

Application of redundancy analysis for aerobiological data

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Abstract An aerobiological survey was conducted through five consecutive years (2006–2010) at Worcester (England). The concentration of 20 allergenic fungal spore types was measured using a 7-day volumetric spore trap. The relationship between investigated fungal spore genera and selected meteorological parameters (maximum, minimum, mean and dew point temperatures, rainfall, relative humidity, air pressure, wind direction) was examined using an ordination method (redundancy analysis) to determine which environmental factors favoured their most abundance in the air and whether it would be possible to detect similarities between different genera in their distribution pattern. Redundancy analysis provided additional information about the biology of the studied fungi through the results of the Spearman's rank correlation. Application of the variance inflation factor in canonical correspondence analysis indicated which explanatory variables were auto-correlated and needed to be excluded from further analyses. Obtained information will be consequently implemented in the selection of factors that will be a foundation for forecasting models for allergenic fungal spores in the future.

Keywords RDA · Fungal spores · Meteorological parameters · Aerobiology · Species-environment relationship

Introduction

With every breath, millions of microorganisms and particles are being inhaled directly into the lungs. Ones of the most important and simultaneously harmful particles, which thoroughly penetrate the respiratory system, are fungal spores (D'Amato and Spieksma 1995). Regardless of their viability, fungal spores contain allergenic components (e.g. Green et al. 2005; Simon-Nobbe et al. 2008) and therefore became a subject of intensive aerobiological monitoring worldwide, which is carried out both in indoor and outdoor environments (e.g. Grinn-Gofroń and Strzelczak 2013; Oliveira et al. 2009; Teixeira Gonçalves et al. 2010).

The relationship between the concentration of airborne allergenic fungal spores and meteorological parameters is commonly presented in a form of descriptive analysis of fungal spore seasons (e.g. Nikkels et al. 1996; Hernández Trejo et al. 2012) or examined using basic statistical tools, such as correlation analysis (e.g. Pearson's test, Spearman's rank correlation coefficient) depending on the data distribution (e.g. Hasnain 1993; Oliveira et al. 2009; Sabariego et al. 2000).

Some authors applied more advanced statistical instruments to predict the concentration of fungal spores, like multiple regression analysis (e.g. Burch and Levetin 2002; Hollins et al. 2004; Recio et al. 2012; Stepalska and Wołek 2005), auto-regressive integrated moving average (ARIMA) models (e.g. Rodríguez-Rajo et al. 2010), artificial neural network (ANN) models (e.g. Grinn-Gofroń and Strzelczak 2011; Grinn-Gofroń and Strzelczak 2013) and multivariate regression tree (MRT) models (e.g. Grinn-Gofroń and Strzelczak 2011).

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However, the ordination techniques, such as canonical correspondence analysis (CCA), detrended correspondence analysis (DCA) and redundancy analysis (RDA), are rarely applied in the examination of aerobiological data (Li and Kendrick 1994, 1995, 1996), although they have been already successfully implemented and commonly used in ecological studies (e.g. Wagner 2004), hydrobiological studies (e.g. Obolewski and Strzelczak 2009), phenological studies (e.g. Mimet et al. 2009) and many others. Recent article dedicated to advanced statistical methods used in the analysis of aerobiological data, such as concentration of fungal spores and pollen grains, called for the exploration of ordination techniques as no study was published regarding this for almost two decades, where ordination would be implemented (Grinn-Gofroń and Bosiacka 2012).

Correlation analysis is usually the third step of the basic statistical testing preceded by an examination of the data distribution and data transformation (del Mar Trigo et al. 2000). Depending on the obtained results, either Pearson's, Spearman's rank or Kendall's tau association test can be performed. Pearson's correlation test can be used only to examine a normally distributed variable, as it is very sensitive to outliers. Even a single outlier affects significantly the robustness of this test (Croux and Dehon 2010). In contrast to Pearson's correlation, Spearman's rank and Kendall's tau are considered as non-parametric tests, although they can be also used to analyse parametric data (Driscoll and Lecky 2001). They have been proved to provide reliable results above 70 % in comparison to the outcomes of the Pearson's correlation test (Croux and Dehon 2010). The popularity of the Spearman's rank test lies at the core of its advantages, which are the following: (1) this test does not require any assumptions about the frequency distribution of the variables, (2) linear relationship between variables and (3) measurement of the variables on interval or ratio scales (Bolboacă and Jäntschi 2006). However, its reliability in comparison with other methods that measure species-environment relationship has not been yet examined. Hence, the results obtained using Spearman's rank test should be verified by application of an alternative statistical tool such as redundancy analysis.

An auto-correlation of the weather parameters is often being neglected in the aerobiological studies, where simultaneously several temperature or wind parameters are being implemented in the construction of the forecast models (e.g. Angelosante Bruno et al. 2007; Puc 2012). The impact of colinearity is also underestimated, although many studies have already proved that colinearity of parameters inter alia led to an increase of variable variance or disabled the numerical solution of a model (Belsley et al. 1980; Greene 1993). Application of the variance inflation factor (VIF) into statistical analysis could be one way of determining a colinearity between investigated variables and of removing interferential factors (Groß 2003; O'Brien 2007).

The aims of this study were as follows: (1) to determine which environmental factors favoured the most abundance of selected allergenic fungal spore taxa in the air of Worcester, England, (2) to detect similarities between different fungi genera in their distribution pattern using both RDA and the Spearman's rank correlation analysis, (3) to detect auto-correlated weather parameters, (4) to examine whether Spearman's rank test and RDA provide complementary, homogenous information or antagonistic, heterogeneous information regarding species-environment relationships.

Materials and methods

Air sample collection and analysis

Air samples were collected continuously using a 7-day volumetric spore trap (Hirst 1952) from 2006 to 2010 at Worcester, England. The sampler was situated at the rooftop of the University of Worcester building (52° 11' N, 2° 14' W) approximately 10 m above the ground level. Drum was changed weekly on Thursday at 09:00 UTC local time. Tape was then removed at sterile laboratory conditions and cut into seven 48-mm-length segments, which corresponded to a 24-h period. Then, they were mounted using a mixture of petroleum jelly and lactophenol cotton blue. This procedure was described in detail by Lacey and Allitt (1995).

Microscope slides were examined using a light microscope under $\times 400$ magnification. Fungal spores were identified by their morphological features up to the genus level and counted along one central longitudinal band following Lacey and West (2006) and Stepalska and Wołek (2009). Several types of fungal spores were enumerated jointly because visual distinction was not possible between genera, i.e. *Aspergillus* spp. and *Penicillium* spp., or they were classified as an artificial "type", i.e. *Drechslera* type and *Leptosphaeria* type or a "group", i.e. coloured basidiospores, rusts and smuts. This is a commonly accepted practice by many aerobiologists (e.g. Ogden et al. 1974; Southworth 1974; Haines et al. 2003).

Weather data

The weather data were obtained from the weather station, which was co-located with a spore trap. Selected meteorological parameters were included in this study, i.e. maximum temperature, minimum temperature, mean temperature, dew point temperature, rainfall, relative humidity, air pressure and wind direction. The wind direction, which had been recorded in 16 cardinal directions, was then recalculated in a degree format using the following formula: N=360, NNE=22.5, NE=45, ENE=67.5, E=90, ESE=112.5, SE=135, SSE=157.5, S=180, SSW=202.5, SW=225, WSW=247.5, W=270, WNW=292.5, NW=315 and NNW=337.5. This change enabled

further transformation of this parameter. All meteorological parameters were recorded from 00:00 to 23:59 hours according to UTC local time.

Statistical analyses

Seasonal distribution of fungal spore concentration can vary between investigated fungal genera; therefore, to conduct an ordination analysis, observations from May to September for each studied year were selected. Initially, the relationship between original fungal spore counts and weather parameters was examined using Spearman's rank test, as the concentration of all spore types was found to be not normally distributed (data not shown). However, prior to the ordination analysis, fungal spore counts and eight meteorological parameters were normalized using $\log(x+1)$ transformation (1) to regularise the distribution of all investigated variables, (2) to minimize the effect of outliers and (3) to enable comparison of all variables, as each of them was measured using a different unit.

The relationship and colinearity (redundancy) of independent variables were examined with the aid of VIF in CCA. Variance inflation factor determined which of the meteorological parameters was auto-correlated and needed to be excluded from further investigation. It is assumed that significant colinearity appears there, where VIF indicates a value above 10 (Groß 2003).

Subsequently, DCA was performed to explore the length of DCA first axis, which denominates the character of relationship between the presence of fungal spores in the air and optimal weather conditions. This association may be either monotonic or unimodal, and hence, it requires either redundancy or canonical correspondence analysis in the concluding examination. According to ter Braak (1995) in most of the ecological studies, species' responses to the environmental conditions are monotonic in their nature, and they are determined by values below 2.

In the end, ANOVA test (Legendre et al. 2011) helped to denote statistically significant axes. CCA, DCA and RDA were conducted using the ComprehensiveR Archive Network (CRAN, R i.386 3.0.1., 2013), vegan (Oksanen et al. 2013) and permute (Simpson 2013) packages and the following functions: cca (Legendre and Legendre 1998), decorana (Hill and Gauch 1980; Oksanen and Minchin 1997), rda (ter Braak 1986; Legendre and Legendre 1998) and anova.cca (Legendre et al. 2011). Spearman's rank association test was done with the aid of STATISTICA StatSoft (v. 2012) software.

The results of RDA were presented graphically on the biplots, where meteorological parameters were shown as arrows+their names, whereas fungal spore types were presented by their Latin names only. The significance of correlation between weather factors and distribution of fungal spores depended on the length of the arrows, the longer arrow, the

stronger is the correlation (ter Braak and Prentice 1988). Vector of relationships (directly proportional or inversely proportional) was interpreted based on the position of the spore types in terms of arrows ends. If spore types were closely located to the end of the arrows, the association was positively correlated. If they were on the opposite site, then the correlation was negative (ter Braak and Prentice 1988).

Results

Contribution of spores to the total load

The distribution of fungal spores varied from year to year, as well as their contribution to the total load of identified and counted fungal genera in the air of the sampling area (Table 1). Based on their 5-year mean input, ten fungal spore types were chosen for further analyses, i.e. *Alternaria* spp., *Aspergillus* spp./*Penicillium* spp., *Botrytis* spp., *Cladosporium* spp., coloured basidiospores, *Didymella* spp., *Ganoderma* spp., *Leptosphaeria* type, rusts and smuts, although the selection of these genera has narrowed down to those with differences in propitious weather conditions (Table 1). *Cladosporium* spp., which contributed most significantly to total spore load between 47.25 and 75.29 %, reached its maximum concentration in 2010. Other taxa, e.g. ascospores and coloured basidiospores, were different. A group of strictly "dry" and "wet" spore types could be also distinguished, based on *Alternaria* spp., *Ganoderma* spp., rusts and smuts, and *Aspergillus* spp./*Penicillium* spp. spores with *Botrytis* spp., respectively.

Spearman's rank test results

Alternaria spp. spores were mainly positively correlated with maximum, minimum and mean temperatures (Table 2). A weaker relationship was found with dew point temperature. The closest relationships were observed in years 2006 and 2009 using Spearman's rank test, e.g. with maximum temperature ($r_s=0.62$ and $r_s=0.60$, respectively). A directly proportional influence on the spore concentration was also observed with the direction of the prevailing winds in 2007. An inversely proportional impact was observed for rainfall and relative humidity in 2006 and 2007. Minor statistically significant relationships were observed for air pressure in 2006 and 2009.

Dependence on the weather factors by *Aspergillus* spp./*Penicillium* spp. spores was not observed (Table 2). A weak statistically significant correlation with dew point temperature, relative humidity, wind direction and air pressure was observed between 2008 and 2010.

The highest impact on the presence and concentration of *Botrytis* spp. spores in the air of Worcester was observed for all temperature parameters, especially dew point temperature (Table 2). The most significant coefficients were obtained in

Table 1 Percent shares of all investigated fungal spore types

Taxon	2006	2007	2008	2009	2010	Mean
<i>Alternaria</i> spp.	0.78	0.80	0.87	0.88	0.50	0.75
<i>Aspergillus</i> spp./ <i>Penicillium</i> spp.	0.91	1.81	1.35	1.02	0.63	1.10
<i>Botrytis</i> spp.	0.42	0.71	0.63	0.60	0.49	0.56
<i>Cladosporium</i> spp.	72.70	55.89	47.25	63.24	75.29	64.10
Coloured basidiospores	11.81	13.92	15.57	11.32	12.24	12.85
<i>Didymella</i> spp.	2.27	13.23	20.56	8.04	2.02	8.44
<i>Drechslera</i> type	0.80	0.68	0.06	0.08	0.25	0.38
<i>Entomophthora</i> spp.	0.20	0.31	0.21	0.16	0.05	0.18
<i>Epicoccum</i> sp.	0.42	0.35	0.38	0.39	0.32	0.37
<i>Erysiphe</i> spp.	0.17	0.19	0.08	0.16	0.08	0.14
<i>Ganoderma</i> spp.	1.61	1.71	1.71	2.13	1.41	1.70
<i>Leptosphaeria</i> type	3.09	4.94	6.79	5.11	2.72	4.36
<i>Periconia</i> spp.	0.02	0.03	0.03	0.04	0.02	0.03
<i>Pithomyces</i> spp.	0.03	0.04	0.02	0.05	0.02	0.03
<i>Pleospora</i> spp.	0.29	0.43	0.14	0.16	0.10	0.22
<i>Polythrincium</i> spp.	0.17	0.36	0.33	0.49	0.25	0.31
Rusts	0.42	1.16	0.73	1.60	0.26	0.78
Smuts	3.71	3.20	3.02	4.36	3.21	3.51
<i>Stemphylium</i> spp.	0.03	0.02	0.04	0.02	0.02	0.03
<i>Torula</i> spp.	0.14	0.25	0.20	0.16	0.12	0.17

The highest obtained values within the 5-year period are set in italics

2009. Occasionally, an influence of wind direction was recorded in 2007 and 2010, as well as relative humidity (2008 and 2010) and air pressure (2006 and 2009).

Distribution of *Cladosporium* spp. spores was very similar to that already reported for *Alternaria* spp. spores, though the dependence on the temperature was more statistically significant ($r_s=0.70$ in 2006 and $r_s=0.68$ in 2009) (Table 2). Weak, negative correlations were also observed with rainfall in 2006 and 2010 and relative humidity in 2006.

Various associations between coloured basidiospores and examined weather parameters were seen (Table 2). Temperature was the most statistically significant, with a very distinctive influence exhibited in 2009 ($r_s=0.64$ for dew point temperature). There was also a directly proportional correlation with relative humidity. Other parameters, such as air pressure and wind direction, affected the concentration of coloured basidiospores only marginally.

The concentration of *Didymella* spp. spores was fluctuated in response to changes of relative humidity, dew point temperature and air pressure (Table 2). The vector of the latest dependency revealed to be negative. The most statistically significant correlations were noted in 2008 and 2010 (e.g. $r_s=0.56$ and $r_s=0.59$ with relative humidity). The impact of other temperature parameters other than dew point temperature was insignificant for the majority of the investigated periods of time (years 2006, 2007 and 2010). In addition, wind direction occasionally played a small role in the increased concentration of trapped *Didymella* spp. spores in

2006 and 2007. In 2008, all examined weather parameters showed statistically significant correlations with the concentration of *Didymella* spp. spores.

Ganoderma spp. spores followed similar distribution patterns to those previously described for *Alternaria* spp. and *Cladosporium* spp. spores (Table 2). Overall, the major factor that affected *Ganoderma* spp. spore concentration was increasing temperature, and dew point temperature in particular (e.g. $r_s=0.69$ in 2009). An inversely proportional relationship was found with rainfall and wind direction (e.g. $r_s=0.35$ and $r_s=-0.23$, respectively). Other environmental factors had a lesser influence, with respect to Spearman's rank coefficient values.

There was occurrence of *Leptosphaeria* type spore which was the only group that was strongly linked to rainfall, with correlation values varying between $r_s=0.47$ and $r_s=0.69$ (Table 2). This resulted in a further close positive association with relative humidity (e.g. $r_s=0.46$) and negative relationship with air pressure (e.g. $r_s=-0.58$) in 2009. In 2010, all examined weather parameters were statistically significant in correlations with the concentration of *Leptosphaeria* type spores.

A complex relationship between variations in fungal spore concentrations and environmental parameters was shown for rust and smut spores (Table 2). Increased numbers of rust spores in the air were observed at higher maximum and minimum temperature conditions, especially in 2009 ($r_s=0.44$), and with smut spores in 2006 ($r_s=0.52$). Dew point temperature was less important, and similarly to rainfall and relative

Table 2 Results of Spearman's rank association test between concentration of fungal spores and meteorological parameters

Taxon	Year	TMA	TMI	TME	DPT	WD	RAIN	RH	AP
Alt	2006	0.62*	0.62*	0.62*	0.23*	0.11	-0.33*	-0.45*	0.17*
	2007	0.54*	0.54*	0.54*	0.20*	0.33*	-0.38*	-0.33*	0.13
	2008	0.38*	0.38*	0.37*	0.34*	-0.01	-0.15	0.03	0.16*
	2009	0.60*	0.60*	0.60*	0.54*	0.01	-0.15	-0.05	0.04
	2010	0.44*	0.44*	0.44*	0.36*	0.15	-0.13	0.04	0.05
Asp/Pen	2006	0.02	0.02	0.02	-0.04	-0.11	-0.13	-0.04	-0.11
	2007	0.12	0.11	0.11	0.13	0.05	-0.08	0.04	0.13
	2008	0.07	0.07	0.07	0.18*	-0.18*	0.03	0.14	0.08
	2009	0.15	0.15	0.15	0.25*	-0.02	0.00	0.17*	-0.17*
	2010	0.05	0.05	0.05	0.16*	0.05	0.10	0.18*	-0.07
Bot	2006	0.13	0.13	0.13	0.20*	0.00	0.00	0.10	-0.17*
	2007	0.36*	0.36*	0.36*	0.38*	0.27*	-0.02	0.08	-0.09
	2008	0.05	0.04	0.04	0.32*	0.09	0.07	0.40*	-0.08
	2009	0.52*	0.52*	0.52*	0.55*	0.05	0.04	0.07	-0.21*
	2010	0.23*	0.23*	0.23*	0.32*	0.27*	0.05	0.25*	-0.12
Cla	2006	0.70*	0.70*	0.70*	0.42*	0.07	-0.20*	-0.33*	0.08
	2007	0.32*	0.33*	0.33*	0.34*	-0.14	-0.03	0.10	-0.05
	2008	0.36*	0.35*	0.35*	0.32*	-0.03	-0.06	0.00	0.01
	2009	0.68*	0.68*	0.68*	0.64*	-0.08	0.08	0.01	-0.13
	2010	0.61*	0.61*	0.61*	0.45*	0.02	-0.16*	-0.08	0.04
Bas	2006	-0.31*	-0.31*	-0.31*	0.05	-0.18*	0.13	0.45*	-0.27*
	2007	0.36*	0.36*	0.36*	0.31*	0.06	0.06	-0.04	-0.29*
	2008	0.05	0.05	0.05	0.29*	0.12	-0.04	0.40*	0.15
	2009	0.60*	0.60*	0.60*	0.64*	0.08	0.06	0.10	-0.17*
	2010	0.05	0.05	0.05	0.24*	0.13	0.06	0.38*	-0.01
Did	2006	0.10	0.10	0.10	0.27*	0.24*	0.33*	0.22*	-0.33*
	2007	0.10	0.10	0.10	0.38*	0.04	0.40*	0.34*	-0.48*
	2008	0.18*	0.17*	0.18*	0.53*	0.17*	0.58*	0.56*	-0.58*
	2009	0.40*	0.40*	0.40*	0.59*	0.09	0.33*	0.33*	-0.38*
	2010	0.11	0.11	0.11	0.44*	0.05	0.41*	0.59*	-0.32*
Gan	2006	0.47*	0.47*	0.47*	0.50*	-0.23*	-0.16*	0.01	-0.02
	2007	0.52*	0.53*	0.52*	0.40*	0.25*	-0.35*	-0.07	0.28*
	2008	0.31*	0.31*	0.31*	0.37*	0.00	-0.17*	0.15	0.30*
	2009	0.66*	0.66*	0.66*	0.69*	-0.03	-0.13	0.11	0.07
	2010	0.65*	0.65*	0.65*	0.65*	-0.02	-0.02	0.18*	0.01
Lep	2006	-0.32*	-0.31*	-0.31*	0.14	0.04	0.54*	0.57*	-0.52*
	2007	-0.04	-0.04	-0.04	0.33*	-0.08	0.47*	0.43*	-0.37*
	2008	-0.09	-0.09	-0.09	0.35*	-0.10	0.60*	0.67*	-0.44*
	2009	0.00	0.00	0.00	0.28*	-0.08	0.69*	0.46*	-0.58*
	2010	-0.19*	-0.19*	-0.19*	0.23*	-0.20*	0.66*	0.63*	-0.54*
Rus	2006	0.22*	0.22*	0.22*	0.26*	-0.05	-0.16	0.03	-0.19*
	2007	0.33*	0.33*	0.33*	0.06	0.46*	-0.43*	-0.29*	0.30*
	2008	0.09	0.09	0.09	0.26*	-0.03	-0.02	0.27*	0.07
	2009	0.44*	0.44*	0.44*	0.44*	0.08	-0.21*	0.06	0.04
	2010	0.19*	0.19*	0.19*	0.31*	0.12	0.15	0.30*	-0.11
Smu	2006	0.52*	0.52*	0.52*	0.30*	-0.12	-0.32*	-0.28*	0.24*
	2007	0.25*	0.25*	0.25*	0.04	-0.10	-0.13	-0.26*	-0.05
	2008	-0.04	-0.04	-0.04	-0.33*	0.12	-0.27*	-0.41*	0.30*
	2009	0.27*	0.27*	0.27*	0.06	-0.06	-0.08	-0.23*	0.07

Table 2 (continued)

Taxon	Year	TMA	TMI	TME	DPT	WD	RAIN	RH	AP
	2010	0.29*	0.29*	0.29*	−0.01	−0.10	−0.32*	−0.43*	0.22*

TMA maximum temperature, *TMI* minimum temperature, *TME* mean temperature, *DPT* dew point temperature, *WD* wind direction, *RH* relative humidity, *RAIN* rainfall, *AP* air pressure, *Alt Alternaria* spp., *Asp/Pen Aspergillus* spp./*Penicillium* spp., *Bas* coloured basidiospores, *Bot Botrytis* spp., *Cla Cladosporium* spp., *Did Didymella* spp., *Gan Ganoderma* spp., *Lep Leptosphaeria* type, *Rus* rusts, *Smu* smuts

* $p < 0.05$ (significance level)

humidity, although in some years, correlations were statistically significant. Almost no impact was found between rust and smut spore concentrations in the air with the direction of the prevailing winds at Worcester.

Canonical correspondence analysis

CCA detected a high auto-correlation within examined weather factors. Hence, it was necessary to remove each time from the data set, three out of four temperature parameters, as their VIF scored above 10. Dew point temperature and minimum temperature were included in data files, in the year 2006 and 2009, respectively, whereas in the remaining years, observation of maximum temperature was analysed. After the applied reduction in the number of used explanatory variables, other parameters obtained low redundancy (Table 3).

Detrended correspondence analysis

Detrended correspondence analysis revealed that relationships between explanatory and investigated variables were monotonic in their nature for each studied year, as the length of DCA first axis analysis resulted in values below 2. Therefore, redundancy analysis was chosen as a final examination tool.

Redundancy analysis

In 2006, the first and second RDA axes explained 16.4 and 7.9 % of the variance, respectively (Table S1). In the following years, the amount of explained variance by the first and

second RDA axes gradually increased, obtaining dually 28.2, 30.3 and 33.3 %, respectively. A slight fall was observed in 2010, when 31.3 % was determined by RDA 1 and RDA 2 axes. Other components were not important in the description of the variance or they did not achieve level of statistical significance (Table S1).

The ANOVA test revealed that during the studied period of time, first three out of five RDA axes exceeded the threshold of statistical significance with a p value below 0.01, with an exception of the year 2008, where additionally RDA 4 was found to be statistically important ($p < 0.05$), (Table 4). Biplot scores for constraining variables and species scores were

Table 4 Results of the ANOVA test

Year	Axis	Degree of freedom	Chi-square test	F	N. perm	Pr>(F)
2006	RDA1	1	0.70	32.58	199	0.01**
	RDA2	1	0.33	15.65	199	0.01**
	RDA3	1	0.12	5.50	199	0.01**
	RDA4	1	0.02	1.11	99	0.31
	RDA5	1	0.01	0.62	99	0.71
2007	RDA1	1	0.64	41.43	199	0.01**
	RDA2	1	0.29	18.95	199	0.01**
	RDA3	1	0.08	4.93	199	0.01**
	RDA4	1	0.02	1.63	99	0.15
	RDA5	1	0.00	0.15	99	0.99
2008	RDA1	1	0.93	54.52	199	0.01**
	RDA2	1	0.22	13.11	199	0.01**
	RDA3	1	0.10	5.65	199	0.01**
	RDA4	1	0.04	2.26	9999	0.05*
	RDA5	1	0.01	0.56	99	0.73
2009	RDA1	1	1.22	60.24	199	0.01**
	RDA2	1	0.30	15.01	199	0.01**
	RDA3	1	0.08	4.11	199	0.01**
	RDA4	1	0.01	0.64	99	0.68
	RDA5	1	0.01	0.31	99	0.94
2010	RDA1	1	0.88	46.37	199	0.01**
	RDA2	1	0.45	23.39	199	0.01**
	RDA3	1	0.11	5.68	199	0.01**
	RDA4	1	0.01	0.47	99	0.82
	RDA5	1	0.01	0.37	99	0.92

* $p < 0.05$; ** $p < 0.01$ (significance level)

Table 3 Variance inflation factors for meteorological parameters after reduction

Year	TMA	TMI	TME	DPT	WD	RH	RAIN	AP
2006	—	—	—	1.12	1.05	1.74	1.47	1.20
2007	1.07	—	—	—	1.12	1.65	1.90	1.31
2008	1.07	—	—	—	1.02	1.61	1.75	1.45
2009	—	1.09	—	—	1.02	1.49	1.72	1.35
2010	1.04	—	—	—	1.08	1.58	1.75	1.22

TMA maximum temperature, *TMI* minimum temperature, *TME* mean temperature, *DPT* dew point temperature, *WD* wind direction, *RH* relative humidity, *RAIN* rainfall, *AP* air pressure

therefore presented for selected axes in the supplementary material section (Table S2 and Table S3, respectively).

The results of RDA were described below and visually presented at Figs. 1, 2, 3, 4 and 5 for each studied year individually. Based on the length of the arrows, meteorological parameters could be classified in the following descending order of importance: relative humidity, dew point temperature, air pressure, rainfall and wind direction (Fig. 1). Presence of *Aspergillus* spp./*Penicillium* spp. spores in the air of Worcester in 2006 did not show dependence on any of the examined environmental factors (Fig. 1). A small dependence on the wind direction revealed only *Didymella* spp. and *Leptosphaeria* type. The concentration of these spores in the air increased along with the degrees of the wind to a certain extent. An influence of dew point temperature and high relative humidity revealed to be the most significantly affecting dispersal of *Didymella* spp. spores, whereas other ascospores were more closely related to rainfall. Changes in the pressure of the air were negatively correlated only with *Didymella* spp. Finally, *Alternaria* spp., *Cladosporium* spp. and smuts constituted a distinctive separate group, which revealed a directly proportional relationship with dew point temperature and inversely proportional with rainfall and relative humidity.

The relative importance of weather conditions changed in 2007, and rainfall was demonstrated to be more important (Fig. 2). In 2007, the importance of rainfall was followed by relative humidity, maximum temperature, air pressure and wind direction. However, similarly to the previous observations,

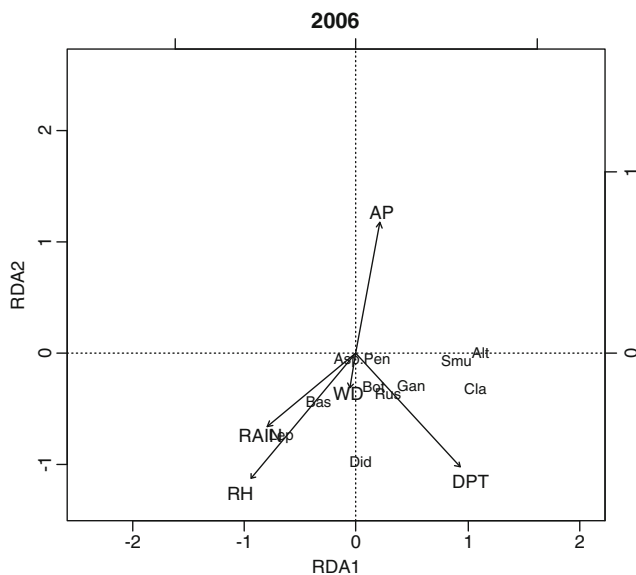


Fig. 1 Results of RDA analysis presenting airborne fungal spores and meteorological parameters from May to September 2006. *TMA* maximum temperature, *TMI* minimum temperature, *TME* mean temperature, *DPT* dew point temperature, *WD* wind direction, *RH* relative humidity, *RAIN* rainfall, *AP* air pressure, *Alt* *Alternaria* spp., *Asp/Pen* *Aspergillus* spp./*Penicillium* spp., *Bas* coloured basidiospores, *Bot* *Botrytis* spp., *Cla* *Cladosporium* spp., *Did* *Didymella* spp., *Gan* *Ganoderma* spp., *Lep* *Leptosphaeria* type, *Rus* rusts, *Smu* smuts

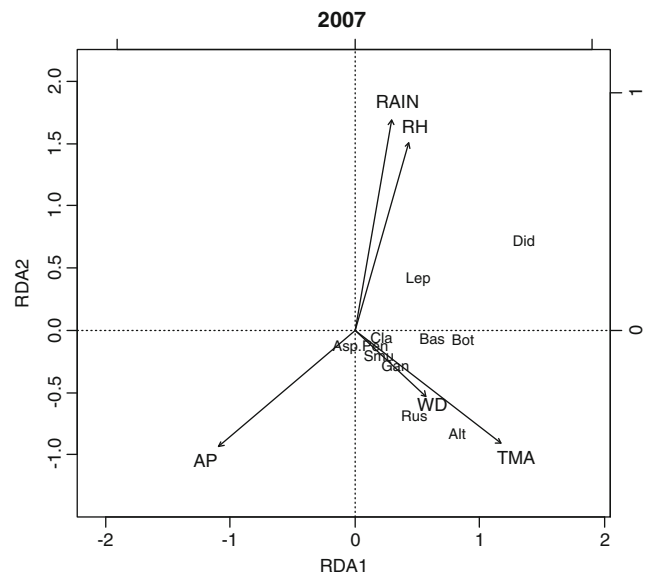


Fig. 2 Results of RDA analysis presenting airborne fungal spores and meteorological parameters from May to September 2007. *TMA* maximum temperature, *TMI* minimum temperature, *TME* mean temperature, *DPT* dew point temperature, *WD* wind direction, *RH* relative humidity, *RAIN* rainfall, *AP* air pressure, *Alt* *Alternaria* spp., *Asp/Pen* *Aspergillus* spp./*Penicillium* spp., *Bas* (coloured basidiospores), *Bot* *Botrytis* spp., *Cla* *Cladosporium* spp., *Did* *Didymella* spp., *Gan* *Ganoderma* spp., *Lep* *Leptosphaeria* type, *Rus* rusts, *Smu* smuts

ascospores were the only types of spores which were directly proportionally correlated with rainfall and high relative humidity conditions and simultaneously inversely proportionally

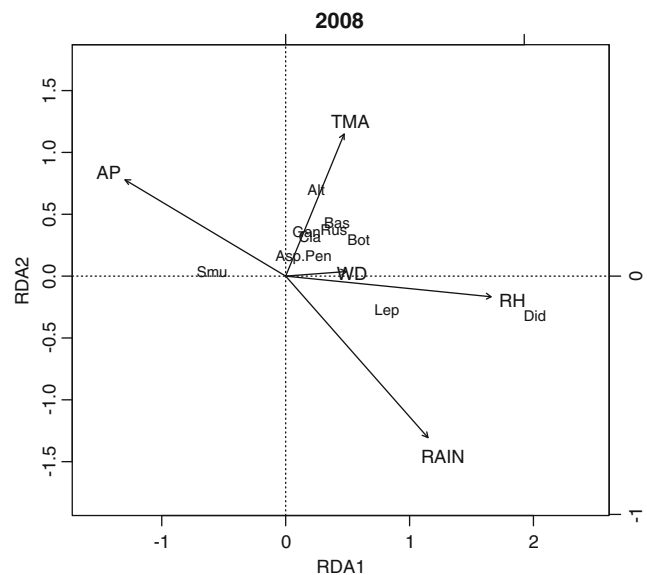


Fig. 3 Results of RDA analysis presenting airborne fungal spores and meteorological parameters from May to September 2008. *TMA* maximum temperature, *TMI* minimum temperature, *TME* mean temperature, *DPT* dew point temperature, *WD* wind direction, *RH* relative humidity, *RAIN* rainfall, *AP* air pressure, *Alt* *Alternaria* spp., *Asp/Pen* *Aspergillus* spp./*Penicillium* spp., *Bas* coloured basidiospores, *Bot* *Botrytis* spp., *Cla* *Cladosporium* spp., *Did* *Didymella* spp., *Gan* *Ganoderma* spp., *Lep* *Leptosphaeria* type, *Rus* rusts, *Smu* smuts

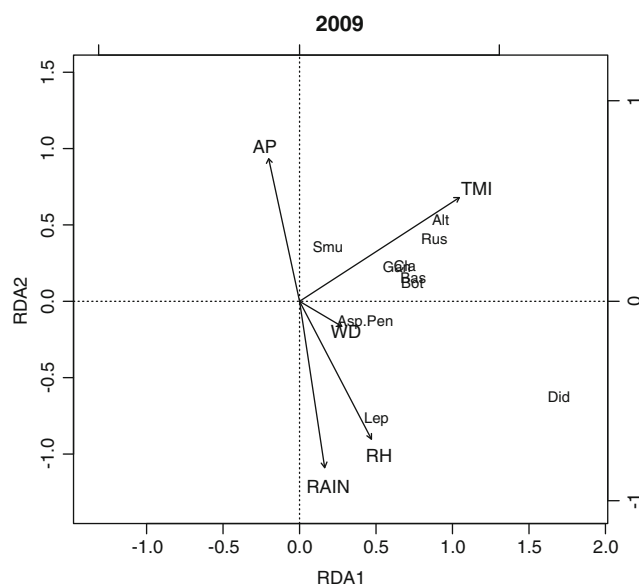


Fig. 4 Results of RDA analysis presenting airborne fungal spores and meteorological parameters from May to September 2009. TMA maximum temperature, TMI minimum temperature, TME mean temperature, DPT dew point temperature, WD wind direction, RH relative humidity, RAIN rainfall, AP air pressure, Alt *Alternaria* spp., Asp/Pen *Aspergillus* spp./*Penicillium* spp., Bas coloured basidiospores, Bot *Botrytis* spp., Cla *Cladosporium* spp., Did *Didymella* spp., Gan *Ganoderma* spp., Lep *Leptosphaeria* type, Rus rusts, Smu smuts

correlated with air pressure (Fig. 2). The concentration of *Aspergillus* spp./*Penicillium* spp. along with *Cladosporium* spp. was not affected entirely by changing weather conditions.

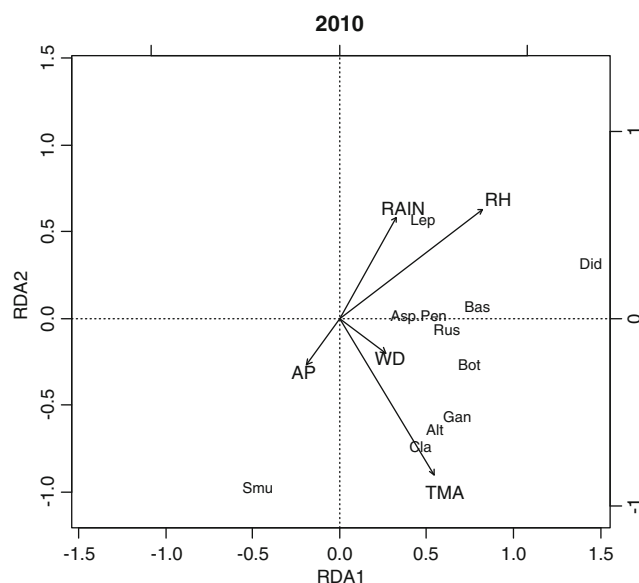


Fig. 5 Results of RDA analysis presenting airborne fungal spores and meteorological parameters from May to September 2010. TMA maximum temperature, TMI minimum temperature, TME mean temperature, DPT dew point temperature, WD wind direction, RH relative humidity, RAIN rainfall, AP air pressure, Alt *Alternaria* spp., Asp/Pen *Aspergillus* spp./*Penicillium* spp., Bas coloured basidiospores, Bot *Botrytis* spp., Cla *Cladosporium* spp., Did *Didymella* spp., Gan *Ganoderma* spp., Lep *Leptosphaeria* type, Rus rusts, Smu smuts

The relationship with wind direction was demonstrated for *Alternaria* spp., *Ganoderma* spp., smut and rust spores. Maximum temperature significantly influenced the abundance of *Alternaria* spp. spores, although some influence was also shown on *Didymella* spp.

Weather conditions in the following year 2008 were almost identical with a significant influence of air pressure and maximum temperature (Fig. 3). The impact of rainfall could be considered as an equally important factor as relative humidity for the distribution of *Leptosphaeria* type spores; however, *Didymella* spp. spores were affected by relative humidity instead. Smuts expressed a clear negative correlation to rainfall and positive correlation with air pressure. Other spore types showed some influence of the wind direction and maximum temperature. The latter parameter once more had a significant impact on *Alternaria* spp. spore distribution.

Year 2009 brought a reshuffle of the weather, and minimum temperature became a major factor in the nearly semi-annual analysis (Fig. 4). For the first time, it was possible to detect an influence of wind direction on the variations in the pattern of *Aspergillus* spp./*Penicillium* spp. spores. Other genera, such as coloured basidiospores and *Ganoderma* spp. with usually closely matching direction of the prevailing winds, remained under the influence of temperature and their concentration increased along with it. *Alternaria* spp. spore concentration was followed nearby by rust spores. Smuts confirmed their dependence on the air pressure, although less distinctively.

Finally, 2010 was characterized by the dominance of the maximum temperature and the higher importance of the wind direction (Fig. 5). This had an impact on the distribution of the fungal spores. The strongest, directly proportional correlation with maximum temperature revealed *Cladosporium* spp., *Ganoderma* spp. and *Alternaria* spp. spores. *Botrytis* spp. were also found to be affected by maximum temperature and partly by wind direction. *Aspergillus* spp./*Penicillium* spp. spores were also slightly affected by wind direction and relative humidity.

Discussion

Prevalence of *Cladosporium* spp. spores among other fungal genera has been reported repeatedly (e.g. Nikkels et al. 1996; Oliveira et al. 2009; Stepalska and Wołek 2005). Weather conditions occurring in England, reported in this study, are from a temperate climatic zone of Europe. Contribution of *Cladosporium* spp. spores to the total spore catch varied from year to year, finally achieving its maximum concentration in 2010 (~75 %), (Table 1). According to D'Amato and Spieksma (1995) *Cladosporium* spp. spores account for 80 % of all spores. This is in agreement with the observations obtained from the Worcester monitoring site. In the European mould

spore calendar, the second and third positions were occupied by basidiospores and ascospores, where concentrations varied between 5–30 % and 5–20 %, respectively (D'Amato and Spieksma 1995). This was in line with the outcome of the survey taken in Worcester, where coloured basidiospores constituted ~11–16 % (Table 1). However, the abundance of *Didymella* spp. spores was twice as high (Table 1) than the European average (D'Amato and Spieksma 1995). Further components of aeromycota at Worcester fell into the classification from 1995 (D'Amato and Spieksma), i.e. *Aspergillus* spp./*Penicillium* spp. spores, *Botrytis* spp. and smuts with their mean contribution equal to ~1, ~1 and ~4 %, respectively (Table 1). Overall, the composition of airborne fungal spores across the temperate zone, including England on the periphery of Northern Europe, was constant.

According to the RDA of Li and Kendrick (1995), *Leptosphaeria* type spores were positively correlated with relative humidity, which was confirmed by RDA from Worcester between 2007 and 2009 (Figs. 2, 3 and 4). This outcome was also in an agreement with Spearman's rank test results with respect to 2008, where maximum abundance of *Leptosphaeria* type spores was recorded (Table 2). However, the same tests indicated higher correlation values for rainfall in 2007, 2009 and 2010 (Table 2, Figs. 1 and 5). Hernández Trejo et al. (2012) and Oliveira et al. (2009) found relative humidity to be the most important factor affecting fluctuations of *Leptosphaeria* type spores in the air of Mérida and Amares, respectively. Multiple regression analysis performed by Grinn-Gofroń and Mika (2008) showed changeable dominance between rainfall and relative humidity in relation to *Leptosphaeria* type spore fluctuation in Szczecin, within a 3-year period of time.

Didymella spp. spores similar to *Leptosphaeria* type peaked in 2008 (Table 2), according to RDA for Worcester, and were mainly influenced by relative humidity between 2007 and 2010 (Figs. 2, 3, 4 and 5) and partly by dew point temperature in 2006 (Fig. 1). Spearman's rank test suggested that rainfall was important. However, in 2006 and 2008, relative humidity played a key role (Table 2). The importance of rainfall rather than relative humidity in relation to observed *Didymella* spp. concentration was also previously reported in Portugal at two sampling sites (Oliveira et al. 2009). Other studies indicated an influence of minimum temperature along with relative humidity using a Spearman's rank test and multiple regression analysis (Grinn-Gofroń and Mika 2008; Stępańska and Wołek 2005; Stępańska et al. 2012). Richardson (1996) using a simple linear regression found that nocturnal peak counts of *Didymella* spp. spores were associated with high humidity and not dependent on rain.

Coloured basidiospores of several fungal genera were jointly counted, i.e. *Agaricus* spp., *Agrocybe* spp., *Boletus* spp., *Bovista* spp., *Coprinus* spp. and *Scleroderma* spp. Spearman's rank test showed heterogeneous results

(Table 2). The most important factor seemed to be the impact of the dew point temperature ($r_s=0.64$), followed by maximum, minimum and mean temperatures ($r_s=0.60$) in 2009. Moreover, a positive yet of less important relationship with relative humidity was recorded ($r_s=0.45$ in 2006 and $r_s=0.40$ in 2008), (Table 2). Similar findings were obtained by Oliveira et al. (2009) for *Coprinus* spp. spores. RDA illustrated that coloured basidiospore occurrence was dependent on maximum and minimum temperatures (Figs. 2, 3 and 4), with a lesser impact of relative humidity in 2006 (Fig. 1) and 2010 (Fig. 5). Li and Kendrick (1995) observed a closer relationship between minimum temperature and concentration of basidiospores, whereas *Coprinus* spp. was more dependent on mean temperature using RDA.

In contrast to ascospores and coloured basidiospores, majority of the fungal spore taxa were very much dependent on the maximum temperature, i.e. *Alternaria* spp., *Cladosporium* spp., *Ganoderma* spp., smuts and rusts, with growth directly proportional to their abundance in the air (Table 2, Figs. 1, 2, 3, 4 and 5).

Li and Kendrick (1995) observed a correlation between the distribution of *Alternaria* spp. and maximum temperature, during the growing season from May to October; however, they have not included dew point temperature in their RDA. Stennet and Beggs (2004) found a positive correlation ($r_s=0.41$, $p<0.001$) between occurrence of *Alternaria* spp. spores in the air at Sydney and maximum dew point temperature, which in a rank of importance was preceded by mean and maximum temperature. This relationship was revealed to be even stronger, when a previous day value was examined ($r_s=0.42$, $p<0.001$), (Stennet and Beggs 2004). Nevertheless, dew point temperature is often being neglected in the environmental analysis of *Alternaria* spp. spore distribution pattern (e.g. Grinn-Gofroń and Rapiejko 2009; Recio et al. 2012). The impact of this parameter requires further statistical examination.

The genus *Cladosporium* spp. required different optimal weather conditions that would favour spore production, release and dispersal in comparison to other investigated fungal taxa as suggested by the results presented in Table 1. Although maximum concentration of *Cladosporium* spp. spores was found in 2010 (Table 1), this year was not unusual with respect to Spearman's rank test results (Table 2). Moreover, higher spore counts would be expected to be observed in the year 2006 or 2009, where the values of coefficients for maximum, minimum and mean temperatures varied between $r_s=0.70$ and $r_s=0.68$ and for dew point temperature $r_s=0.42$ and $r_s=0.64$ (Table 2). RDA showed that there was lack of influence of any investigated meteorological parameters. Multiple regression analysis performed by Stępańska and Wołek (2005) confirmed the impact of the maximum temperature as the only explanatory variable for the post-peak period; however, for the pre-peak period, it indicated a joint influence of sunshine, relative

humidity and rainfall. The same type of analysis conducted by Grinn-Gofroń and Rapiejko (2009) found mean temperature to be the main, if not the only explanatory, factor for the fluctuations of *Cladosporium* spp. spores in the air at three locations. It should be noted that maximum temperature was not included in that study; therefore, it could be assumed that actually, maximum temperature was the key factor there (Recio et al. 2012).

Dependence on the temperature in general by *Ganoderma* spp. was shown by both Spearman's rank test (Table 2) and RDA (Figs. 1, 2, 3, 4 and 5). The highest concentration of *Ganoderma* spp. spores was observed in 2009, where again the strongest correlations were with maximum, minimum and mean temperatures ($r_s=0.66$), as well as with dew point temperature ($r_s=0.69$), (Table 2). The same results were obtained by Li and Kendrick (1995) using RDA on the data collected in Waterloo, Canada and using Spearman's rank test and for the data sampled in Amares, Portugal by Oliveira et al. (2009) and in Szczecin, Poland by Grinn-Gofroń and Strzelczak (2011). Other weather parameters, although from time to time approached a statistical level of significance, exhibited minimal effect on *Ganoderma* spp. spore variations in the air of Worcester (Table 2). Different results were found in Kraków, where in the pre-peak period multiple regression analysis indicated minimum temperature, relative humidity and sunshine as major factors. In the post-peak period, minimum temperature and sunshine (Stępalska and Wolek 2005) and, at Porto, minimum temperature were detected to be the most significant explanatory variables for *Ganoderma* spp. spore observations using Spearman's rank test (Oliveira et al. 2009).

Ustilago spp. and *Tilletia* spp. spores were jointly counted under the grouping name of "smuts". In 2009, they comprised more than 4 % share in the total load of spores (Table 1). Spearman's rank test could not provide an explanation for the sudden increase in fungal spore concentration in that particular year, as stronger correlations with weather parameters, such as maximum, minimum and mean temperatures, were observed. Other authors found that wind speed and sunshine were the most important factors that influenced the concentration of airborne *Ustilago* spp. spores, i.e. $r_s=-0.58$ and $r_s=-0.36$ at the significance level of $p<0.01$ (Sabariego et al. 2000). The concentration of smut spores in the air of Worcester and investigated weather parameters either must have a complex character or there were other explanatory variables, which had not been included in this study.

Likewise to smuts, rust spore numbers were highest in 2009, although with twice lower input (Table 1). However, in this case, Spearman's rank test provided an explanation for the rise of rust spores in the air. The most significant rust spores were associated with higher temperatures (maximum, minimum, mean and dew point), which achieved the highest correlation in 2009 ($r_s=0.44$), (Table 2). A survey conducted in Portugal could only confirm ascendancy of temperatures

above other weather parameters. The Spearman's rank correlation scored lower than in Worcester (Oliveira et al. 2009). Attention should be also given to a positive influence of wind direction in 2007 ($r_s=0.46$) and negative impact of rainfall ($r_s=-0.43$), (Table 2) that were also seen in RDA (Fig. 2).

Botrytis spp. and *Aspergillus* spp./*Penicillium* spp. spores were the only fungal genera, where maximum concentration was found in 2007 (Table 1). High *Botrytis* spp. concentration could not be explained by Spearman's rank test (Table 2) nor RDA results (Fig. 2). The most significant correlations occurred in 2009 for maximum, minimum and mean temperatures and for dew point temperature and air pressure (Table 2). RDA confirmed these relationships for maximum temperature (Figs. 3 and 5), minimum temperature (Fig. 4) and dew point temperature (Fig. 1). Rodríguez-Rajo et al. (2010) examined similar set of variables, and dew point temperature was found to be the most important explanatory variable for *Botrytis* spp. presence (in 2004 $r_s=0.42$, $p<0.01$), whilst Stępalska and Wolek (2005) indicated minimum temperature, sunshine and relative humidity in the pre-peak period and minimum temperature and sunshine in the post-peak period as the only parameters that affected *Botrytis* spp. distribution in the air using multiple regression analysis.

The highest numbers of *Aspergillus* spp./*Penicillium* spp. spores were observed in 2007 (Table 1). No significant influence of meteorological factors could be detected in 2007 and 2006 using both tests (Table 2, Figs. 1 and 2). These observations were similar to the measurements taken from Porto and Amares in Portugal, where Spearman's rank test did not find any statistically significant correlation between *Aspergillus* spp./*Penicillium* spp. spore concentration and examined meteorological parameters (Oliveira et al. 2009). At Worcester, Spearman's rank test showed that in 2009, spore concentration was positively correlated with dew point temperature ($r_s=0.25$) and relative humidity ($r_s=0.17$) and negatively with air pressure ($r_s=-0.17$). Grinn-Gofroń (2011) also found dew point temperature to be the most closely related explanatory variable with an r_s coefficient value equal to 0.34 ($p<0.001$). Although these relationships were statistically significant, they could be hardly considered as important (Table 1), (Grinn-Gofroń 2011). Other studies detected a correlation between *Aspergillus* spp./*Penicillium* spp. with a maximum wind speed during the growing season from May to October (Li and Kendrick 1995), which was not included in this study.

The detection of colinearity of independent variables may sometimes cause difficulties. However, an application of the variance inflation factor in CCA indicated readily which explanatory variables were auto-correlated and needed to be excluded from further analyses. Reduction of investigated factors to absolute minimum resulted in avoidance of factors, whose implementation could lead to erroneous conclusion. Li

and Kendrick (1995) for instance included simultaneously daily maximum, minimum and mean temperatures into their analysis, which must end the interference of the environmental factors' influence.

Grouping fungal genera using a criterion of their response towards particular environmental factors has brought a lot of confusion, seeing that observed species-environment reactions were different to the expected in certain cases, like the results of Spearman's rank association test and significant spore count during specific years. Even the application of one form of statistical examination, like redundancy analysis, showed various responses of the same fungal genus in relation to one explanatory variable. In other situations, both RDA and Spearman's rank test could not provide a definitive clear answer to the following question: Which weather parameters influenced the presence of individual fungal genera spores in the air?

Although RDA provided supplementary information about biology of the studied fungi in comparison with the results of Spearman's rank test, it has also shown its own limitations. Fungal spore types that exposed correlation with wind direction could not be directly linked with location of their sources. Similarly, no threshold of any other tested factor could be established, like the optimum maximum temperature for the presence of *Alternaria* spp. spores in the air. A more detailed RDA could be however performed, if certain thresholds would be previously established.

Conclusion

Overall, the obtained information using RDA can help in understanding the complicated relationships between presence and abundance of fungal spores in the air and environmental factors that affected them. It would be much recommended to include RDA/CCA into statistical examination of aerobiological data as well as use it as a supplementary instrument in the selection of parameters that will be a foundation for forecasting models for allergenic fungal spores in the future.

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