

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	
Corresponding Author:	Magdalena Sadyś, Ph.D. Rothamsted Research Harpenden, Hertfordshire UNITED KINGDOM	
Corresponding Author Secondary Information:		
Corresponding Author's Institution:	Rothamsted Research	
Corresponding Author's Secondary Institution:		
First Author:	Magdalena Sadyś, Ph.D.	
First Author Secondary Information:		
Order of Authors:	Magdalena Sadyś, Ph.D.	
Order of Authors Secondary Information:		
Funding Information:	University of Worcester (Ph.D. scholarship)	Dr. Magdalena Sadyś
Abstract:	<p>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.</p>	
Suggested Reviewers:	Agnieszka Grinn-Gofroń, Ph.D. Uniwersytet Szczeciński agofr@univ.szczecin.pl	
	Irene Câmara Camacho, Ph.D. Universidade da Madeira camire@uma.pt	
	Santiago Fernandez-Rodriguez, Ph.D. Universidad de Extremadura santiferro@unex.es	
Response to Reviewers:	Ref.: Ms. No. AERO-D-17-00008	

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 be 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.

[Click here to view linked References](#)

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

3

4

5 Magdalena Sadyś^{1,2}

6

7

8 ¹ Institute of Science and the Environment, University of Worcester, Henwick Grove,
9 Worcester, WR2 6AJ, United Kingdom

10

11 ² (✉) Rothamsted Research, West Common, Harpenden, AL5 2JQ, United Kingdom,
12 e-mail: magdalena.sadys@rothamsted.ac.uk, tel. +44 (0) 1582 938 471

13

14

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31

32

33

34 **Abstract**

35

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

52

53

54

55

56

57

58

59

60

61

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

64

65 1. Introduction

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

83 The outcomes of these clinical surveys are in agreement with the observations
84 previously made by Lacey (1981) who reviewed a number of aerobiological reports and
85 established the dominance of *Cladosporium* spores in the air of areas characterized by cooler
86 humid continental climates with warm summers; in Madrid (Spain) overall contribution of
87 *Cladosporium* spores to the total air spora was estimated for 41%, while in Worcester (UK)
88 this can reach up to 75% (Sadyś et al. 2015a; Díez Herrero et al. 2006). Other sites, such as
89 Krasne (Poland) reported contributions up to 92% (Kasprzyk and Worek 2006). Harvey
90 (1970) estimated spore production in six species of *Cladosporium* to be within a range from
91 7.3×10^2 to 2.61×10^4 s mg⁻¹ dry weight of mycelium. Spore production by individual
92 species turned out to be independent of spore frequency in the air (Harvey 1970). Frankland
93 and Davies (1965) established a threshold value of spore concentration above which
94 susceptible individuals exhibit symptoms of sensitization, *i.e.* 3,000 s m⁻³ in the United
95 Kingdom. Different threshold values for *Cladosporium* were estimated in Finland (4,000 s m⁻³
96 ³) and Poland (2,800 s m⁻³), (Ranta and Pessi 2006; Rapiejko et al. 2004).

97

98 *Cladosporium* spores were found to be present in the upper atmosphere layer at 3.3
99 km above the ground and together with spores of *Alternaria* spp. and *Aspergillus* spp., they
100 constituted 75% of total collected fungal spores (Fulton 1966). However, the vertical
101 stratification in *Cladosporium* spore concentration measured at 300 m and at 1,650 m above
102 ground level revealed to be similar (Hirst et al. 1967). Subsequently, Hirst (1973) concluded
103 that *Cladosporium* spores may be suspended in the atmosphere for a period longer than a
104 week, based on the analysis of air samples collected during several flights, and indicated their
105 potential for a long-distance transport. A case study from Taiwan has confirmed this when
106 *Cladosporium* was found as a major biological component of the dust blown from China (Wu
107 et al. 2004). A qualitative and quantitative study of free tropospheric air in the North America
108 has also detected *Cladosporium* conidia among identified fungal taxa that originated from
109 inoculum sources located in Asia (Smith et al. 2012). The transport of bioaerosols in the
110 atmosphere can be studied with the aid of atmospheric models, such as CALifornia PUFF
111 Model (CALPUFF 1990), HYbrid Single Particle Lagrangian Integrated Trajectory
112 (HYSPLIT; Draxler and Rolph 2014; Rolph 2014) and System for Integrated modeLling of
113 Atmospheric composition (SILAM; Sofiev and Siljamo 2004; Sofiev et al. 2006). However, a
114 limited number of surveys investigated regional and long-distance airborne transmission of
115 fungal spores, e.g. rust spores (Isard et al. 2005), *Leptosphaeria biglobosa* (Grinn-Gofroń et
116 al. 2015), *Alternaria* spp. (Fernández-Rodríguez et al. 2015; Sadyś et al. 2015b; Skjøth et al.
117 2012). To date only one article was focused exclusively on *Cladosporium* (Sadyś et al.
118 2015c) and many more are needed since this is one of the most important fungal
119 aeroallergens. Grinn-Gofroń (2009) reviewed a large number of reports which examined the
120 dependence of *Cladosporium* on the meteorological variables. She found positive statistically
121 significant correlations with maximum, minimum, and mean temperature, sunshine hours
122 while negative statistically significant relationships with dew point temperature and air
123 pressure. Contrary results were found for rainfall, relative humidity and wind speed by other
124 researchers (Grinn-Gofroń 2008; Herrero and Zaldivar 1997; Hjelmroos 1993; Katial et al.
125 1997; Kurkela 1997; Mediavilla Molina et al. 1998; Mitakakis et al. 1997; Oliveira et al.
126 2009a; Stępańska and Wołek 2005; Troutt and Levetin 2001). However, this review did not
127 include a wind direction analysis as this is a rarely studied parameter (Recio et al. 2012;
128 Sánchez Reyes et al. 2009).

129 The aim of this study was to analyze the impact of local wind and air mass flow over
130 an urban area (Worcester, UK) in connection with the monthly concentration pattern in
131 *Cladosporium* conidia in the air of a chosen location. This has been accomplished by (1)

132 collecting air samples throughout a 5-year period (2006-2010) using air sampler of the Hirst
133 design, (2) microscopy analysis, (3) atmospheric modelling using the Hybrid Single Particle
134 Lagrangian Integrated Trajectory model, (4) circular statistics and (5) geospatial evaluations
135 of collected data.

136

137 **2. Materials and methods**

138 **2.1. Bioaerosol specimen**

139 The volumetric air sampler (Burkard Manufacturing Co. Ltd., Rickmansworth, UK)
140 was operating continuously from 2006 to 2010. The spore trap (Hirst 1952) was installed
141 permanently at a height of 10 m above ground level, on the rooftop of the University of
142 Worcester building (52° 11' 48" N, 2° 14' 31" W). Measurements were taken following the
143 guidance given by Lacey and West (2006). Drums with trapping surface were changed every
144 Thursday at 09:00 UTC, and then processed at the laboratory as described by Lacey and West
145 (2006).

146 The shape of the *Cladosporium* spores varies from cylindrical, through ellipsoidal and
147 ovoid up to sub-spherical. They are usually small in size (40-60 $\mu\text{m} \times 3-22 \mu\text{m}$) from
148 olivaceous to brown in colour, frequently observed in branched chains. Hence, depending on
149 the location, spores may have a shield or round shape at the ends, visible black scars of
150 attachment points. Spores towards the end of chain are smaller and aseptate (Bensch et al.
151 2012). The surface of the wall may be either smooth or rough (verruculose or echinulate),
152 (Ellis 1971).

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

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

166 30th of April 2007, was discarded as it would not constitute a representative value for the
167 entire month.

168

169 **2.2. Meteorological data and atmospheric modelling**

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

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

191

192 **2.3. Statistical analyses**

193 Directions of local wind and air mass were investigated using the circular statistics.
194 The linear-circular correlation analysis between spore occurrence in the atmosphere and wind
195 direction was possible thanks to "cassociation" module available in GenStat (v. 17) software.
196 This methodology was described in more detail by Sadyś et al. (2015c) and Maya-Manzano
197 et al. (*in press*). In addition to that, Spearman's rank test was applied. The level of statistical
198 association was classified following Mukaka (2012). Hours, when calm was recorded in local
199 wind data, were excluded in order to perform a correlation analysis. From June to September

200 the contribution of calm hours did not exceed 2% of a total number of records. Upon the
201 primary results that would show the greater influence either of local wind or air mass
202 direction, a further velocity analysis was performed.

203

204 **3. Results**

205 **3.1. Distribution of *Cladosporium* spores**

206 Concentrations of *Cladosporium* spores varied significantly between months (Fig. 1a,
207 Fig. 1b). Overall the greatest number of spores was trapped in July and these constituted
208 31.79% of the total 5-year spore catch. August (22.97%) and June (15.87%) were the second
209 and third months in order when large numbers of conidia were observed in the air of
210 Worcester (Fig. 1a). However, this pattern was not repeated each year, as in 2007 the greatest
211 concentration of *Cladosporium* spores was collected in June, followed then by July and
212 September (Fig. 1b). In 2008, once again spore counts recorded in September outnumbered
213 counts observed in June the same year (Fig. 1b). An interesting situation also occurred in
214 2009, when the second largest monthly sum of daily mean spore concentration was found in
215 October, not in August (Fig. 1b). A total number of spores collected between December and
216 April contributed less than 4% of the total number of spores recorded within five years of
217 investigation (Fig. 1a). The number of high spore concentration days was also a subject of
218 change. A number of days, when daily mean spore concentration was $\geq 6,000 \text{ s m}^{-3}$, turned
219 out to be within a range from 8 in 2007 to 47 in 2006. The year 2010 showed a lot of
220 similarities to 2006, as 44 high spore concentration days were found and exactly the same
221 order in monthly contribution occurred (Fig. 1b).

222

223 **3.2. Influence of wind direction on spore counts**

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

232 The analysis of spore dependence on wind direction examined using linear-circular
233 correlation is also presented in Table 1. Both associations with local wind and air mass

234 directions with *Cladosporium* spores were statistically significant in each investigated month
235 with an exception of August (Table 1). The vector of these relationships was found to be
236 proportional, yet weak to moderate (Table 1). In October, a slightly larger impact on spore
237 occurrence revealed local wind above the air masses, while this has changed in favour of the
238 air mass in remaining months.

239

240 **3.3. Air mass analysis**

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

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

258

259 **3.4. Local wind analysis**

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

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

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

282 Upon these results, it was decided to perform a further analysis of the local wind
283 speed recorded during high spore count days, individually for each month (Fig. 4). Obtained
284 histograms showed that regardless the time of the year and the spore concentration levels,
285 95% of observations were made when daily mean wind speed was equal to or lower than 2.5
286 m s⁻¹ (Fig. 4). A Spearman's rank test did not find this relationship to be statistically
287 significant ($r_s=0.019$, $p=0.847$).

288

289 **4. Discussion**

290 This study indicated an unimodal distribution in *Cladosporium* spore frequency, with
291 a single peak occurring mostly in July. Morrow Brown and Jackson (1978a) reported that at 8
292 locations across England, *i.e.*: Derby, Birmingham, Ashby, Church Broughton, Hartington,
293 Crich, Attenborough, Sutton Bonington, *Cladosporium* spores similarly showed a single peak
294 between the end of July and mid-August although the spore counts did not vary greatly
295 between sampling sites. In contrast, a concentration of *Cladosporium* spores in Spain
296 (Madrid, Malaga, Valladolid) and Turkey (Sivrihisar) was reported to follow a bimodal
297 distribution with first peak occurring either by the end of spring or at the beginning of
298 summer (May-June) and second peak observed in autumn (September-October), (Díez
299 Herrero et al. 2006; Sánchez Reyes et al. 2009; Recio et al. 2012; Erkara et al. 2009). The
300 magnitude of spring-summer and autumn peaks differed between locations, and the latter
301 peaks in Madrid were more important than in Malaga or Valladolid. With regard to the

302 monthly sum of daily mean spore concentrations, October catch was a factor of 2 higher than
303 June (Díez Herrero et al. 2006). Such high contribution of autumn months was explained by
304 more susceptible environmental conditions for the fungal growth while too high daily
305 maximum temperature and lack of precipitation over the summer prevented numerous spore
306 production and dissemination (Díez Herrero et al. 2006; Sánchez Reyes et al. 2009; Erkara et
307 al. 2009). However, none of the authors reported which *Cladosporium* species were
308 responsible for the spring-summer and autumn peaks.

309 A study of Harvey (1967) determined that spores produced by six *Cladosporium*
310 species dominated in the air of Cardiff (Wales), *i.e.*: *C. herbarum*, *C. cladosporioides*, *C.*
311 *sphaerospermum*, *C. macrocarpum*, *C. elatum* and *C. resinae*. They jointly constituted more
312 than 97% of the total *Cladosporium* spore catch (Harvey 1967). Similar results were reported
313 by Calvo Torras et al. (1981) who isolated from the air in Barcelona (Spain) the same species
314 of *Cladosporium* with the exception of *C. resinae*. Also, the frequency of *C. macrocarpum*
315 was found to be greater than that of *C. sphaerospermum* (Calvo Torras et al. 1981). In the UK
316 conidia of *Cladosporium herbarum* are present in the air mainly between June and October,
317 and usually, they peak twice during the vegetation season, *i.e.*: (1) in June-July, (2) in
318 August-October. The presence of *Cladosporium cladosporioides* overlaps with *C. herbarum*,
319 as spores are found from July to September with peak protruding mostly in August. Increased
320 concentrations of *Cladosporium sphaerospermum* are observed largely in colder, autumnal
321 months (September-November). Finally, *Cladosporium macrocarpum* sporulates
322 simultaneously with *C. herbarum*, thus its presence is difficult to detect (Harvey 1967).
323 Moreover, Oliveira et al. (2009b) examined spore levels in urban and rural areas of Portugal
324 and showed that throughout a 3-year survey the highest levels of *Cladosporium* spores in a
325 rural area were found in autumn (September-October). This trend was not the same for an
326 urban area where spores peaked primarily during summer time (July-August). The latter
327 results were, however, similar to those recorded at Worcester sampling station. This study
328 also showed that overall percentage directions of the local wind remained constant
329 throughout the examined period of time (Fig. 3). Despite this, Spearman's rank test indicated
330 statistically significant correlations between very high spore concentration days and local
331 wind direction only in July and September (Table 1). These results were not confirmed by
332 circular statistics, as the dominance of local winds was found only in October (Table 1).
333 Considering the overall local wind direction distribution, it seems that this could be explained
334 by the greatest contribution of wind blowing from E-SE directions (26%) in comparison with
335 other examined months (Fig. 3). With regard to the impact of air mass on fungal spore levels,

336 Spearman's rank test showed statistically significant associations for the same months as with
337 local wind direction (Table 1). Lack of any sort of correlation between *Cladosporium* and
338 both local wind and air mass directions was observed in August (Table 1). Despite that during
339 this month, the air mass spent over the non-UK areas the lowest amount of their time (Table
340 S1). Also, the relationship between local wind and air mass direction showed to be the
341 weakest in August ($r_s=0.41$) out of five examined months (Table 3). Taking into account the
342 overall pattern in local wind direction, August was notable for a significant decrease in the
343 contribution of E-SE wind direction (Fig. 3). Hence, within a span of 91° - 180° there must be
344 a considerable source area of *Cladosporium* spores. Studies that investigate the impact of
345 wind direction on bioaerosol concentration are very scarce (Sadyś et al. 2015c). An exception
346 is a study of Sánchez Reyes et al. (2009) who examined the impact of wind direction on the
347 presence and concentration levels of *Cladosporium* spores in the air of Valladolid (Spain).
348 Another exception is a survey made by Recio et al. (2012) who performed the same analysis
349 in Malaga (Spain). Sánchez Reyes et al. (2009) found that although NE direction was
350 dominant throughout two years of sampling (37.4% and 31.4%, respectively), Spearman's
351 rank test indicated statistically significant correlation with SE wind direction only. Sánchez
352 Reyes et al. (2009) reported that along this direction an extensive grassland area was found
353 that most likely constituted an inoculum source of *Cladosporium* spores (Sánchez Reyes et al.
354 2009). Contrary findings were reported by Recio et al. (2012) who found statistically
355 significant relationships between dominant wind directions (SW and NE) and an increase in
356 fungal spore concentration. Moreover, wind blowing from the sea (SE) was correlated
357 negatively with the presence of *Cladosporium* spores in the air of Malaga (Recio et al. 2012).

358 Morrow Brown and Jackson (1978b) investigated the difference in the contribution of
359 local wind direction to the overall fungal spore (including *Cladosporium*) and pollen grains
360 (grass, nettle) concentration recorded at three coastal sites (Point Lynas, Withernsea, Cromer)
361 and one inland (Derby). In general, the lowest pollen and spore concentration were found in
362 Point Lynas (West coast) where the wind from the sea dominated over the wind from the
363 land. In contrast, the highest counts of biological particles were detected in Derby located in
364 the center of the East Midlands of England. Similar high concentration of *Cladosporium*
365 conidia was observed in Cromer (East coast), where wind blowing from the land contributed
366 more significantly than the wind originating from the North Sea, thus over passing potential
367 source areas of the fungus. Likewise, Rodríguez-Rajo et al. (2005) reported a rise in
368 *Cladosporium cladosporioides* type proportionally to the increase of the Continental Index,
369 and inversely proportionally to the effect of the sea. Out of three sampling stations, the

370 coastal site (Vigo) exhibited a strong positive correlation ($r_s=0.45-0.52$), simultaneously with
371 a mountainous site in Trives ($r_s=0.29-0.58$) between *Cladosporium* concentration and wind
372 calm at the significance level of $p \leq 0.001$ (Rodríguez-Rajo et al. 2005). The inland site
373 located in Ourense demonstrated the greatest contribution in spore concentration when NE-S
374 wind direction occurred ($r_s=0.32, p \leq 0.001$).

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

381

382 **Conclusion**

383 The major findings of this aerobiological survey were following: (1) the greatest
384 numbers of spore concentrations were recorded in July and August when *Cladosporium*
385 *herbarum*, *C. cladosporioides* and *C. macrocarpum* sporulate; (2) sources of *Cladosporium*
386 conidia must have an origin within the UK territory; (3) local wind had a greater impact on
387 *Cladosporium* conidia occurrence in the air of Worcester than the air masses; (4) taking into
388 account the strength of statistical significance of detected dependencies of *Cladosporium* on
389 local wind, it must be stressed that the origin of conidia had a rather regional than local
390 character; (5) the most contributing sources of the fungus were located in the SE to SW
391 directions; (6) very high daily mean concentrations of *Cladosporium* spores, *i.e.* between
392 6,000 and 32,000 spores per cubic meter of air, were observed when daily mean local wind
393 speed was $v_s \leq 2.5 \text{ m s}^{-1}$ indicating warm days with light breeze.

394

395 **Acknowledgements**

396

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

404

405 **References**

- 406 Bensch, K., Braun, U., Groenewald, J.Z., Crous, P.W. (2012) The genus *Cladosporium*. *Stud Mycol*,
407 72, 1-401.
- 408 Bouziane, H., Latge, J.P., Fitting, C., Mecheri, S., Lelong, M., David, B. (2005) Comparison of the
409 allergenic potency of spores and mycelium of *Cladosporium*. *Allergol Immunopathol*, 33,
410 125-130.
- 411 Bouziane, H., Latge, J.P., Lelong, M. (2006) Immunochemical comparison of the allergenic potency
412 of spores and mycelium of *Cladosporium cladosporioides* extracts by a nitrocellulose
413 electroblotting technique. *Allergol Immunopathol*, 34, 64-69.
- 414 CALPUFF Modeling System (1990) Atmospheric Studies Group (ASG),
415 <http://www.src.com/calpuff/calpuff1.htm>. Accessed 3 March 2017.
- 416 Calvo Torras, M.A., Guarro Artigas, J., Suarez Fernandez, G. (1981) Air-borne fungi in the air of
417 Barcelona (Spain). 4. The genus *Cladosporium*. *Mycopathologia*, 74, 19-24.
- 418 Díez Herrero, A., Sabariego Ruiz, S., Gutiérrez Bustillo, M., Cervigón Morales, P. (2006) Study of
419 airborne fungal spores in Madrid, Spain. *Aerobiologia*, 22, 135-142.
- 420 Draxler, R.R., Rolph, G.D. (2014) HYSPLIT (HYbrid Single-Particle Lagrangian Integrated
421 Trajectory) Model. NOAA Air Resources Laboratory.
422 <http://ready.arl.noaa.gov/HYSPLIT.php>.
- 423 Ellis, M.B. (1971) Dematiaceous Hyphomycetes. London: The Eastern Press, Ltd.
- 424 Erkara, I.P., Ilhan, S., Oner, S. (2009) Monitoring and assessment of airborne *Cladosporium* Link and
425 *Alternaria* Nees spores in Sivrihisar (Eskisehir), Turkey. *Environ Monit Assess*, 48, 477-484.
- 426 Fernández-Rodríguez, S., Sadyś, M., Smith, M., Tormo-Molina, R., Skjøth, C.A., Maya-Manzano, et
427 al. (2015) Potential sources of airborne *Alternaria* spp. spores in South-west Spain. *Sci Total*
428 *Environ*, 533, 165-176.
- 429 Frankland, A.W., Davies, R.R. (1965) Allergy to mold spores in England. *Poumon Coeur*, 21:11-31.
- 430 Fulton, J.D. (1966) Microorganisms in the upper atmosphere. 3. Relationship between altitude and
431 micropopulation. *J Appl Microbiol*, 14, 237-240.
- 432 Green, B.J., Mitakakis, T.Z., Tovey, E.R. (2003) Allergen detection from 11 fungal species before and
433 after germination. *J Allergy Clin Immunol*, 11, 285-289.
- 434 Grinn-Gofroń, A. (2008) The variation in spore concentrations of selected fungal taxa associated with
435 weather conditions in Szczecin, Poland, 2004-2006. *Grana*, 47, 139-146.
- 436 Grinn-Gofroń, A. (2009) The occurrence of *Cladosporium* spores in the air and their relationships with
437 meteorological parameters. *Acta Agrobot*, 62, 111-116.
- 438 Grinn-Gofroń, A., Sadyś, M., Kaczmarek, J., Bednarczyk, A., Pawłowska, S., Jedryczka, M. (2016) Back-
439 trajectory modelling and DNA-based species-specific detection methods allow tracking of
440 fungal spore transport in air masses. *Sci Total Environ*, 571, 658-669.
- 441 Harvey, R. (1967) Air-spora studies at Cardiff. I. *Cladosporium*. *Trans Br Mycol Soc*, 50, 479-&.
- 442 Harvey, R. (1970) Spore productivity in *Cladosporium*. *Mycopathol Mycol Appl*, 41, 251-256.
- 443 Heinzerling, L., Frew, A.J., Bindslev-Jensen, C., Bonini, S., Bousquet, J., Bresciani, M., et al. (2005)
444 Standard skin prick testing and sensitization to inhalant allergens across Europe - a survey
445 from the GALEN network. *Allergy*, 60, 1287-300.
- 446 Hernández-Ceballos, M.A., Skjøth, C.A., García-Mozo, H., Bolívar, J.P., Galán, C. (2014)
447 Improvement in the accuracy of back trajectories using WRF to identify pollen sources in
448 southern Iberian Peninsula. *Int J Biometeorol*, 58, 2031-2043.
- 449 Herrero, B., Zaldivar, P. (1997) Effects of meteorological factors on the levels of *Alternaria* and
450 *Cladosporium* spores in the atmosphere of Palencia, 1990-92. *Grana*, 36, 180-184.
- 451 Hirst, J.M. (1952) An automatic volumetric spore trap. *Ann Appl Biol*, 39, 257-265.
- 452 Hirst, J.M. (1973) Spore transport and vertical profiles, vol 18. *Bulletins from the Ecological Research*
453 *Committee*.
- 454 Hirst, J.M., Stedman, O.J., Hurst, G.W. (1967) Long-distance spore transport - vertical sections of
455 spore clouds over sea. *J Gen Microbiol*, 48, 357-&.

- 456 Hjelmroos, M. (1993) Relationship between airborne fungal spore presence and weather variables -
457 *Cladosporium* and *Alternaria*. Grana, 32:40-47.
- 458 Hyde, H.A., Richards, M., Williams, D.A. (1956) Allergy to mould spores in Britain. Br Med J, 1,
459 886-890.
- 460 Isard, S.A., Gage, S.H., Comtois, P., Russo, J.M. (2005) Principles of the atmospheric pathway for
461 invasive species applied to soybean rust. Bioscience, 55, 851-861.
- 462 Kasprzyk, I., Worek, M. (2006) Airborne fungal spores in urban and rural environments in Poland.
463 Aerobiologia, 22, 169-176.
- 464 Katial, R.K., Zhang, Y.M., Jones, R.H., Dyer, P.D. (1997) Atmospheric mold spore counts in relation
465 to meteorological parameters. Int J Biometeorol, 41, 17-22.
- 466 Kurkela, T. (1997) The number of *Cladosporium* conidia in the air in different weather conditions.
467 Grana, 36, 54-61.
- 468 Lacey, J. (1981) The aerobiology of conidial fungi. In: Cole GT, editor. Biology of conidial fungi.
469 New York: Academic Press; p. 373-416.
- 470 Lacey, M.E., West, J.S. (2006) Air spora: A manual for catching and identifying airborne biological
471 particles. Verlag GmbH: Springer.
- 472 Maya-Manzano, J.M., Sadyś, M., Tormo-Molina, R., Fernández-Rodríguez, S., Oteros, J., Silva-
473 Palacios, I., et al. 2017. Relationships between airborne pollen grains, wind direction and land
474 cover using GIS and circular statistics. Sci Total Environ, *in press*.
- 475 Mediavilla Molina, A., Angulo Romero, J., García-Pantaleón, F.I., Comtois, P., Domínguez Vilches,
476 E. (1998) Preliminary statistical modelling of the presence of two conidial types of
477 *Cladosporium* in the atmosphere of Córdoba, Spain. Aerobiologia, 14, 229-234.
- 478 Mitakakis, T.Z., Kok Ong, E., Stevens, A., Guest, D., Knox, R.B. (1997) Incidence of *Cladosporium*,
479 *Alternaria* and total fungal spores in the atmosphere of Melbourne (Australia) over three
480 years. Aerobiologia, 13, 83-90.
- 481 Morrow Brown, H., Jackson, F.A. (1978a) Aerobiological studies based in Derby. 2. Simultaneous
482 pollen and spore sampling at eight sites within a 60 km radius. Clin Allergy, 8, 599-609.
- 483 Morrow Brown, H., Jackson, F.A. (1978b) Aerobiological studies based in Derby. 3. Comparison of
484 simultaneous pollen and spore counts from East coast, Midlands and West coast of England
485 and Wales. Clin Allergy, 8, 611-619.
- 486 Mukaka, M.M. (2012) A guide to appropriate use of correlation coefficient in medical research.
487 Malawi Med J, 24, 69-71.
- 488 Oliveira, M., Ribeiro, H., Delgado, J.L., Abreu, I. (2009a) The effects of meteorological factors on
489 airborne fungal spore concentration in two areas differing in urbanisation level. Int J
490 Biometeorol, 53, 61-73.
- 491 Oliveira, M., Ribeiro, H., Delgado, J.L., Abreu, I. (2009b) Seasonal and intradiurnal variation of
492 allergenic fungal spores in urban and rural areas of the North of Portugal. Aerobiologia, 25,
493 85-98.
- 494 Ranta, H., Pessi, A.-M. (2006) Pollen Bulletin Summary 2005. The Finnish Pollen Bulletin, 30, 1-12.
- 495 Rapiejko, P., Lipiec, A., Wojadas, A., Jurkiewicz, D. (2004) Threshold pollen concentration necessary
496 to evoke allergic symptoms. Internat Rev Allergol Clin Immunol, 10, 91-94.
- 497 Recio, M., del Mar Trigo, M., Docampo, S., Melgar, M., Garcia-Sanchez, J., Bootello, L., et al. (2012)
498 Analysis of the predicting variables for daily and weekly fluctuations of two airborne fungal
499 spores: *Alternaria* and *Cladosporium*. Int J Biometeorol, 56, 983-991.
- 500 Reynolds, A.M., Bohan, D.A., Bell, J.R. (2007) Ballooning dispersal in arthropod taxa: conditions at
501 take-off. Biol Letters, 3, 237-240.
- 502 Rodríguez-Rajo, F.J., Iglesias, I., Jato, V. (2005) Variation assessment of airborne *Alternaria* and
503 *Cladosporium* spores at different bioclimatical conditions. Mycol Res, 109, 497-507.
- 504 Rolph, G.D. (2014) Real-time Environmental Applications and Display sYstem (READY). NOAA Air
505 Resources Laboratory. <http://ready.arl.noaa.gov>.
- 506 Sadyś, M., Strzelczak, A., Grinn-Gofroń, A., Kennedy, R. (2015a) Application of redundancy analysis
507 for aerobiological data. Int J Biometeorol, 59, 25-36.
- 508 Sadyś, M., Kennedy, R., Skjøth, C.A. (2015b) Determination of *Alternaria* spp. habitats using 7-day
509 volumetric spore trap, Hybrid Single Particle Lagrangian Integrated Trajectory model and
510 geographic information system. Urban Climate, 14, 429-440.

511 Sadyś, M., Kennedy, R., Skjøth, C.A. (2015c) An analysis of local wind and air mass directions and
512 their impact on *Cladosporium* distribution using HYSPLIT and circular statistics. *Fungal*
513 *Ecol*, 18, 56-66.

514 Sadyś, M., Adams-Groom, B., Herbert, R.J., Kennedy, R. (2016) Comparisons of fungal spore
515 distributions using air sampling at Worcester, England (2006–2010). *Aerobiologia*, 32, 619-
516 634.

517 Sánchez Reyes, E., Rodríguez de la Cruz, D., Sanchís Merino, M.E., Sánchez, J. (2009)
518 Meteorological and agricultural effects on airborne *Alternaria* and *Cladosporium* spores and
519 clinical aspects in Valladolid (Spain). *Ann Agric Environ*, 16, 53-61.

520 Skjøth, C.A., Sommer, J., Frederiksen, L., Karlson, U.G. (2012) Crop harvest in Denmark and Central
521 Europe contributes to the local load of airborne *Alternaria* spore concentrations in
522 Copenhagen. *Atmos Chem Phys*, 12, 11107-11123.

523 Smith, D.J., Jaffe, D.A., Birmele, M.N., Griffin, D.W., Schuerger, A.C., Hee, J., et al. (2012) Free
524 tropospheric transport of microorganisms from Asia to North America. *Microb Ecol*, 64, 973-
525 985.

526 Sofiev, M., Siljamo, P. (2004) Forward and inverse simulations with Finnish emergency model
527 SILAM. In C. Borrego, S., Incecik (Ed.), *Air Pollution Modelling and its Applications* (pp.
528 417-425). New York: Springer.

529 Sofiev, M., Siljamo, P., Valkama, I., Ilvonen, M., Kukkonen, J. (2006) A dispersion modelling system
530 SILAM and its evaluation against ETEX data. *Atmos Environ*, 40, 674-685.

531 Stępańska, D., Wołek, J. (2005) Variation in fungal spore concentrations of selected taxa associated to
532 weather conditions in Cracow, Poland, in 1997. *Aerobiologia*, 21, 43-52.

533 Troutt, C., Levetin, E. (2001) Correlation of spring spore concentrations and meteorological
534 conditions in Tulsa, Oklahoma. *Int J Biometeorol*, 45, 64-74.

535 Wu, P.C., Tsai, J.C., Li, F.C., Lung, S.C., Su, H.J. (2004) Increased levels of ambient fungal spores in
536 Taiwan are associated with dust events from China. *Atmos Environ*, 38, 4879-4886.

537 Zureik, M., Neukirch, C., Leynaert, B., Liard, R., Bousquet, J., Neukirch, F. (2002) Sensitisation to
538 airborne moulds and severity of asthma: cross sectional study from European Community
539 respiratory health survey. *BMJ*, 325, 1-7.

540

541

542

543 Figure captions

544

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

548

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

551

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

554

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

558

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

565

566 Table captions

567

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

570

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

573

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

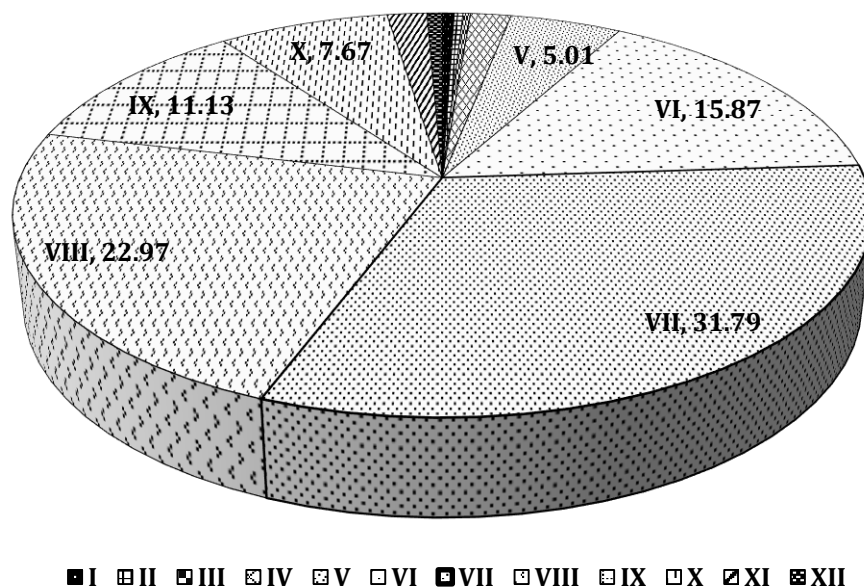


Fig. 1a Five year sums of daily mean concentration of *Cladosporium* spores, recorded monthly, measured in Worcester, UK (2006-2010) and expressed in percentage. Contribution of less than 5% was not shown.

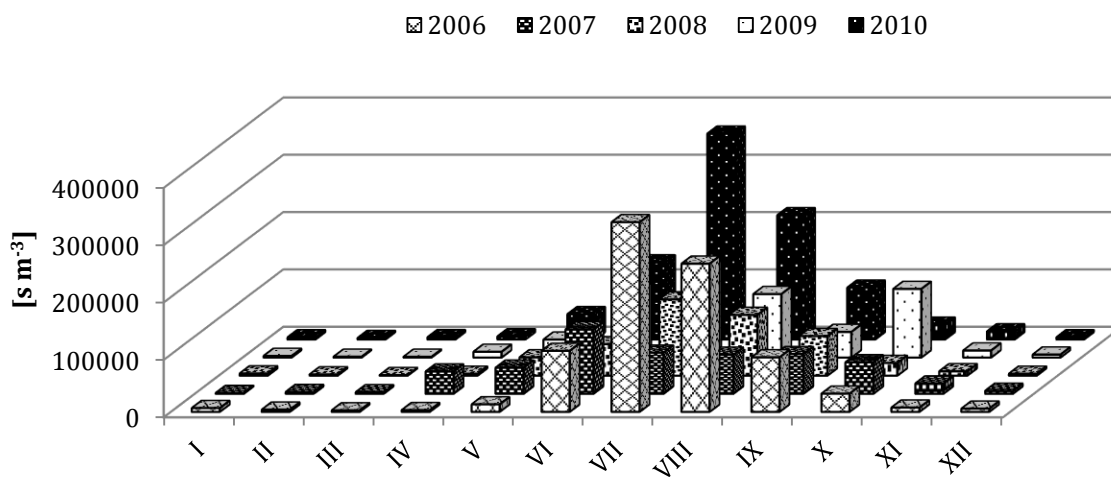


Fig. 1b Monthly sums of daily mean concentration of *Cladosporium* spores measured in Worcester, UK (2006-2010).

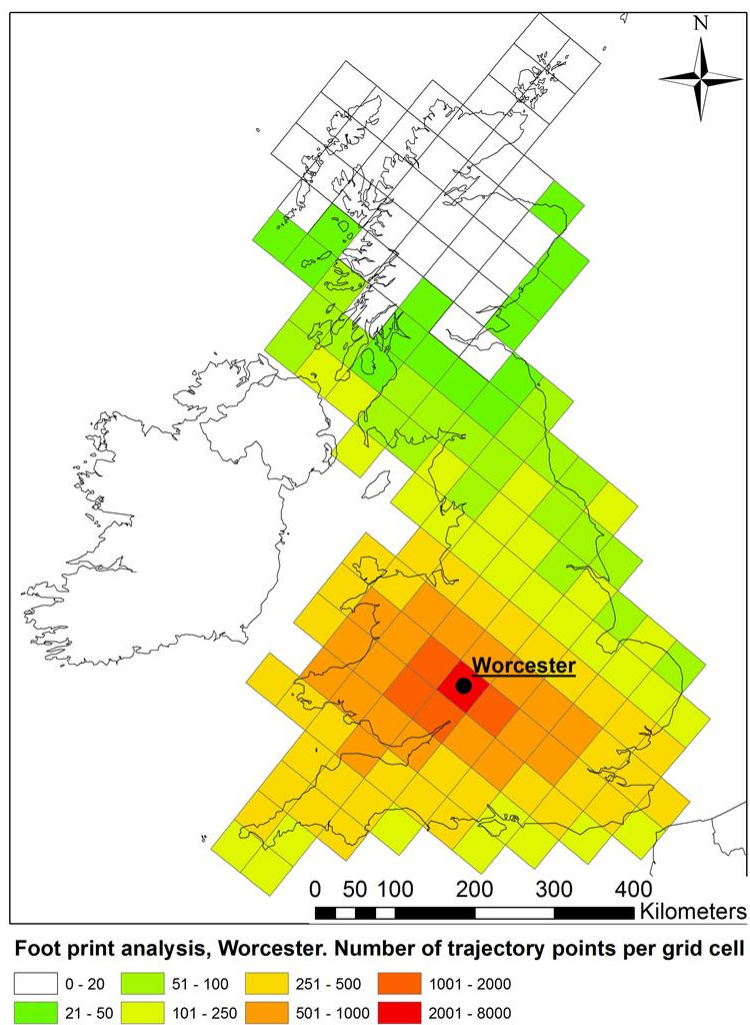


Fig. 2 Foot print area computed upon frequency distribution of the air mass trajectories recorded during very high *Cladosporium* spore count days ($\geq 6,000 \text{ s m}^{-3}$).

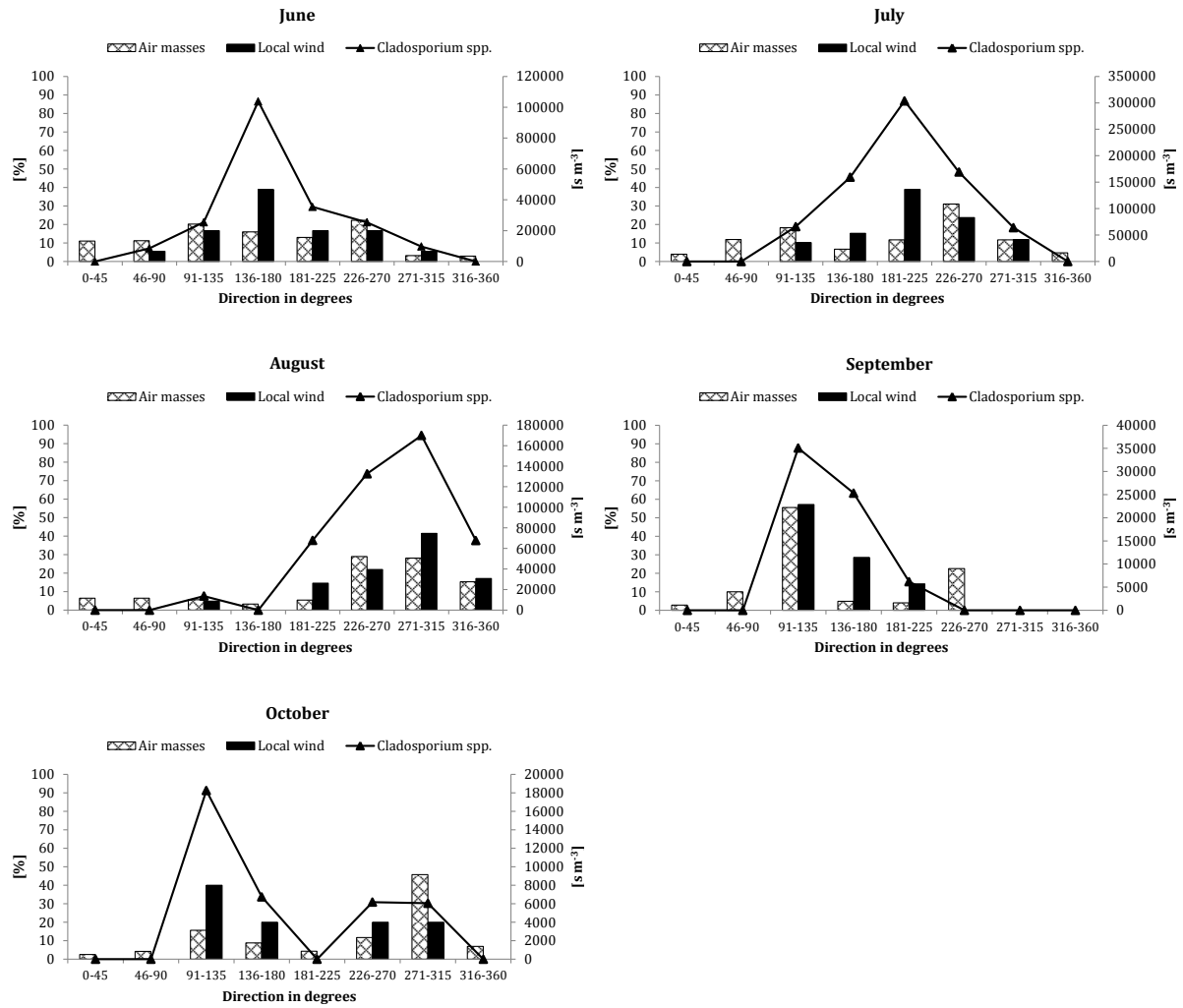


Fig. 3 Histograms showing a distribution pattern of the air masses, local wind direction both expressed in percent and *Cladosporium* spore concentrations recorded during high spore count days between June and October in Worcester, UK.

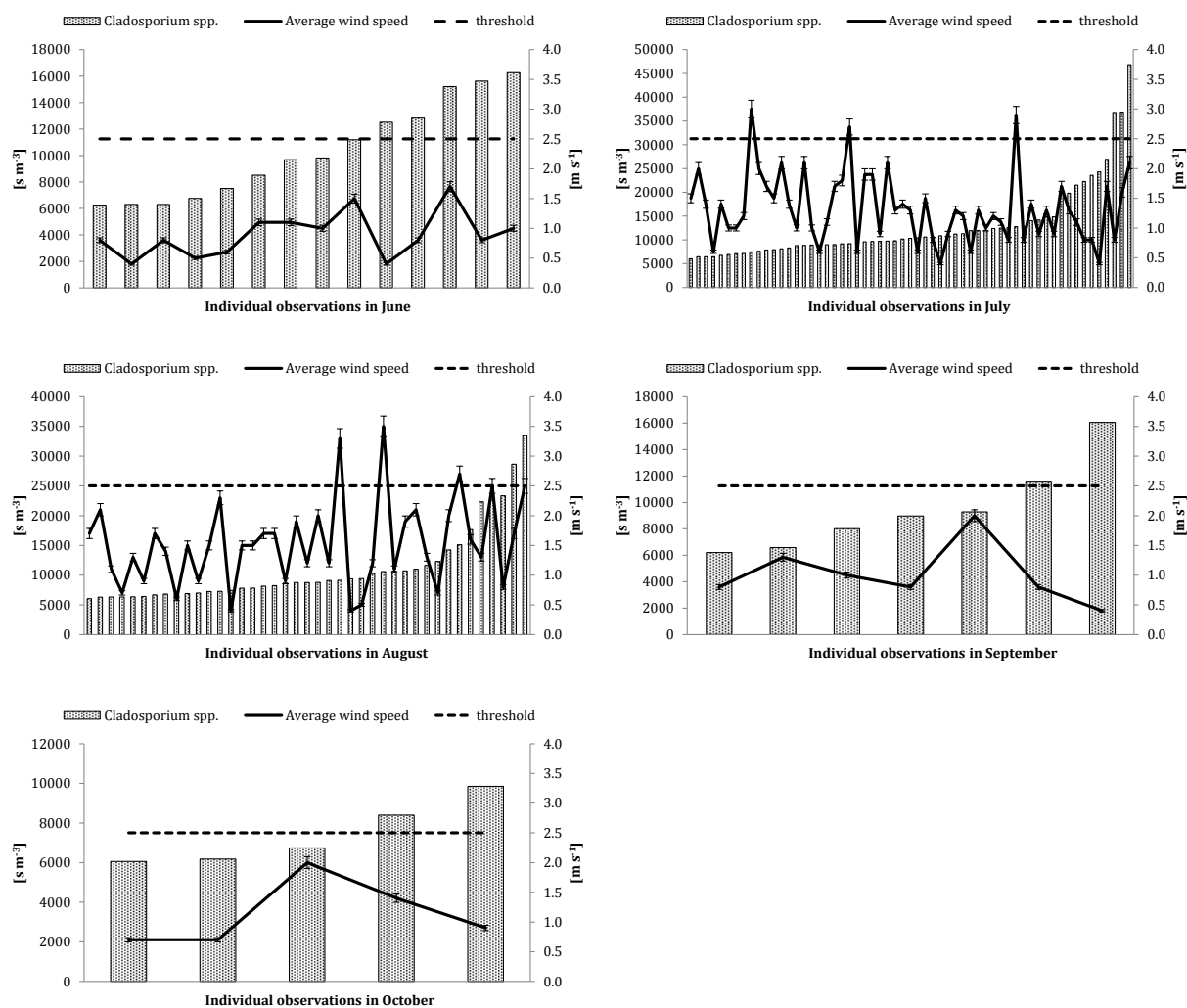


Fig. 4 Histograms showing (a) a daily mean concentration of *Cladosporium* recorded between June and October in Worcester, UK, (b) daily mean local wind speed with an indication of 5% error bars, (c) threshold line – 95% of observations were found when daily mean wind speed was $\leq 2.5\ m\ s^{-1}$. 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 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
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 $p \leq 0.001$

attachment to manuscript

[Click here to view linked References](#)



[Click here to view linked References](#)

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

3

4

5 Magdalena Sadyś^{1,2}

6

7

8 ¹ Institute of Science and the Environment, University of Worcester, Henwick Grove,
9 Worcester, WR2 6AJ, United Kingdom

10

11 ² (✉) Rothamsted Research, West Common, Harpenden, AL5 2JQ, United Kingdom,
12 e-mail: magdalena.sadys@rothamsted.ac.uk, tel. +44 (0) 1582 938 471

13

14

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31

32

33

34 **Abstract**

35

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

52

53

54

55

56

57

58

59

60

61

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

64

65 1. Introduction

66 *Cladosporium* spp. (~~hereafter *Cladosporium*~~) conidia have become of special interest
67 to scientists since 1932 when Cobe first reported their allergenic properties ~~were reported for~~
68 ~~the first time in 1932 by Cobe~~ (Hyde et al. 1956). Bouziane et al. (2005) and Bouziane et al.
69 (2006) found that conidia of *Cladosporium cladosporioides* contained a larger concentration
70 of allergens than mycelia. Furthermore, Green et al. (2003) showed that increased allergen
71 production varying from 5% to 40% was observed during germination by *Cladosporium*
72 *herbarum*. A cross-sectional study of Zureik et al. (2002) examined sensitization rates in
73 1,132 patients living in six regions, i.e., northern Europe, central Europe, southern Europe,
74 United Kingdom/Republic of Ireland, Portland (US) and Australia/New Zealand using
75 allergen extracts from two fungi (*Alternaria* spp., *Cladosporium* spp.), five pollen types
76 (grass, birch, ragweed, olive, pellitory of the wall), cat and house dust mites. Their survey
77 showed that the sensitization rates to fungi increased along with the severity of asthma. In
78 Europe, the sensitization to *Cladosporium* spp. (hereafter *Cladosporium*) was found within
79 the range of 0.7-9.9% with an upward trend towards the North; in the case of British and Irish
80 population this was equal to 6.8% (Zureik et al. 2002). Another study conducted in 16
81 European countries confirmed the highest sensitization rate to *Cladosporium herbarum* to
82 occur in Ireland, UK and other northern countries, and an average sensitization rate to this
83 type of spores equal to 5.8% (Heinzerling et al. 2005).

84 The outcomes of these clinical surveys are in agreement with the observations
85 previously made by Lacey (1981) who reviewed a number of aerobiological reports and
86 established the dominance of *Cladosporium* spores in the air of areas characterized by cooler
87 humid continental climates with warm summers; in Madrid (Spain) overall contribution of
88 *Cladosporium* spores to the total air spora was estimated for 41%, while in Worcester (UK)
89 this can reach up to 75% (Sadyś et al. 2015a; Díez Herrero et al. 2006). Other sites, such as
90 Krasne (Poland) reported contributions up to 92% (Kasprzyk and Worek 2006). ~~Based on~~
91 ~~analysis of a number of aerobiological reports Lacey (1981) concluded that *Cladosporium*~~
92 ~~spores dominated in cooler humid continental climates with warm summers; in Madrid~~
93 ~~(Spain) overall contribution of *Cladosporium* spores to the total air spora was estimated for~~
94 ~~41%, while in Worcester (UK) this can reach up to 75% (Sadyś et al. 2015a; Díez Herrero et~~
95 ~~al. 2006). Other sites, such as Krasne (Poland) reported contributions up to 92% (Kasprzyk~~
96 ~~and Worek 2006).~~ Harvey (1970) estimated spore production in six species of *Cladosporium*
97 to be within a range from 7.3×10^2 to 2.61×10^4 s mg^{-1} dry weight of mycelium. Spore
98 production by individual species turned out to be independent of spore frequency in the air

99 (Harvey 1970). Frankland and Davies (1965) established a threshold value of spore
100 concentration above which susceptible individuals exhibit symptoms of sensitization, *i.e.*
101 3,000 s m⁻³ in the United Kingdom. Different threshold values for *Cladosporium* were
102 estimated in Finland (4,000 s m⁻³) and Poland (2,800 s m⁻³), (Ranta and Pessi 2006; Rapiejko
103 et al. 2004). ~~Bouziane et al. (2005) and Bouziane et al. (2006) found that conidia of~~
104 ~~*Cladosporium cladosporioides* contained a larger concentration of allergens than mycelia.~~
105 ~~Furthermore, Green et al. (2003) showed that increased allergen production varying from 5%~~
106 ~~to 40% was observed during germination by *Cladosporium herbarum*.~~

107 ~~Grinn Gofroń (2009) reviewed a large number of reports which examined the~~
108 ~~dependence of *Cladosporium* on the meteorological variables. She found positive statistically~~
109 ~~significant correlations with maximum, minimum, and mean temperature, sunshine hours~~
110 ~~while negative statistically significant relationships with dew point temperature and air~~
111 ~~pressure. Contrary results were found for rainfall, relative humidity and wind speed by other~~
112 ~~researchers (Grinn Gofroń 2008; Herrero and Zaldivar 1997; Hjelmroos 1993; Katial et al.~~
113 ~~1997; Kurkela 1997; Mediavilla Molina et al. 1998; Mitakakis et al. 1997; Oliveira et al.~~
114 ~~2009a; Stepalska and Wołek 2005; Troutt and Levetin 2001). However, this review did not~~
115 ~~include a wind direction analysis as this is a rarely studied parameter (Grinn Gofroń 2009).~~

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

133 Siljamo 2004; Sofiev et al. 2006). However, a limited number of surveys investigated
134 regional and long-distance airborne transmission of fungal spores, e.g. rust spores (Isard et al.
135 2005), *Leptosphaeria biglobosa* (Grinn-Gofroń et al. 2015), *Alternaria* spp. (Fernández-
136 Rodríguez et al. 2015; Sadyś et al. 2015b; Skjøth et al. 2012). To date only one article was
137 focused exclusively on *Cladosporium* (Sadyś et al. 2015c) and many more are needed since
138 this is one of the most important fungal aeroallergens. Grinn-Gofroń (2009) reviewed a large
139 number of reports which examined the dependence of *Cladosporium* on the meteorological
140 variables. She found positive statistically significant correlations with maximum, minimum,
141 and mean temperature, sunshine hours while negative statistically significant relationships
142 with dew point temperature and air pressure. Contrary results were found for rainfall, relative
143 humidity and wind speed by other researchers (Grinn-Gofroń 2008; Herrero and Zaldivar
144 1997; Hjelmroos 1993; Katial et al. 1997; Kurkela 1997; Mediavilla Molina et al. 1998;
145 Mitakakis et al. 1997; Oliveira et al. 2009a; Stępańska and Wołek 2005; Troutt and Levetin
146 2001). However, this review did not include a wind direction analysis as this is a rarely
147 studied parameter (Recio et al. 2012; Sánchez Reyes et al. 2009).

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

156 2. Materials and methods

157 2.1. Bioaerosol specimen

158 The volumetric air sampler (Burkard Manufacturing Co. Ltd., Rickmansworth, UK)
159 was operating continuously from 2006 to 2010. The spore trap (Hirst 1952) was installed
160 permanently at a height of 10 m above ground level, on the rooftop of the University of
161 Worcester building (52° 11' 48" N, 2° 14' 31" W). Measurements were taken following the
162 guidance given by Lacey and West (2006). Drums with trapping surface were changed every
163 Thursday at 09:00 UTC, and then processed at the laboratory as described by Lacey and West
164 (2006).

165 The shape of the *Cladosporium* spores varies from cylindrical, through ellipsoidal and
166 ovoid up to sub-spherical. They are usually small in size (40-60 µm × 3-22 µm) from

167 olivaceous to brown in colour, frequently observed in branched chains. Hence, depending on
168 the location, spores may have a shield or round shape at the ends, visible black scars of
169 attachment points. Spores towards the end of chain are smaller and aseptate (Bensch et al.
170 2012). The surface of the wall may be either smooth or rough (verruculose or echinulate),
171 (Ellis 1971).

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

177 Throughout a 5-year period the clinical threshold of $\geq 3,000 \text{ s m}^{-3}$ established for
178 *Cladosporium* conidia (Frankland and Davies 1965) was recorded on 330 days and varied
179 from 47 to 88 days in a single year (Sadyś et al. 2016). Such atmospheric concentrations
180 achieved by *Cladosporium* can be produced by a local source. Thus, in order to examine the
181 impact of local wind and air mass transport on *Cladosporium* concentrations, and possible
182 transport of conidia at a regional scale, days when clinical threshold was two-folded were
183 selected for this study ($n=131$). As this study focused on monthly fluctuations in bioaerosol
184 distribution, data for a single day with above-mentioned concentration, which occurred on the
185 30th of April 2007, was discarded as it would not constitute a representative value for the
186 entire month.

187

188 **2.2. Meteorological data and atmospheric modelling**

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

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

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

210

211 **2.3. Statistical analyses**

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

222

223 **3. Results**

224 **3.1. Distribution of *Cladosporium* spores**

225 Concentrations of *Cladosporium* spores varied significantly between months (Fig. 1a,
226 Fig. 1b). Overall the greatest number of spores was trapped in July and these constituted
227 31.79% of the total 5-year spore catch. August (22.97%) and June (15.87%) were the second
228 and third months in order when large numbers of conidia were observed in the air of
229 Worcester (Fig. 1a). However, this pattern was not repeated each year, as in 2007 the greatest
230 concentration of *Cladosporium* spores was collected in June, followed then by July and
231 September (Fig. 1b). In 2008, once again spore counts recorded in September outnumbered
232 counts observed in June the same year (Fig. 1b). An interesting situation also occurred in
233 2009, when the second largest monthly sum of daily mean spore concentration was found in
234 October, not in August (Fig. 1b). A total number of spores collected between December and

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

241

242 **3.2. Influence of wind direction on spore counts**

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

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

258

259 **3.3. Air mass analysis**

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

265 Figure 3 presented the distribution of the air mass for each studied month when the
266 daily mean concentration of *Cladosporium* spores was equal to or above $6,000 \text{ s m}^{-3}$. Overall,
267 the air masses were coming from the SSE to the WNW directions, while none or very little
268 contribution was detected from N-E bearings (Figs 2, 3). Obtained results were in agreement

269 with an analysis of clustered trajectories points, which showed that majority of the air masses
270 originated from the southern directions (SW-SE) when increased levels of *Cladosporium*
271 spores were trapped at Worcester station (Table 2, Fig. 3). Throughout the period of study,
272 the mean angle remained within a range of 135°-293° (Table 2). A lack of uniformity in the
273 sampled data was confirmed jointly by von Mises and Rayleigh tests (Table 2). The values of
274 kappa also greatly varied, with an agreement from 0.60 (October) to 2.14 (September), (Table
275 2). The relationship between local wind direction and air mass directions (Table 3) varied
276 from low (August) to high (October) level of association.

277

278 **3.4. Local wind analysis**

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

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

301 Upon these results, it was decided to perform a further analysis of the local wind
302 speed recorded during high spore count days, individually for each month (Fig. 4). Obtained

303 histograms showed that regardless the time of the year and the spore concentration levels,
304 95% of observations were made when daily mean wind speed was equal to or lower than 2.5
305 m s⁻¹ (Fig. 4). A Spearman's rank test did not find this relationship to be statistically
306 significant ($r_s=0.019$, $p=0.847$).

307

308 4. Discussion

309 ~~Obtained results~~ This study indicated an unimodal distribution in *Cladosporium* spore
310 frequency, with a single peak occurring mostly in July (~~Fig. 1b~~). Morrow Brown and Jackson
311 (1978a) reported that at 8 locations across England, i.e.: (Derby, Birmingham, Ashby, Church
312 Broughton, Hartington, Crich, Attenborough, Sutton Bonington), Cladosporium spores
313 similarly showed a single peak between the end of July and mid-August although the spore
314 counts did not vary greatly between sampling sites. In contrast, a concentration of
315 *Cladosporium* spores in Spain (Madrid, Malaga, Valladolid) and Turkey (Sivrihisar) was
316 reported to follow a bimodal distribution with first peak occurring either by the end of spring
317 or at the beginning of summer (May-June) and second peak observed in autumn (September-
318 October), (Díez Herrero et al. 2006; Sánchez Reyes et al. 2009; Recio et al. 2012; Erkara et
319 al. 2009). The magnitude of spring-summer and autumn peaks differed between locations,
320 and the latter peaks in Madrid were more important than in Malaga or Valladolid. With
321 regard to the monthly sum of daily mean spore concentrations, October catch was a factor of
322 2 higher than June (Díez Herrero et al. 2006). Such high contribution of autumn months was
323 explained by more susceptible environmental conditions for the fungal growth while too high
324 daily maximum temperature and lack of precipitation over the summer prevented numerous
325 spore production and dissemination (Díez Herrero et al. 2006; Sánchez Reyes et al. 2009;
326 Erkara et al. 2009). However, none of the authors reported which *Cladosporium* species were
327 responsible for the spring-summer and autumn peaks.

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

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

348 This study also showed that overall percentage directions of the local wind remained
349 constant throughout the examined period of time (Fig. 3). ~~The prevailing wind directions~~
350 ~~originated from the SE-SW bearing (Fig. 3).~~ Despite this, Spearman's rank test indicated
351 statistically significant correlations between very high spore concentration days and local
352 wind direction only in July and September (Table 1). These results were not confirmed by
353 circular statistics, as the dominance of local winds was found only in October (Table 1).
354 Considering the overall local wind direction distribution, it seems that this could be explained
355 by the greatest contribution of wind blowing from E-SE directions (26%) in comparison with
356 other examined months (Fig. 3). With regard to the impact of air mass on fungal spore levels,
357 Spearman's rank test showed statistically significant associations for the same months as with
358 local wind direction (Table 1). Lack of any sort of correlation between *Cladosporium* and
359 both local wind and air mass directions was observed in August (Table 1). Despite that during
360 this month, the air mass spent over the non-UK areas the lowest amount of their time (Table
361 S1). Also, the relationship between local wind and air mass direction showed to be the
362 weakest in August ($r_s=0.41$) out of five examined months (Table 3). Taking into account the
363 overall pattern in local wind direction, August was notable for a significant decrease in the
364 contribution of E-SE wind direction (Fig. 3). Hence, within a span of 91° - 180° there must be
365 a considerable source area of *Cladosporium* spores. Studies that investigate the impact of
366 wind direction on bioaerosol concentration are very scarce (Sadyś et al. ~~2015b~~2015c). An
367 exception is a study of Sánchez Reyes et al. (2009) who examined the impact of wind
368 direction on the presence and concentration levels of *Cladosporium* spores in the air of
369 Valladolid (Spain). Another exception is a survey made by Recio et al. (2012) who performed
370 the same analysis in Malaga (Spain). Sánchez Reyes et al. (2009) found that although NE

371 direction was dominant throughout two years of sampling (37.4% and 31.4%, respectively),
372 Spearman's rank test indicated statistically significant correlation with SE wind direction
373 only. Sánchez Reyes et al. (2009) reported that along this direction an extensive grassland
374 area was found that most likely constituted an inoculum source of *Cladosporium* spores
375 (Sánchez Reyes et al. 2009). Contrary findings were reported by Recio et al. (2012) who
376 found statistically significant relationships between dominant wind directions (SW and NE)
377 and an increase in fungal spore concentration. Moreover, wind blowing from the sea (SE)
378 was correlated negatively with the presence of *Cladosporium* spores in the air of Malaga
379 (Recio et al. 2012).

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

397 Finally, although the correlation between local wind and high concentration of
398 *Cladosporium* spores was not found to be statistically significant, yet 95% of observations
399 were made when daily average wind speed was $v_s \leq 2.5 \text{ m s}^{-1}$. This result-finding is in
400 agreement with previously reported a value of $v_s \leq 3 \text{ m s}^{-1}$ in relation to the overall dispersal
401 of biological particles in the atmosphere (Reynolds et al. 2007), as well as v_s varying between
402 2 and 3.5 m s^{-1} established in particular for *Cladosporium* spores (Kurkela 1997).

403

404 **Conclusion**

405 The major findings of this aerobiological survey were following: (1) the greatest
406 numbers of spore concentrations were recorded in July and August when *Cladosporium*
407 *herbarum*, *C. cladosporioides* and *C. macrocarpum* sporulate; (2) sources of *Cladosporium*
408 conidia must have an origin within the UK territory; (3) local wind had a greater impact on
409 *Cladosporium* conidia occurrence in the air of Worcester than the air masses; (4) taking into
410 account the strength of statistical significance of detected dependencies of *Cladosporium* on
411 local wind, it must be stressed that the origin of conidia had a rather regional than local
412 character; (5) the most contributing sources of the fungus were located in the SE to SW
413 directions; (6) very high daily mean concentrations of *Cladosporium* spores, *i.e.* between
414 6,000 and 32,000 spores per cubic meter of air, were observed when daily mean local wind
415 speed was $v_s \leq 2.5 \text{ m s}^{-1}$ indicating warm days with light breeze.

416

417 **Acknowledgements**

418

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

426

427 **References**

- 428 Bensch, K., Braun, U., Groenewald, J.Z., Crous, P.W. (2012) The genus *Cladosporium*. *Stud Mycol*,
429 72, 1-401.
- 430 Bouziane, H., Latge, J.P., Fitting, C., Mecheri, S., Lelong, M., David, B. (2005) Comparison of the
431 allergenic potency of spores and mycelium of *Cladosporium*. *Allergol Immunopathol*, 33,
432 125-130.
- 433 Bouziane, H., Latge, J.P., Lelong, M. (2006) Immunochemical comparison of the allergenic potency
434 of spores and mycelium of *Cladosporium cladosporioides* extracts by a nitrocellulose
435 electroblotting technique. *Allergol Immunopathol*, 34, 64-69.
- 436 [CALPUFF Modeling System \(1990\) Atmospheric Studies Group \(ASG\),](http://www.src.com/calpuff/calpuff1.htm)
437 <http://www.src.com/calpuff/calpuff1.htm>. Accessed 3 March 2017.
- 438 Calvo Torras, M.A., Guarro Artigas, J., Suarez Fernandez, G. (1981) Air-borne fungi in the air of
439 Barcelona (Spain). 4. The genus *Cladosporium*. *Mycopathologia*, 74, 19-24.
- 440 Díez Herrero, A., Sabariego Ruiz, S., Gutiérrez Bustillo, M., Cervigón Morales, P. (2006) Study of
441 airborne fungal spores in Madrid, Spain. *Aerobiologia*, 22, 135-142.

442 Draxler, R.R., Rolph, G.D. (2014) HYSPLIT (HYbrid Single-Particle Lagrangian Integrated
443 Trajectory) Model. NOAA Air Resources Laboratory.
444 <http://ready.arl.noaa.gov/HYSPLIT.php>.

445 Ellis, M.B. (1971) Dematiaceous Hyphomycetes. London: The Eastern Press, Ltd.

446 Erkara, I.P., Ilhan, S., Oner, S. (2009) Monitoring and assessment of airborne *Cladosporium* Link and
447 *Alternaria* Nees spores in Sivrihisar (Eskisehir), Turkey. Environ Monit Assess, 48, 477-484.

448 Fernández-Rodríguez, S., Sadyś, M., Smith, M., Tormo-Molina, R., Skjøth, C.A., Maya-Manzano,
449 ~~J.M., Silva-Palacios, I., Gonzalo-Garijo, Á.~~ et al. (2015) Potential sources of airborne
450 *Alternaria* spp. spores in South-west Spain. Sci Total Environ, 533, 165-176.

451 Frankland, A.W., Davies, R.R. (1965) Allergy to mold spores in England. Poumon Coeur, 21:11-31.

452 Fulton, J.D. (1966) Microorganisms in the upper atmosphere. 3. Relationship between altitude and
453 micropopulation. J Appl Microbiol, 14, 237-240.

454 Green, B.J., Mitakakis, T.Z., Tovey, E.R. (2003) Allergen detection from 11 fungal species before and
455 after germination. J Allergy Clin Immunol, 11, 285-289.

456 Grinn-Gofroń, A. (2008) The variation in spore concentrations of selected fungal taxa associated with
457 weather conditions in Szczecin, Poland, 2004-2006. Grana, 47, 139-146.

458 Grinn-Gofroń, A. (2009) The occurrence of *Cladosporium* spores in the air and their relationships with
459 meteorological parameters. Acta Agrobot, 62, 111-116.

460 [Grinn-Gofroń, A., Sadyś, M., Kaczmarek, J., Bednarz, A., Pawłowska, S., Jedryczka, M. \(2016\) Back-](#)
461 [trajectory modelling and DNA-based species-specific detection methods allow tracking of](#)
462 [fungal spore transport in air masses. Sci Total Environ, 571, 658-669.](#)

463 Harvey, R. (1967) Air-spora studies at Cardiff. I. *Cladosporium*. Trans Br Mycol Soc, 50, 479-&.

464 Harvey, R. (1970) Spore productivity in *Cladosporium*. Mycopathol Mycol Appl, 41, 251-256.

465 [Heinzerling, L., Frew, A.J., Bindslev-Jensen, C., Bonini, S., Bousquet, J., Bresciani, M., et al. \(2005\)](#)
466 [Standard skin prick testing and sensitization to inhalant allergens across Europe - a survey](#)
467 [from the GALEN network. Allergy, 60, 1287-300.](#)

468 Hernández-Ceballos, M.A., Skjøth, C.A., García-Mozo, H., Bolívar, J.P., Galán, C. (2014)
469 Improvement in the accuracy of back trajectories using WRF to identify pollen sources in
470 southern Iberian Peninsula. Int J Biometeorol, 58, 2031-2043.

471 Herrero, B., Zaldivar, P. (1997) Effects of meteorological factors on the levels of *Alternaria* and
472 *Cladosporium* spores in the atmosphere of Palencia, 1990-92. Grana, 36, 180-184.

473 Hirst, J.M. (1952) An automatic volumetric spore trap. Ann Appl Biol, 39, 257-265.

474 Hirst, J.M. (1973) Spore transport and vertical profiles, vol 18. Bulletins from the Ecological Research
475 Committee.

476 Hirst, J.M., Stedman, O.J., Hurst, G.W. (1967) Long-distance spore transport - vertical sections of
477 spore clouds over sea. J Gen Microbiol, 48, 357-&.

478 Hjelmroos, M. (1993) Relationship between airborne fungal spore presence and weather variables -
479 *Cladosporium* and *Alternaria*. Grana, 32:40-47.

480 Hyde, H.A., Richards, M., Williams, D.A. (1956) Allergy to mould spores in Britain. Br Med J, 1,
481 886-890.

482 [Isard, S.A., Gage, S.H., Comtois, P., Russo, J.M. \(2005\) Principles of the atmospheric pathway for](#)
483 [invasive species applied to soybean rust. Bioscience, 55, 851-861.](#)

484 Kasprzyk, I., Worek, M. (2006) Airborne fungal spores in urban and rural environments in Poland.
485 Aerobiologia, 22, 169-176.

486 Katial, R.K., Zhang, Y.M., Jones, R.H., Dyer, P.D. (1997) Atmospheric mold spore counts in relation
487 to meteorological parameters. Int J Biometeorol, 41, 17-22.

488 Kurkela, T. (1997) The number of *Cladosporium* conidia in the air in different weather conditions.
489 Grana, 36, 54-61.

490 Lacey, J. (1981) The aerobiology of conidial fungi. In: Cole GT, editor. Biology of conidial fungi.
491 New York: Academic Press; p. 373-416.

492 Lacey, M.E., West, J.S. (2006) Air spora: A manual for catching and identifying airborne biological
493 particles. Verlag GmbH: Springer.

494 Maya-Manzano, J.M., Sadyś, M., Tormo-Molina, R., Fernández-Rodríguez, S., Oteros, J., Silva-
495 ~~Palacios, I., Gonzalo-Garijo, Á.~~ et al. 2017. Relationships between airborne pollen grains, wind
496 direction and land cover using GIS and circular statistics. Sci Total Environ, *in press*.

497 Mediavilla Molina, A., Angulo Romero, J., García-Pantaleón, F.I., Comtois, P., Domínguez Vilches,
498 E. (1998) Preliminary statistical modelling of the presence of two conidial types of
499 *Cladosporium* in the atmosphere of Córdoba, Spain. *Aerobiologia*, 14, 229-234.

500 Mitakakis, T.Z., Kok Ong, E., Stevens, A., Guest, D., Knox, R.B. (1997) Incidence of *Cladosporium*,
501 *Alternaria* and total fungal spores in the atmosphere of Melbourne (Australia) over three
502 years. *Aerobiologia*, 13, 83-90.

503 Morrow Brown, H., Jackson, F.A. (1978a) Aerobiological studies based in Derby. 2. Simultaneous
504 pollen and spore sampling at eight sites within a 60 km radius. *Clin Allergy*, 8, 599-609.

505 Morrow Brown, H., Jackson, F.A. (1978b) Aerobiological studies based in Derby. 3. Comparison of
506 simultaneous pollen and spore counts from East coast, Midlands and West coast of England
507 and Wales. *Clin Allergy*, 8, 611-619.

508 Mukaka, M.M. (2012) A guide to appropriate use of correlation coefficient in medical research.
509 *Malawi Med J*, 24, 69-71.

510 Oliveira, M., Ribeiro, H., Delgado, J.L., Abreu, I. (2009a) The effects of meteorological factors on
511 airborne fungal spore concentration in two areas differing in urbanisation level. *Int J*
512 *Biometeorol*, 53, 61-73.

513 Oliveira, M., Ribeiro, H., Delgado, J.L., Abreu, I. (2009b) Seasonal and intradiurnal variation of
514 allergenic fungal spores in urban and rural areas of the North of Portugal. *Aerobiologia*, 25,
515 85-98.

516 Ranta, H., Pessi, A.-M. (2006) Pollen Bulletin Summary 2005. *The Finnish Pollen Bulletin*, 30, 1-12.

517 Rapiejko, P., Lipiec, A., Wojadas, A., Jurkiewicz, D. (2004) Threshold pollen concentration necessary
518 to evoke allergic symptoms. *Internat Rev Allergol Clin Immunol*, 10, 91-94.

519 Recio, M., del Mar Trigo, M., Docampo, S., Melgar, M., Garcia-Sanchez, J., Bootello, L., ~~Cabezudo,~~
520 ~~B-et al.~~ (2012) Analysis of the predicting variables for daily and weekly fluctuations of two
521 airborne fungal spores: *Alternaria* and *Cladosporium*. *Int J Biometeorol*, 56, 983-991.

522 Reynolds, A.M., Bohan, D.A., Bell, J.R. (2007) Ballooning dispersal in arthropod taxa: conditions at
523 take-off. *Biol Letters*, 3, 237-240.

524 Rodríguez-Rajo, F.J., Iglesias, I., Jato, V. (2005) Variation assessment of airborne *Alternaria* and
525 *Cladosporium* spores at different bioclimatical conditions. *Mycol Res*, 109, 497-507.

526 Rolph, G.D. (2014) Real-time Environmental Applications and Display sYstem (READY). NOAA Air
527 Resources Laboratory. <http://ready.arl.noaa.gov>.

528 Sadyś, M., Strzelczak, A., Grinn-Gofroń, A., Kennedy, R. (2015a) Application of redundancy analysis
529 for aerobiological data. *Int J Biometeorol*, 59, 25-36.

530 Sadyś, M., Kennedy, R., Skjøth, C.A. (2015b) Determination of *Alternaria* spp. habitats using 7-day
531 volumetric spore trap, Hybrid Single Particle Lagrangian Integrated Trajectory model and
532 geographic information system. *Urban Climate*, 14, 429-440.

533 Sadyś, M., Kennedy, R., Skjøth, C.A. (2015b) (2015c) An analysis of local wind and air mass directions
534 and their impact on *Cladosporium* distribution using HYSPLIT and circular statistics. *Fungal*
535 *Ecol*, 18, 56-66.

536 Sadyś, M., Adams-Groom, B., Herbert, R.J., Kennedy, R. (2016) Comparisons of fungal spore
537 distributions using air sampling at Worcester, England (2006–2010). *Aerobiologia*, 32, 619-
538 634.

539 Sánchez Reyes, E., Rodríguez de la Cruz, D., Sanchís Merino, M.E., Sánchez, J. (2009)
540 Meteorological and agricultural effects on airborne *Alternaria* and *Cladosporium* spores and
541 clinical aspects in Valladolid (Spain). *Ann Agric Environ*, 16, 53-61.

542 Skjøth, C.A., Sommer, J., Frederiksen, L., Karlson, U.G. (2012) Crop harvest in Denmark and Central
543 Europe contributes to the local load of airborne *Alternaria* spore concentrations in
544 Copenhagen. *Atmos Chem Phys*, 12, 11107-11123.

545 Smith, D.J., Jaffe, D.A., Birmele, M.N., Griffin, D.W., Schuerger, A.C., Hee, J., et al. (2012) Free
546 tropospheric transport of microorganisms from Asia to North America. *Microb Ecol*, 64, 973-
547 985.

548 Sofiev, M., Siljamo, P. (2004) Forward and inverse simulations with Finnish emergency model
549 SILAM. In C. Borrego, S., Incecik (Ed.), *Air Pollution Modelling and its Applications* (pp.
550 417-425). New York: Springer.

551 [Sofiev, M., Siljamo, P., Valkama, I., Ilvonen, M., Kukkonen, J. \(2006\) A dispersion modelling system](#)
552 [SILAM and its evaluation against ETEX data. Atmos Environ, 40, 674-685.](#)
553 Stępańska, D., Wołek, J. (2005) Variation in fungal spore concentrations of selected taxa associated to
554 weather conditions in Cracow, Poland, in 1997. *Aerobiologia*, 21, 43-52.
555 Troutt, C., Levetin, E. (2001) Correlation of spring spore concentrations and meteorological
556 conditions in Tulsa, Oklahoma. *Int J Biometeorol*, 45, 64-74.
557 Wu, P.C., Tsai, J.C., Li, F.C., Lung, S.C., Su, H.J. (2004) Increased levels of ambient fungal spores in
558 Taiwan are associated with dust events from China. *Atmos Environ*, 38, 4879-4886.
559 [Zureik, M., Neukirch, C., Leynaert, B., Liard, R., Bousquet, J., Neukirch, F. \(2002\) Sensitisation to](#)
560 [airborne moulds and severity of asthma: cross sectional study from European Community](#)
561 [respiratory health survey. BMJ, 325, 1-7.](#)
562
563
564

565 Figure captions

566

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

570

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

573

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

576

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

580

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

587

588 Table captions

589

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

592

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

595

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