparison between years of sampling as well as a comparison of fungal spore counts with clusters of backward trajectories.

The HYSPLIT model was employed in order to calculate the clusters of back trajectories graphically presenting transport of the air masses during the examined period of time (Draxler and Rolph 2014; Rolph 2014). The Global Data Analysis System (GDAS), which has been made available by the National Oceanic and Atmospheric Administration

(NOAA) Air Resources Laboratory (ARL), formed the foundation of the back trajectories. The temporal resolution was chosen to equal to 1 h while the total of 24 trajectories was generated for the period of 24 h. Due to the design of trajectory models, such as HYSPLIT, it is therefore recommended to use a receptor height from 200 m to 1,000 m to simulate the overall transport in the planetary boundary layer (Fernández-Rodríguez et al. 2015; Hernández-Ceballos et al. 2014), which includes convection and dispersion near the source. In this study, the back trajectories were computed at the height of 500 m above ground. Further analysis of the air masses transport was performed using the Geographic Information System (GIS) techniques (ArcMap v. 10.0).

2.3. Statistical analyses

Directions of local wind and air mass were investigated using the circular statistics. The linear-circular correlation analysis between spore occurrence in the atmosphere and wind direction was possible thanks to "cassociation" module available in GenStat (v. 17) software. This methodology was described in more detail by Sadyś et al. (~~2015b~~2015c) and Maya-Manzano et al. (*in press*). In addition to that, Spearman’s rank test was applied. The level of statistical association was classified following Mukaka (2012). Hours, when calm was recorded in local wind data, were excluded in order to perform a correlation analysis. From June to September the contribution of calm hours did not exceed 2% of a total number of records. Upon the primary results that would show the greater influence either of local wind or air mass direction, a further velocity analysis was performed.

3. Results

3.1. Distribution of *Cladosporium* spores

Concentrations of *Cladosporium* spores varied significantly between months (Fig. 1a, Fig. 1b). Overall the greatest number of spores was trapped in July and these constituted 31.79% of the total 5-year spore catch. August (22.97%) and June (15.87%) were the second and third months in order when large numbers of conidia were observed in the air of Worcester (Fig. 1a). However, this pattern was not repeated each year, as in 2007 the greatest concentration of *Cladosporium* spores was collected in June, followed then by July and September (Fig. 1b). In 2008, once again spore counts recorded in September outnumbered counts observed in June the same year (Fig. 1b). An interesting situation also occurred in 2009, when the second largest monthly sum of daily mean spore concentration was found in October, not in August (Fig. 1b). A total number of spores collected between December and

April contributed less than 4% of the total number of spores recorded within five years of investigation (Fig. 1a). The number of high spore concentration days was also a subject of change. A number of days, when daily mean spore concentration was ≥ 6,000 s m^{-3}, turned out to be within a range from 8 in 2007 to 47 in 2006. The year 2010 showed a lot of similarities to 2006, as 44 high spore concentration days were found and exactly the same order in monthly contribution occurred (Fig. 1b).

3.2. Influence of wind direction on spore counts

The Spearman's rank test (Table 1) showed that the relationship between hourly mean spore concentration and local wind direction was inversely proportional, and it reached the level of statistical significance ($p \leq 0.05$) only in July and September. The highest correlation coefficient of r_s= -0.47 was found in September (Table 1). With regard to air mass, the relationship with spore concentration also revealed to be inversely proportional (Table 1). The highest correlation coefficient value arose in September (r_s= -0.43). No statistically significant association was found between *Cladosporium* presence and air mass direction in June, August and October (Table 1).

The analysis of spore dependence on wind direction examined using linear-circular correlation is also presented in Table 1. Both associations with local wind and air mass directions with *Cladosporium* spores were statistically significant in each investigated month with an exception of August (Table 1). The vector of these relationships was found to be proportional, yet weak to moderate (Table 1). In October, a slightly larger impact on spore occurrence revealed local wind above the air masses, while this has changed in favour of the air mass in remaining months.

3.3. Air mass analysis

The analysis of the back trajectories revealed that the durations air masses spent over the non-UK areas were only a minor fraction of the time within the 24 h before they reached Worcester (Table S1, Fig. 2). In the annual summaries for high spore concentration days, this fraction of the time was found to be ≤ 16%. The influence of possible sources of *Cladosporium* spores from Ireland was estimated at 4% or less (Table S1).

Figure 3 presented the distribution of the air mass for each studied month when the daily mean concentration of *Cladosporium* spores was equal to or above 6,000 s m^{-3}. Overall, the air masses were coming from the SSE to the WNW directions, while none or very little contribution was detected from N-E bearings (Figs 2, 3). Obtained results were in agreement with an analysis of clustered trajectories points, which showed that majority of the air masses originated from the southern directions (SW-SE) when increased levels of *Cladosporium* spores were trapped at Worcester station (Table 2, Fig. 3). Throughout the period of study, the mean angle remained within a range of 135°-293° (Table 2). A lack of uniformity in the sampled data was confirmed jointly by von Mises and Rayleigh tests (Table 2). The values of kappa also greatly varied, with an agreement from 0.60 (October) to 2.14 (September), (Table 2). The relationship between local wind direction and air mass directions (Table 3) varied from low (August) to high (October) level of association.

3.4. Local wind analysis

The overall distribution of local wind direction was examined using daily mean values recorded within five years of study. Results of this analysis are presented in Fig. 3. No influence of the northern direction was observed from the end of spring and throughout summer. Its contribution started to be apparent with the advent of autumnal months (Fig. 3). A similar pattern was found for NE direction. Wind blowing from the eastern bearings (E-SE) was mainly recorded in spring (June) and autumn (September-October) while its contribution decreased to a minimum of 1% input in August (Fig. 3). The dominance of southern directions (S-SW) was pervasive and reached a maximum of 35% in July (Fig. 3). Western wind constituted the second fraction with regard to its impact on the overall distribution of the local wind measured in Worcester. The greatest input was noted in August when it scored 22% while its importance diminished with the beginning of autumn (Fig. 3). Similar results were true for NW wind direction.

A more detailed analysis of local wind direction during high spore concentration days is given in Table 2. Both the Chi-square von Mises and the Rayleigh tests revealed that the null hypothesis must be rejected, and hence local wind direction did not have a uniform circular distribution. The correlation between observed and expected from a von Mises distribution, expressed as kappa, varied monthly from 0.64 (June) to 1.44 (September). Figure 3 showed wind histograms for each individually examined month, produced upon spore concentration threshold equal to or above 6,000 s m^{-3}. Both the size of analyzed samples varied, as well as the monthly fluctuations in local wind direction (Fig. 3). High spore counts of *Cladosporium* conidia were recorded, when wind direction was observed within the span of ESE to the WNW directions (121°-294°), (Table 2, Fig. 3).

Upon these results, it was decided to perform a further analysis of the local wind speed recorded during high spore count days, individually for each month (Fig. 4). Obtained histograms showed that regardless the time of the year and the spore concentration levels, 95% of observations were made when daily mean wind speed was equal to or lower than 2.5 m s^{-1} (Fig. 4). A Spearman's rank test did not find this relationship to be statistically significant (r_s=0.019, p=0.847).

4. Discussion

~~Obtained results~~This study indicated an unimodal distribution in *Cladosporium* spore frequency, with a single peak occurring mostly in July~~ (Fig. 1b)~~. Morrow Brown and Jackson (1978a) reported that at 8 locations across England, *i.e.*: ~~(~~Derby, Birmingham, Ashby, Church Broughton, Hartington, Crich, Attenborough, Sutton Bonington~~) -~~, *Cladosporium* spores similarly showed a single peak between the end of July and mid-August although the spore counts did not vary greatly between sampling sites. In contrast, a concentration of *Cladosporium* spores in Spain (Madrid, Malaga, Valladolid) and Turkey (Sivrihisar) was reported to follow a bimodal distribution with first peak occurring either by the end of spring or at the beginning of summer (May-June) and second peak observed in autumn (September-October), (Díez Herrero et al. 2006; Sánchez Reyes et al. 2009; Recio et al. 2012; Erkara et al. 2009). The magnitude of spring-summer and autumn peaks differed between locations, and the latter peaks in Madrid were more important than in Malaga or Valladolid. With regard to the monthly sum of daily mean spore concentrations, October catch was a factor of 2 higher than June (Díez Herrero et al. 2006). Such high contribution of autumn months was explained by more susceptible environmental conditions for the fungal growth while too high daily maximum temperature and lack of precipitation over the summer prevented numerous spore production and dissemination (Díez Herrero et al. 2006; Sánchez Reyes et al. 2009; Erkara et al. 2009). However, none of the authors reported which *Cladosporium* species were responsible for the spring-summer and autumn peaks.

A study of Harvey (1967) determined that spores produced by six *Cladosporium* species dominated in the air of Cardiff (Wales), *i.e.*: *C. herbarum, C. cladosporioides, C. sphaerospermum, C. macrocarpum, C. elatum* and *C. resinae*. They jointly constituted more than 97% of the total *Cladosporium* spore catch (Harvey 1967). Similar results were reported by Calvo Torras et al. (1981) who isolated from the air in Barcelona (Spain) the same species of *Cladosporium* with the exception of *C. resinae.* Also, the frequency of *C. macrocarpum* was found to be greater than that of *C. sphaerospermum* (Calvo Torras et al. 1981). In the UK conidia of *Cladosporium herbarum* are present in the air mainly between June and October, and usually, they peak twice during the vegetation season, *i.e.*: (1) in June-July, (2) in

August-October. The presence of *Cladosporium cladosporioides* overlaps with *C. herbarum*, as spores are found from July to September with peak protruding mostly in August. Increased concentrations of *Cladosporium sphaerospermum* are observed largely in colder, autumnal months (September-November). Finally, *Cladosporium macrocarpum* sporulates simultaneously with *C. herbarum*, thus its presence is difficult to detect (Harvey 1967). Moreover, Oliveira et al. (2009b) examined spore levels in urban and rural areas of Portugal and showed that throughout a 3-year survey the highest levels of *Cladosporium* spores in a rural area were found in autumn (September-October). This trend was not the same for an urban area where spores peaked primarily during summer time (July-August). The latter results were, however, similar to those recorded at Worcester sampling station. ~~Hence, they could be considered as typical for this type of habitat (Fig. 1).~~

This study also showed that overall percentage directions of the local wind remained constant throughout the examined period of time (Fig. 3). ~~The prevailing wind directions originated from the SE-SW bearing (Fig. 3).~~ Despite this, Spearman's rank test indicated statistically significant correlations between very high spore concentration days and local wind direction only in July and September (Table 1). These results were not confirmed by circular statistics, as the dominance of local winds was found only in October (Table 1). Considering the overall local wind direction distribution, it seems that this could be explained by the greatest contribution of wind blowing from E-SE directions (26%) in comparison with other examined months (Fig. 3). With regard to the impact of air mass on fungal spore levels, Spearman's rank test showed statistically significant associations for the same months as with local wind direction (Table 1). Lack of any sort of correlation between *Cladosporium* and both local wind and air mass directions was observed in August (Table 1). Despite that during this month, the air mass spent over the non-UK areas the lowest amount of their time (Table S1). Also, the relationship between local wind and air mass direction showed to be the weakest in August (r_s=0.41) out of five examined months (Table 3). Taking into account the overall pattern in local wind direction, August was notable for a significant decrease in the contribution of E-SE wind direction (Fig. 3). Hence, within a span of 91°-180° there must be a considerable source area of *Cladosporium* spores. Studies that investigate the impact of wind direction on bioaerosol concentration are very scarce (Sadyś et al. ~~2015b~~2015c). An exception is a study of Sánchez Reyes et al. (2009) who examined the impact of wind direction on the presence and concentration levels of *Cladosporium* spores in the air of Valladolid (Spain). Another exception is a survey made by Recio et al. (2012) who performed the same analysis in Malaga (Spain). Sánchez Reyes et al. (2009) found that although NE direction was dominant throughout two years of sampling (37.4% and 31.4%, respectively), Spearman's rank test indicated statistically significant correlation with SE wind direction only. Sánchez Reyes et al. (2009) reported that along this direction an extensive grassland area was found that most likely constituted an inoculum source of *Cladosporium* spores (Sánchez Reyes et al. 2009). Contrary findings were reported by Recio et al. (2012) who found statistically significant relationships between dominant wind directions (SW and NE) and an increase in fungal spore concentration. Moreover, wind blowing from the sea (SE) was correlated negatively with the presence of *Cladosporium* spores in the air of Malaga (Recio et al. 2012).

Morrow Brown and Jackson (1978b) investigated the difference in the contribution of local wind direction to the overall fungal spore (including *Cladosporium*) and pollen grains (grass, nettle) concentration recorded at three coastal sites (Point Lynas, Withernsea, Cromer) and one inland (Derby). In general, the lowest pollen and spore concentration were found in Point Lynas (West coast) where the wind from the sea dominated over the wind from the land. In contrast, the highest counts of biological particles were detected in Derby located in the center of the East Midlands of England. Similar high concentration of *Cladosporium* conidia was observed in Cromer (East coast), where wind blowing from the land contributed more significantly than the wind originating from the North Sea, thus over passing potential source areas of the fungus. Likewise, Rodríguez-Rajo et al. (2005) reported a rise in *Cladosporium cladosporioides* type proportionally to the increase of the Continental Index, and inversely proportionally to the effect of the sea. Out of three sampling stations, the coastal site (Vigo) exhibited a strong positive correlation (r_s=0.45-0.52), simultaneously with a mountainous site in Trives (r_s=0.29-0.58) between *Cladosporium* concentration and wind calm at the significance level of $p \leq 0.001$ (Rodríguez-Rajo et al. 2005). The inland site located in Ourense demonstrated the greatest contribution in spore concentration when NE-S wind direction occurred (r_s=0.32, $p \leq 0.001$).

Finally, although the correlation between local wind and high concentration of *Cladosporium* spores was not found to be statistically significant, yet 95% of observations were made when daily average wind speed was $v_s \leq 2.5$ m s^{-1}. This ~~result~~ finding is in agreement with previously reported a value of $v_s \leq 3$ m s^{-1} in relation to the overall dispersal of biological particles in the atmosphere (Reynolds et al. 2007), as well as v_s varying between 2 and 3.5 m s^{-1} established in particular for *Cladosporium* spores (Kurkela 1997).

Conclusion

The major findings of this aerobiological survey were following: (1) the greatest numbers of spore concentrations were recorded in July and August when *Cladosporium herbarum*, *C. cladosporioides* and *C. macrocarpum* sporulate; (2) sources of *Cladosporium* conidia must have an origin within the UK territory; (3) local wind had a greater impact on *Cladosporium* conidia occurrence in the air of Worcester than the air masses; (4) taking into account the strength of statistical significance of detected dependencies of *Cladosporium* on local wind, it must be stressed that the origin of conidia had a rather regional than local character; (5) the most contributing sources of the fungus were located in the SE to SW directions; (6) very high daily mean concentrations of *Cladosporium* spores, *i.e.* between 6,000 and 32,000 spores per cubic meter of air, were observed when daily mean local wind speed was $v_s \leq 2.5$ m s^{-1} indicating warm days with light breeze.

Acknowledgements

This project was funded by the University of Worcester and conducted within the framework of the doctoral studies. The author would like to thank Dr. ~~Andy~~ Andrew M. Reynolds ~~from the~~ (Rothamsted Research) for a critical evaluation of the manuscript. Subsequently, thanks go to Dr. Carsten Ambelas Skjøth ~~from the~~ (University of Worcester) for producing Fig. 2 used in this study. Finally, the author would like to acknowledge the NOAA ARL for the provision of the HYSPLIT model used in this publication as well as access to input data (GDAS archive) for running the HYSPLIT model.

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Figure captions

Fig. 1a Five year sums of daily mean concentration of *Cladosporium* spores, recorded monthly, measured in Worcester, UK (2006-2010) and expressed in percentage. Contribution of less than 5% was not shown.

Fig. 1b Monthly sums of daily mean concentration of *Cladosporium* spores measured in Worcester, UK (2006-2010).

Fig. 2 Foot print area computed upon frequency distribution of the air mass trajectories recorded during very high *Cladosporium* spore count days (≥ 6,000 s m^{-3}).

Fig. 3 Histograms showing a distribution pattern of the air masses, local wind direction both expressed in percent and *Cladosporium* spore concentrations recorded during high spore count days between June and October in Worcester, UK.

Fig. 4 Histograms showing (a) a daily mean concentration of *Cladosporium* recorded between June and October in Worcester, UK, (b) daily mean local wind speed with an indication of 5% error bars, (c) threshold line – 95% of observations were found when daily mean wind speed was ≤ 2.5 m s^{-1}~~a threshold line equal to 2.5 m s^{-1}~~. In all examined cases concentration of *Cladosporium* was equal to or higher than 6,000 spores per cubic meter of air. The number of examined cases varied between months.

Table captions

Table 1 Results of Spearman's rank test (r_s) and linear-circular correlation (r_c) between *Cladosporium* spore concentration and local wind (a) and air mass (b) directions

Table 2 Results of descriptive circular statistics for local wind and air mass direction, when high *Cladosporium* spore count occurred (n=130)

Table 3 Results of Spearman's rank test (r_s) between local wind and air mass directions