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ORIGINAL PAPER

Atmospheric concentrations of *Alternaria*, *Cladosporium*, *Ganoderma* and *Didymella* spores monitored in Cork (Ireland) and Worcester (England) during the summer of 2010

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Abstract This study represents the first international intercomparison of fungal spore observations since 1990, focusing on atmospheric concentrations of *Alternaria*, *Cladosporium*, *Ganoderma* and *Didymella* spores. The campaigns were performed at sites located in Cork (Ireland) and Worcester (England) during summer 2010. Observations were made using Hirst-type volumetric spore traps and corresponding optical identification at the genus level by microscope. The measurements at both sites (including meteorological parameters) were compared and contrasted. The relationships between the fungal spore concentrations with selected meteorological parameters were investigated using statistical methods and multivariate regression trees (MRT). The results showed high correlations between the two sites with respect to daily variations. Statistically significant higher spore concentrations for *Alternaria*, *Cladosporium* and *Ganoderma* were monitored at the Worcester site. This result was most likely due to the differences in precipitation and local fungal spore sources at the two sites. *Alternaria* and *Cladosporium* reached their maxima a month earlier in Cork than in Worcester, and *Didymella* with *Ganoderma* peaked simultaneously with similar diurnal trends found for all the investigated spore types. MRT analysis helped to determine threshold values of the meteorological parameters that exerted most influence on the presence of spores: they were found to vary at the two sites. Our results suggest that the aeromycological profile is quite uniform over the British Isles, but a description of bioaerosols with respect to overall load and daily concentration can be quite diverse although the geographical difference between sites is relatively small. These variations in the concentrations therefore need to be explored at the national level.

Keywords Aerobiology · Fungal spores · Air monitoring · Meteorological parameters · Outdoor environment · Multivariate regression tree

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1 Introduction

Airborne concentrations of certain fungal spore species have been linked with detrimental effects on human health (e.g. Horner et al. 1995; Lacey 1997). For example, *Alternaria* and *Cladosporium* genera are considered to be the most allergenic taxa and together may constitute up to 93 % of the total fungal spores found in the air (Ataygul et al. 2007) and are associated with allergic respiratory disease, especially asthma in susceptible atopic patients (Horner et al. 1995; Ostro et al. 2001; Douwes et al. 2003). Similarly, *Ganoderma* has been the subject of many studies relevant to human sensitisation for both its cap and spore extracts (e.g. Tarlo et al. 1979). Thus, *Ganoderma* represents a potential source of allergens involved in asthma and rhinitis (Horner et al. 1995; Craig and Levetin 2000). Furthermore, *Ganoderma* is an important pathogen that attacks healthy forest ecosystems (e.g. Pegler and Young 1973). A further genus, *Didymella*, has been monitored in certain places due to its high abundance in the air and harmful properties. Such spores have been shown to induce allergenic response in sensitised individuals (Harries et al. 1985; Nasser and Pulimood 2009). These genera therefore form an important part of the overall bioaerosol load with the implications for ecosystems, crops and human health.

Given the significant effect of fungal spores, it is surprising how little work has been performed on their identification and measurement of their ambient airborne concentrations in Ireland. A few studies on pollen and spores have investigated atmospheric concentrations of selected particles, i.e. grass pollen, *Cladosporium*, basidiospores and other spores (where *Aspergillus*, *Penicillium* and *Botrytis* were counted jointly) (McDonald 1980; McDonald and O'Driscoll 1980). These investigations centred on airborne species were detected in Galway City in the late 1970s. At a similar period of time, collections of *Cladosporium* and basidiospores were made in Dublin with the results analysed much later, in 1990 (Stephen et al. 1990). A more recent study has focused on the use of culture techniques for sampling ambient fungal spore concentrations in Galway (O'Gorman and Fuller 2008). Since then, no further aerobiological surveys have been carried out in the Republic of Ireland. Hence, a large gap in the knowledge with regard to spore concentrations and types remains to be investigated. The work reported here is the first survey in the Cork region, indeed in Ireland, to cover four of the most important fungal spores and also the first study that explores the diurnal pattern of these species.

The UK, in contrast, has many established aerobiological monitoring stations, often organised in the British Aerobiological Federation (BAF). Therefore, far greater numbers of studies relevant to spore concentrations, their distributions and correlation with meteorological parameters have been performed (e.g. Harvey 1967; Corden and Millington 1994; Hollins et al. 2004). For example, monitoring campaigns for ambient *Cladosporium* concentrations and also the fungal genus, *Ganoderma*, have been carried out throughout the country (Sreeramulu 1963; Lewis et al. 2000; Hollins et al. 2004). Furthermore, related studies have highlighted the significance of *Alternaria*, in conjunction with other primary biological aerosol particles (PBAP), for the exacerbation of symptoms in asthma sufferers (Langenberg et al. 1977; Corden et al. 2003). However, very few studies have focused on the diurnal variation of the spores, although these variations can be very large and therefore relevant with respect to exposure.

The surveys presented here (Cork, Ireland and Worcester, England) are also important because they present a unique international comparison of spore levels, in two regions with similar climate and topography, and so are biogeographically similar.

Thus, we present here results from the two sampling sites in Ireland and England and contrast observed concentrations in order to explore both similarities and differences in the spatial load of bioaerosols. The selected taxa require contrary weather conditions for spore release, which can be assumed to have a direct effect on the observed concentrations. The spores have been grouped as "dry", *Alternaria* and *Cladosporium*, and "wet", *Didymella* (e.g. Elbert et al. 2007; Stępalska and Wołek 2009). In contrast, *Ganoderma* release is generally observed with increasing humidity but not in conjunction with rainfall (Breitenbach et al. 2002). Statistical approaches including the use of multivariate regression trees (MRT) and simple linear regression were used for the correlation between meteorological parameters and fungal spore concentrations of the four types investigated.

2 Materials and methods

2.1 Study sites

The study of airborne *Alternaria*, *Cladosporium*, *Didymella* and *Ganoderma* spores was carried out during a 3-month period between 1 May and 31 July 2010 using two 7-day volumetric spore samplers of the

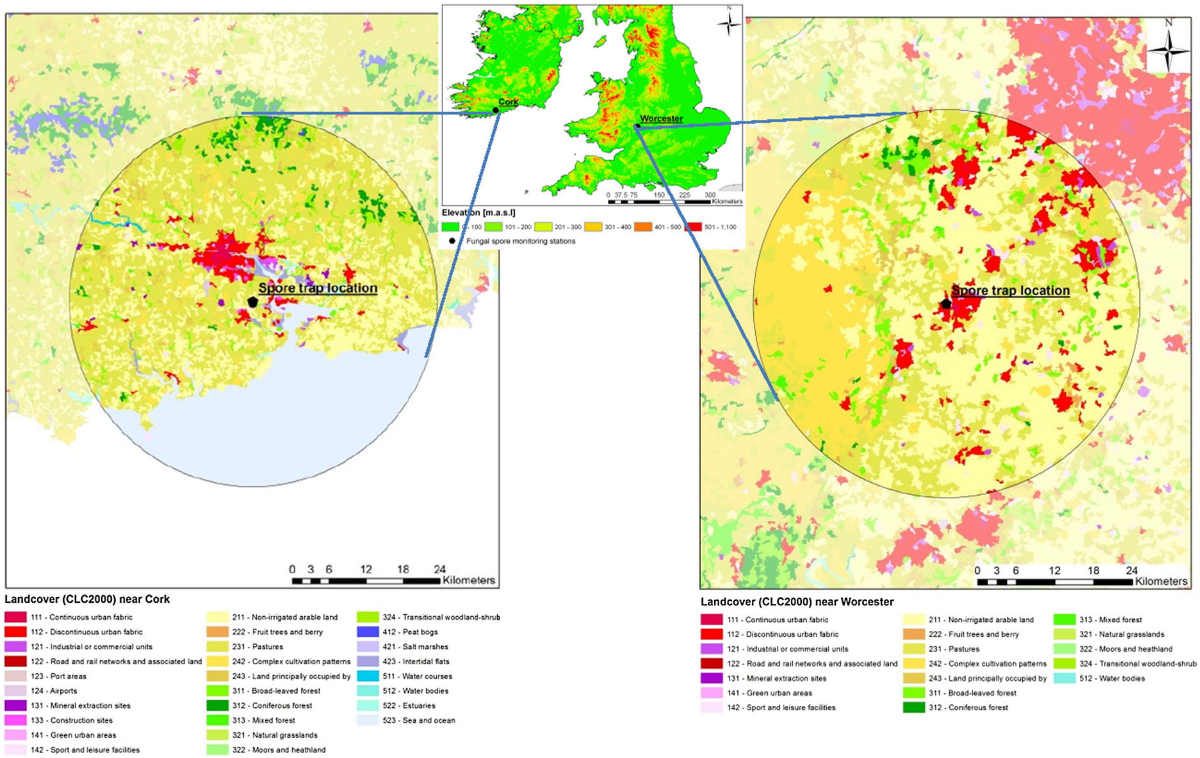

Fig. 1 *Small* site map including the location of the monitoring sites Cork and Worcester and an elevation map. *Enlarged* detailed site map including land cover and with the typical catchment area for a roof-top-based Hirst trap (30 km distance) highlighted. Numerical distribution of land cover is found in the associated Table S1

Hirst design (Hirst 1952). The Irish trap was placed at Raffeen Hill, a rural location situated outside Cork City ca. 86 m above sea level (51°50′25″N, 8°22′31″W). The UK sampler was installed on the roof of the University of Worcester building approximately 25 m above sea level (52°11′48″N, 2°14′31″W) (Fig. 1). This method of investigation in general can be assumed to capture the profile of pollen and spores and from sources within a 30 km distance (Skjøth et al. 2010). During the Cork investigation, power cuts twice interrupted sampling and no data were available for 6 days (i.e. 13–14 May and 6–10 June). On 31 July, the air sampler was moved from Raffeen Hill to Killarney National Park, which is located approximately 90 km west from Cork; therefore, no sampling was carried out on that day.

Cork City is situated in the south-east of Ireland and represents the second largest urban centre outside the capital city, Dublin, with a population of ~200,000 inhabitants (Fig. 1). Cork has a mild and changeable climate with an abundance of rainfall events experienced throughout the year. The annual mean temperature is measured to be 9.9 °C with a mean annual rainfall of 1,227.9 mm. Indeed, on average, 152 days of the year exhibit rainfall with 11 days of snow or sleet. South-westerly winds are generally prevalent although south-easterly winds are also often recorded. The site was situated 4 km from Cork Harbour. Within a 30 km distance, the dominating land covers are as follows: pastures (39.9 %), water (27.4 %) and agricultural land under rotation (18.7 %). The amount of forest areas only constitutes 4.5 % (Fig. 1 and Table S1).

Worcester is located in the West Midlands region of England (Fig. 1). Together with surrounding suburban regions, Worcestershire plays an important role in the horticulture and agriculture of the country. The city population is approaching 100,000 inhabitants, and population density is almost equal to 3,000 people per km^2 (Rice 2011). The climate of Worcester is characterised as “temperate maritime” exhibiting both mild winters and warm summers. The annual mean

Table 1 Monthly means for meteorological parameters at Cork and Worcester

	Cork, Ireland			Worcester, England		
Month	May	June	July	May	June	July
Temp. mean (°C)	11.15	15.28	15.50	12.12	16.34	17.66
Humidity (%)	77.62	79.41	83.94	70.17	70.92	74.63
Rainfall (mm)	41.20	49.20	132.80	51.40	80.00	47.00
Wind dir. (°)	182.33	170.68	182.53	202.67	205.68	227.57
Air press. (hPa)	1,010.43	1,009.35	1,004.98	1,017.43	1,017.11	1,014.12
Solar rad. (W/m^2)	181.48	207.24	159.78	198.29	230.67	174.69

temperature of the city is around 9.5 °C with an annual mean precipitation of approximately 669 mm and cloud coverage for 70 % of the year (Cavan et al. 2004). There are only ~17 days with snow cover. The south-westerly winds are the most frequent, as monitored at the closest meteorological station to Worcester, at Birmingham Airport (Elmdon) (Met Office 2012). Within a 30 km distance, the dominating land covers are as follows: agricultural land under rotation (40.7 %), pastures (23.9 %) and small parcels forming a complex agricultural landscape (19.3 %). The amount of forest areas only constitutes 7.4 % as shown in Fig. 1 and Table S1.

2.2 Spore identification and daily counts

Microscope slides from the Hirst-type samplers (Hirst 1952) were stained with basic fuchsine (Cork) or lactophenol cotton blue (Worcester), and the samples were examined under 400× magnification in accordance with the British Aerobiology Federation regulations (Lacey and Allitt 1995). Fungal spores were counted along 12 horizontal transects (Cork) and 1 central longitudinal transect (Worcester). Both data sets were calculated in bi-hourly concentrations for each of the fungal spore types to enable comparison. Fungal spore concentrations are presented as the number of spores per cubic metre of air. Hence, 12.2 % of each slide at the Cork site and 3.9 % of each slide at the Worcester site were counted. Of course, it should be noted that the greater areas of the slide that were counted in Cork than in Worcester would suggest higher precision at the former site. Indeed, the whole area of each slide would need to be counted to ensure completely accurate results, and so the potential for inaccuracies in both data sets remains.

2.3 Meteorological data

The meteorological data were provided by weather stations co-located with the spore traps. At the Cork site, a Casella Nomad Weather Station (CASELLA CEL, Regent House, Wolseley Road, Kempston, Brentford) was used, and at Worcester, a Weather Link (v.9.0) Vantage Pro2 (Davis, Davis Instruments, Hayward, California, United States) was operated. Six of the most common meteorological factors were investigated, i.e. air temperature, air pressure, relative humidity, rainfall, solar radiation and wind direction. A summary of the monthly means of weather variables for both study sites is presented in Table 1.

2.4 Statistical analyses

Statistical analyses were performed using Microsoft Excel (2010) and Statistica StatSoft (30-day trial version, v.10) programmes. The distribution of all the investigated spore types for both study sites was examined using Kolmogorov–Smirnov and Shapiro–Wilk tests for normality. The linearity of any relationships between spore and meteorological data was tested by plotting scatter graphs. Spearman's rank correlation was applied to measure the relationship between daily fungal spore counts and the various meteorological parameters.

In 2000, the use of MRT as a new method for analysing the relationship between environmental parameters and species was proposed (De'ath and Fabricius 2000). This method is commonly applied in ecological studies due to the lack of assumptions regarding relationships between examined variables. Subsequently, the approach has been successfully implemented in various aerobiological studies, e.g. for examining spore distributions of *Alternaria* (Grinn-Gofroń and Strzelczak 2009), *Cladosporium* (Grinn-Gofroń and Strzelczak 2009) and *Ganoderma* (Grinn-Gofroń and Strzelczak 2011; Kasprzyk et al. 2011) at different locations. Similar studies on *Didymella* have not yet been published.

Multivariate regression tree models were constructed with the aid of R (v.i386 2.15.3) software to detect threshold values above which spore concentration in the air of study sites significantly increased. For this purpose, the mvpart (Multivariate Partitioning) package was used (De'ath 2002). Two thousand multiple cross-validations were applied to stabilise the cross-validated relative error (CVRE). The final MRT models were selected based on the lowest obtained CVRE, following De'ath and Fabricius (2000), Grinn-Gofroń and Strzelczak (2009, 2011). The overall results obtained for Cork and Worcester were analysed using *U* Mann–Whitney nonparametric test according to the published procedures (Trigo del Mar et al. 2000).

3 Results and discussion

3.1 Seasonal trends, correlations and concentration peaks in relation to meteorological parameters

The sums of the daily mean spore concentrations were higher at the Worcester site, in comparison with Cork, for the whole period under investigation (Table 2). Out of the four spore types evaluated, *Cladosporium* was the most prevalent at both sites (Table 2). Average *Cladosporium* levels at Worcester (5,837 spores/m^3) were about four times higher than the ambient concentrations in Cork (1,516 spores/m^3). Similar findings were also noted with regard to both the *Didymella* and *Alternaria* concentrations (Table 2). Progressively increasing monthly concentrations of *Didymella* were monitored from May to July with peak levels detected during July at both sites (Table 2). The trend was also seen for the *Alternaria*, *Ganoderma* and *Cladosporium* collections in Worcester, where again the highest average concentrations were monitored in July 2010. These observations are not unexpected given that mean temperature increases clearly followed a similar pattern (Table 1). However, the trend was not followed at the Cork site, where the highest monthly spore concentrations for *Alternaria*, *Cladosporium* and *Ganoderma* were identified in June (Table 2). This finding was most likely due to the far higher levels of rainfall observed at the Cork site in the month of July by comparison with May and June (Table 1).

Spearman's rank correlation was used to investigate the relationships between spore concentration and meteorological factors (Table 3). Both mean temperature and rainfall were seen to have significant correlations with all fungal genera at Cork (Table 3), while negative correlations were observed for *Alternaria*, *Ganoderma* and *Cladosporium* with regard to rainfall. July rainfall data registered at a factor almost three times greater than the two previous months in Cork. Given that fungal spores such as *Alternaria* and *Cladosporium* are considered "dry" weather spores (Breitenbach et al. 2002; Lacey and West 2006), lower concentrations were to be expected. Indeed, 21 of the days in July displayed notable rainfall, and the concentrations of both these spores were low. Similarly, *Ganoderma*, while not released under the same meteorological conditions as *Alternaria* and *Cladosporium*, has also not been linked with release during, or directly after, rainfall (Breitenbach et al. 2002).

Table 2 Characteristics of campaign May–July 2010 at Cork (n = 84) and Worcester (n = 92)

	Cork, Ireland				Worcester, England			
Spore type	*Alt*	*Cla*	*Did*	*Gan*	*Alt*	*Cla*	*Did*	*Gan*
May (sum of daily means)	46	29,216	170	929	44	43,945	82	717
June (sum of daily means)	283	60,228	298	1,643	222	134,178	760	2,420
July (sum of daily means)	127	37,923	1,912	1,541	914	358,854	5,827	3,929
Daily mean (fungal spores/m^3)	5	1,516	28	49	16	5,837	91	78
Peak value (fungal spores/m^3)	39	11,397	175	163	83	46,831	911	218
Date of peak	21st Jun	29th Jun	20th Jul	25th Jul	29th Jul	14th Jul	22nd Jul	9th Jul

Table 3 Spearman's rank correlation coefficients between spore counts and meteorological parameters at Cork and Worcester

	Cork, Ireland				Worcester, England			
Spore type	*Alt*	*Cla*	*Did*	*Gan*	*Alt*	*Cla*	*Did*	*Gan*
Air temp. (°C)	**0.404**	**0.393**	**0.378**	**0.367**	**0.643**	**0.625**	0.168	**0.816**
Humidity (%)	−0.105	−0.016	**0.466**	−0.114	−0.005	0.120	**0.542**	−0.015
Rainfall (mm)	**−0.317**	**−0.245**	**0.513**	**−0.419**	−0.184	−0.063	**0.307**	−0.136
Wind dir. (°)	−0.140	−0.178	0.003	0.136	0.091	−0.004	0.008	0.159
Air press. (hPa)	0.027	−0.028	**−0.294**	0.185	0.057	−0.108	**−0.304**	0.066
Solar rad. (W/m^2)	0.211	**0.411**	**−0.234**	0.143	0.061	0.037	**−0.364**	0.087

Significance level: $p < 0.05$ (values in bold)

Hence, in a similar manner to *Alternaria*, the *Cladosporium* and *Ganoderma* concentrations decreased in July (Table 2). Interestingly, only *Didymella* monthly concentrations collected at Cork showed an analogous pattern to that seen at Worcester (Table 2). *Didymella* spores are known to correlate with high relative humidity (e.g. Richardson 1996; Stępalska et al. 2012), a property noted at both sites during this campaign (Table 3). Thus, it was not surprising to see peak concentrations in July at both sites (Table 2). However, it should be noted that given Cork is a coastal site, wind direction may also have an effect on fungal concentrations. The possibility also remains that rainfall in tandem with wind direction leads to the monitoring observations made at the site.

Alternaria and *Cladosporium* levels correlated significantly with mean temperature at both sites; this behaviour has been observed previously (Rodríguez-Rajo et al. 2005) (Table 3). Moreover, mean temperature was shown by the MRT analyses to be the major variable influencing *Alternaria* spore concentration for both the Irish and English sites with a threshold of 15 °C for Cork and 16 °C at Worcester (Fig. 2). Both these thresholds were found to be above the average daily temperatures measured during the campaigns at both Cork (13.9 °C) and Worcester (15.3 °C). When these conditions were fulfilled, maximum concentrations of *Alternaria* spores were observed. Similar results were obtained for *Cladosporium*, where the presence of spores was also dependent on the threshold value of mean temperature, which for both sites was equal to approximately 16 °C (Fig. 3). However, when *Cladosporium* levels peaked, the mean temperature oscillated around 17 °C. Previous MRT performed for a study in Szczecin have revealed contrary meteorological parameters influencing both the *Alternaria* and *Cladosporium* spores in the air (Grinn-Gofroń and Strzelczak 2009).

In the current campaigns, air pressure and relative humidity were found to be the most important factors for *Alternaria* release (1,011 hPa and 37 %, respectively). For *Cladosporium*, air pressure and time of day were important (1,008 hPa and 04:00 h, respectively). Of final note, light rain showers (0.02–0.90 mm) were observed to accompany the highest concentrations of *Cladosporium* spores detected for both Cork and Worcester. This contrasts with the results indicated by Spearman's association test, where a negative, statistically significant relationship between *Cladosporium* spores and rainfall was found for the Cork data (Table 3).

Interestingly, *Didymella* spores peaked at both study sites towards the end of July, with a difference of only 2 days (Table 2). This type of spore was shown to be significantly associated with the highest number of meteorological parameters in Worcester, and most importantly with rainfall and relative humidity (Table 3). The impact of relative humidity was directly shown by the MRT constructed for Worcester, which indicated that it was the major factor influencing the presence of *Didymella* with a threshold value equal to 77 % (Fig. 4). The MRT constructed for Cork confirmed this relationship indirectly, through the consideration of the air pressure. Hence, a threshold value of 990 hPa in Cork was apparent (Fig. 4). In meteorology, the range of air pressures between 995 and 1,005 hPa is considered as the border band between low- and high-pressure systems (Wallace and Hobbs 1977). Therefore, the Cork results indicate the presence of a low-pressure system above the campaign site, which is likely associated with falling air temperatures and increase in rainfall. Many researchers have

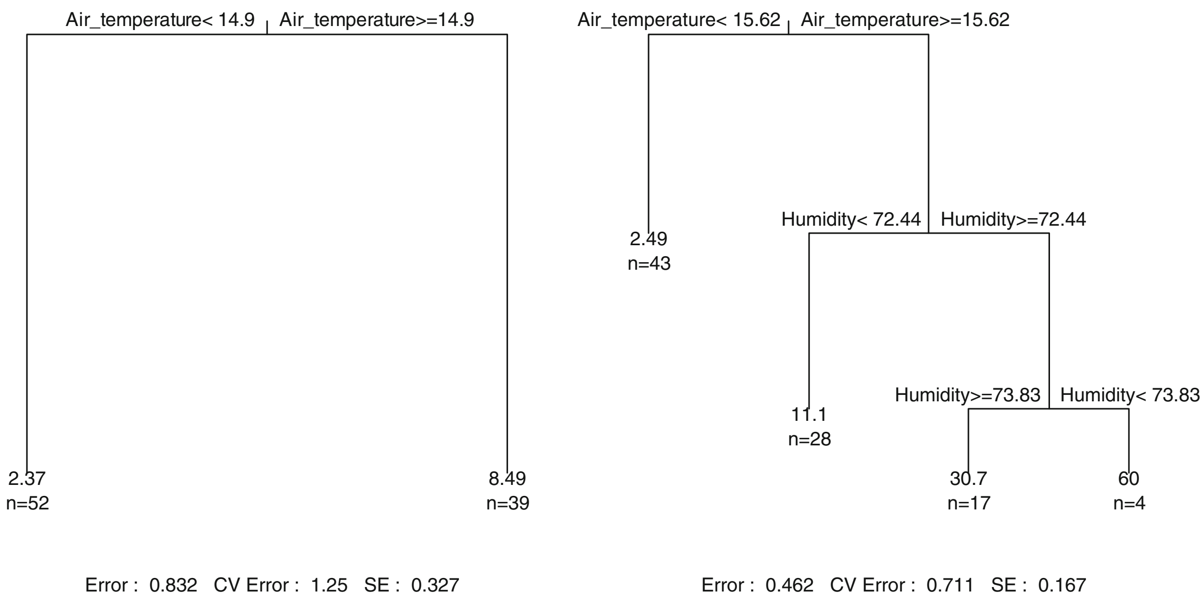

Fig. 2 Multivariate regression trees for *Alternaria* at Cork (*left*) and Worcester (*right*)

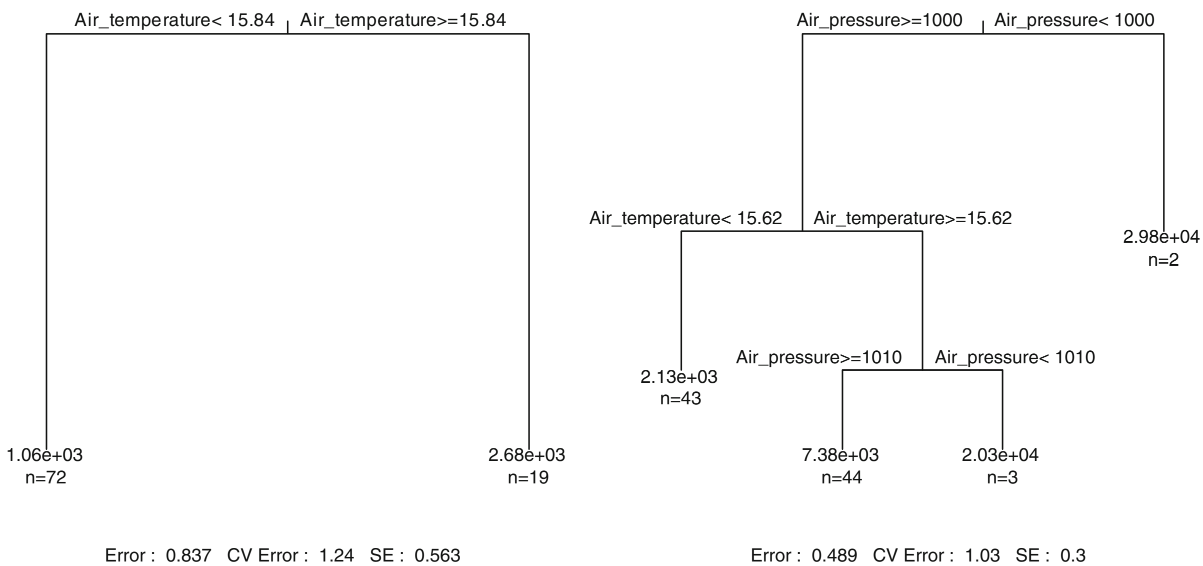

Fig. 3 Multivariate regression trees for *Cladosporium* at Cork (*left*) and Worcester (*right*)

suggested that the impact of relative humidity was more important than the direct influence of rainfall on *Didymella* spore concentration (e.g. Richardson 1996; Stępalska et al. 2012). This behaviour has been confirmed by the results presented here. Mean temperature was computed as the second significant factor affecting *Didymella* spore occurrence in Worcester: the threshold value being 18 °C (Fig. 4). However, slightly lower mean temperatures were, in fact, observed for Cork (15 °C) compared to Worcester (16 °C), at which points *Didymella* concentrations approached their maxima. Corden and Millington (1994) confirmed some previous findings of Packe and Ayres (1985) that a hot dry spell (20 °C) before rainfall had a significant impact on further outbreaks of *Didymella* spores. This suggestion would also be in

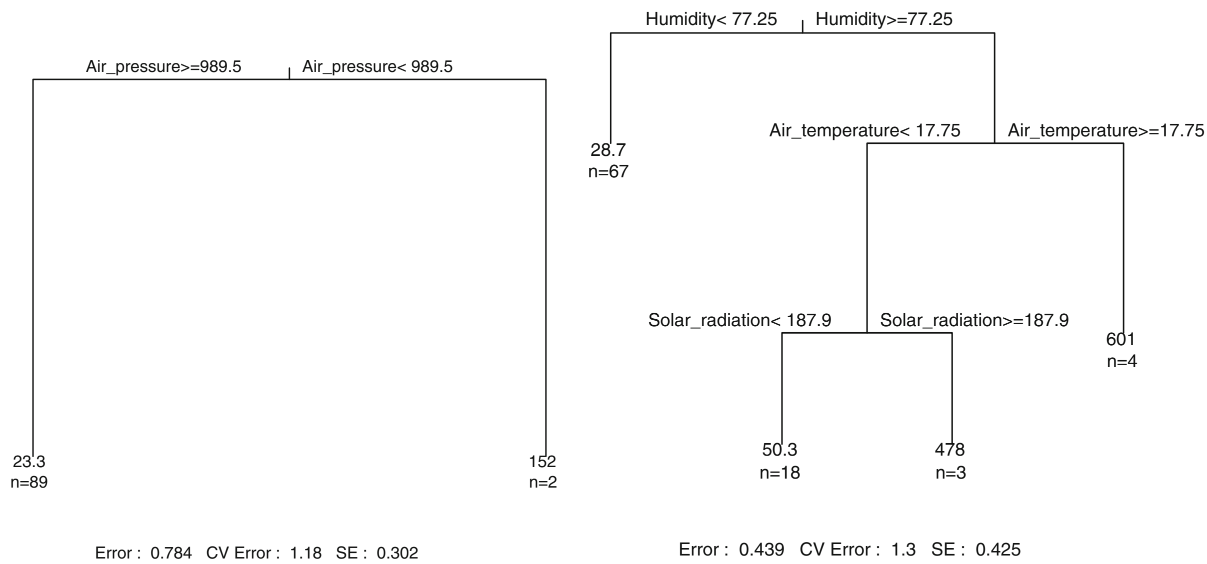

Fig. 4 Multivariate regression trees for *Didymella* at Cork (*left*) and Worcester (*right*)

agreement with the threshold value, equal to 18 °C, obtained by MRT analysis for Worcester (Fig. 4).

Ganoderma has been classified many times as a "wet" type of spore (e.g. Elbert et al. 2007; Stępalska and Wołek 2009). However, the results obtained in the international comparison campaign reported here did not confirm this status and is in agreement with an earlier reported finding (Stępalska and Wołek 2005). According to MRT analysis, the most important variable among the examined meteorological factors was found to be mean temperature, with values ranging between 17 °C (Worcester) and 18 °C (Cork) (Fig. 5). In fact, MRT computed for *Ganoderma* spores collected in Szczecin, Poland, have indicated that primarily, dew point temperature and secondary mean temperature with threshold values of 9 and 15 °C respectively are important (Grinn-Gofroń and Strzelczak 2011). These values are somewhat lower than those registered for Cork and Worcester in this study. The MRT obtained from a campaign in Rzeszów, Poland, suggest that only the time of day controlled capture by the air sampler with the highest spore counts being observed between 21:00 and 08:00 (Kasprzyk et al. 2011). Higher mean air temperatures were recorded during the *Ganoderma* peak periods detected in Cork (19 °C) and Worcester (20 °C), and this relationship was confirmed by Spearman's rank test (Table 3). In contrast, rainfall did not appear to have an influence on increased numbers of spores in the air (Table 3).

The *U* Mann–Whitney test indicated statistically significant differences ($p < 0.05$) in *Alternaria*, *Cladosporium* and *Ganoderma* spore concentrations measured at Cork and Worcester (Table 4). Median values for these spore types were found to be higher at Worcester. Furthermore, statistically significant differences were also obtained for all of the examined meteorological parameters, with the exception of solar radiation. Rainfall and relative humidity were significantly higher at Cork than Worcester, as confirmed by the *U* Mann–Whitney test (Table 4).

3.2 Diurnal trends

All spore genera under investigation exhibited definite diurnal trends. Peak concentrations of *Alternaria* and *Cladosporium* were measured during daytime hours and correlated with decreasing relative humidity and increasing temperature (Fig. 6a, b). Such a finding has been noted previously (e.g. Davies 1957; Harvey 1967; Langenberg et al. 1977; Vittal and Krishnamoorthi 1989; Peternel et al. 2004; Kasprzyk 2006; Stępalska and Wołek 2009, Skjøth et al. 2012). The highest concentrations of *Alternaria* were recorded at 16:00 for both sites with *Cladosporium* peaks at 14:00 for Cork and two hours earlier at Worcester (Fig. 6a, b). The opposite behaviour was apparent regarding monitored *Ganoderma* and *Didymella* levels. Both of these genera were predominantly sampled during

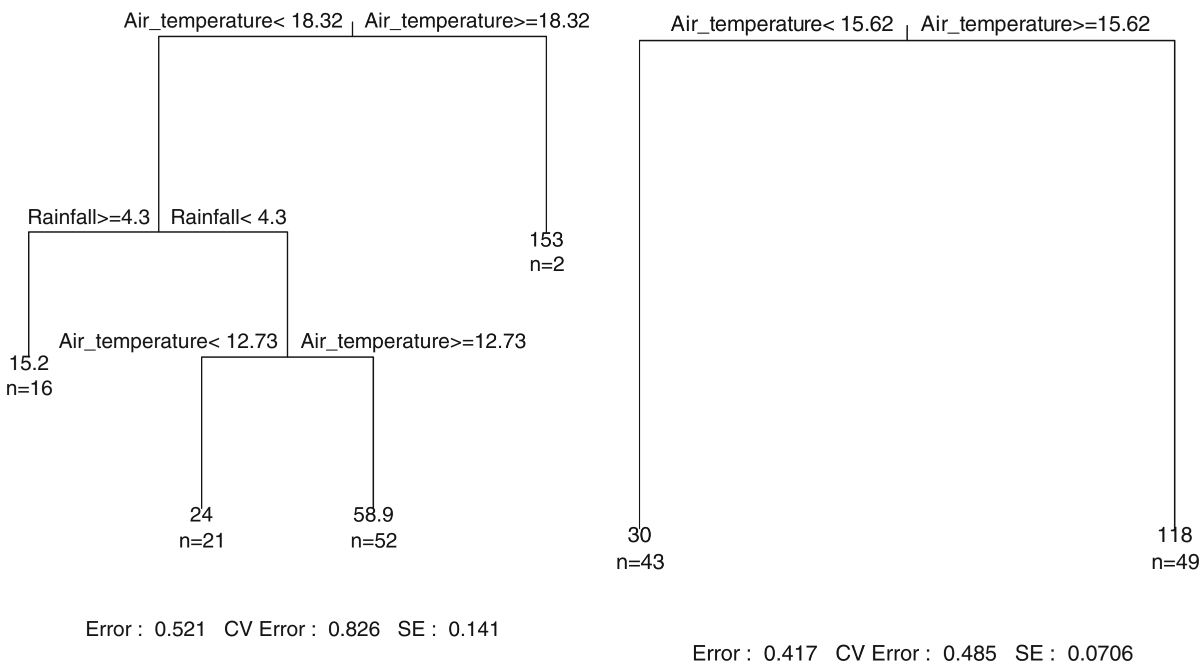

Fig. 5 Multivariate regression trees for *Ganoderma* at Cork (*left*) and Worcester (*right*)

Table 4 *U* Mann–Whitney test for *Alternaria*, *Cladosporium*, *Didymella* and *Ganoderma* spores and meteorological parameters between Cork and Worcester

Variable	Median Cork	Median Worcester	*U*	*Z*	*p*
Alternaria (s/m^3)	**2.22**	**5.00**	**3,050.00**	**−3.07**	**0.00**
Cladosporium (s/m^3)	**1,223.60**	**3,992.00**	**1,824.00**	**−6.52**	**0.00**
Didymella (s/m^3)	7.76	4.00	4,047.00	0.26	0.79
Ganoderma (s/m^3)	**36.58**	**64.00**	**2,876.50**	**−3.56**	**0.00**
Air temperature (°C)	**14.61**	**16.00**	**2,989.00**	**−3.24**	**0.00**
Humidity (%)	**80.07**	**70.69**	**1,881.00**	**6.36**	**0.00**
Rainfall (mm)	**0.20**	**0.00**	**3,385.50**	**2.12**	**0.03**
Wind direction (°)	**177.19**	**225.00**	**2,790.00**	**−3.80**	**0.00**
Air pressure (hPa)	**1,008.43**	**1,016.55**	**1,617.00**	**−7.10**	**0.00**
Solar radiation (W/m^2)	192.12	202.71	3,608.00	−1.50	0.13

Significance level: $p < 0.05$ (values in bold)

night-time hours where humidity increases and temperature drops (Fig. 6c, d). *Didymella* peaked at 04:00 at Cork and two hours later at Worcester (Fig. 6c). *Ganoderma* reached its maximum at 02:00 at Cork and 2 h later at Worcester (Fig. 6d). This observation is again similar to previous findings related to these spore types (e.g. Frankland and Gregory 1973; Lacey 1962; Sreeramulu 1963; Tarlo et al. 1979; Harries et al. 1985; von Wahl and Kersten 1991; Corden and Millington 1994; Richardson 1996; Kasprzyk 2006; Stępalska and Wołek 2009).

3.3 Wind direction analysis

Figures 7 and 8 show the percentage sampling frequency of the selected fungal spore in relation to the average monthly wind directions registered over the course of the campaign at each sampling site. The Cork

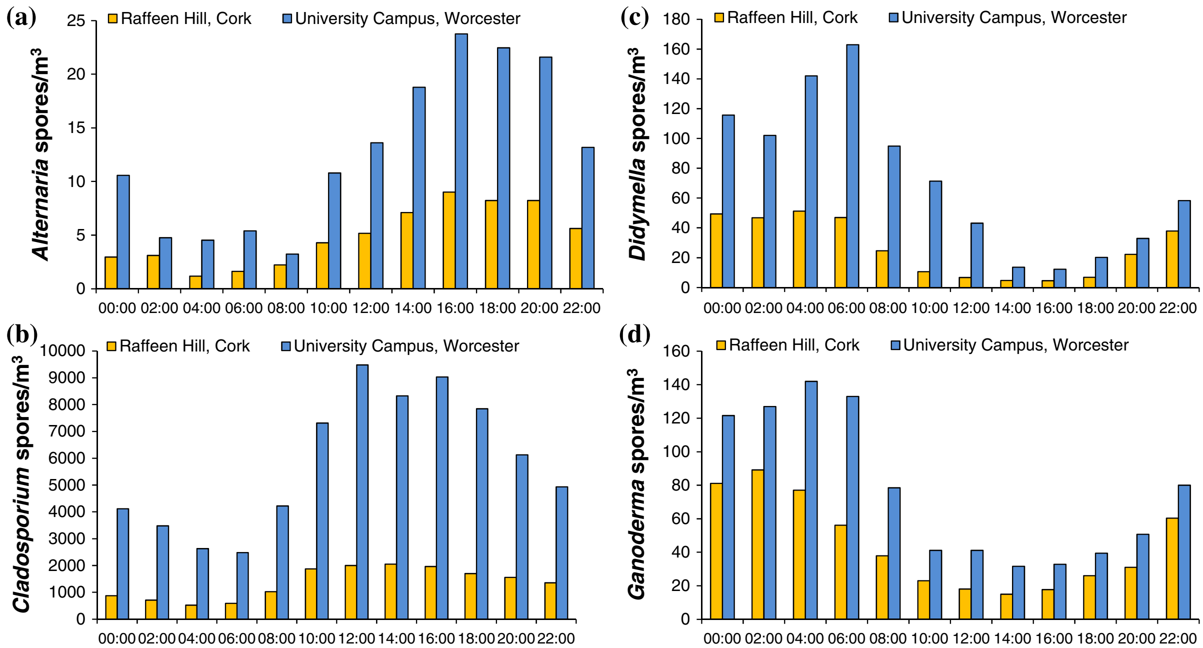

Fig. 6 Diurnal variations (bi-hourly observations) for *Alternaria* (**a**), *Cladosporium* (**b**), *Didymella* (**c**) and *Ganoderma* (**d**) at Cork and Worcester

location appears to be more consistent with winds that originate predominantly from the southern quadrants, in particular the south-east, for all months. Little wind was apparent from the other quadrants. July data showed a slight deviation in the wind profiles by comparison with the previous months, with a greater influence from south and south-westerly directions.

The Worcester site could attribute the majority of the observed winds to northern directions for the months of May and June, with little or no wind originating from other directions (Fig. 7). However, July showed a quite different wind direction profile with the bulk of the wind coming from the south-western quadrant.

Using the wind direction data collected throughout the campaign, it was possible to link the percentage sampling frequency of fungal spore types to the corresponding wind directions at the time of their sampling. Thus, directional profiles for the fungal spore types under investigation were constructed and are shown in Figs. 7 and 8. The bar scales incorporated into the graphs reflect the percentage sampling frequency of each direction. Scale maxima equal the radius percentage frequency with the scale progressions reflecting the percentages associated with the concentric circle eminating from the origin.

Figure 8 shows the wind directions for which each fungal spore type was sampled throughout the three-month campaign in Raffeen Hill, Cork. It can be concluded that *Alternaria* presented a strong association with the south-easterly direction. This observation is, of course, likely because this direction was seen to represent the most prominent wind bearing, as well as demonstrating the greatest wind speeds (data not shown). Given the relatively large size of *Alternaria*, this behaviour is also not unexpected as such conditions enable efficient transport of this spore type. For the months of May and June, a similar trend was seen for *Cladosporium* (Fig. 8). The largest percentage sampling frequency was most apparent from the south-eastern quadrant. However, there was a slight deviation during July with south/south-western directions also contributing significant quantities of the fungal spores. Both spore types were in general found to have higher concentrations at daytime hours under drier conditions, low humidity and greater wind speeds. Recently, it has been shown that periods of dry weather during the harvesting season can promote high concentrations of fungal spores from the mechanical release of spores through harvesting machines (Skjøth et al. 2012), where a considerable fraction of them can be transported several hundred kilometres away. This

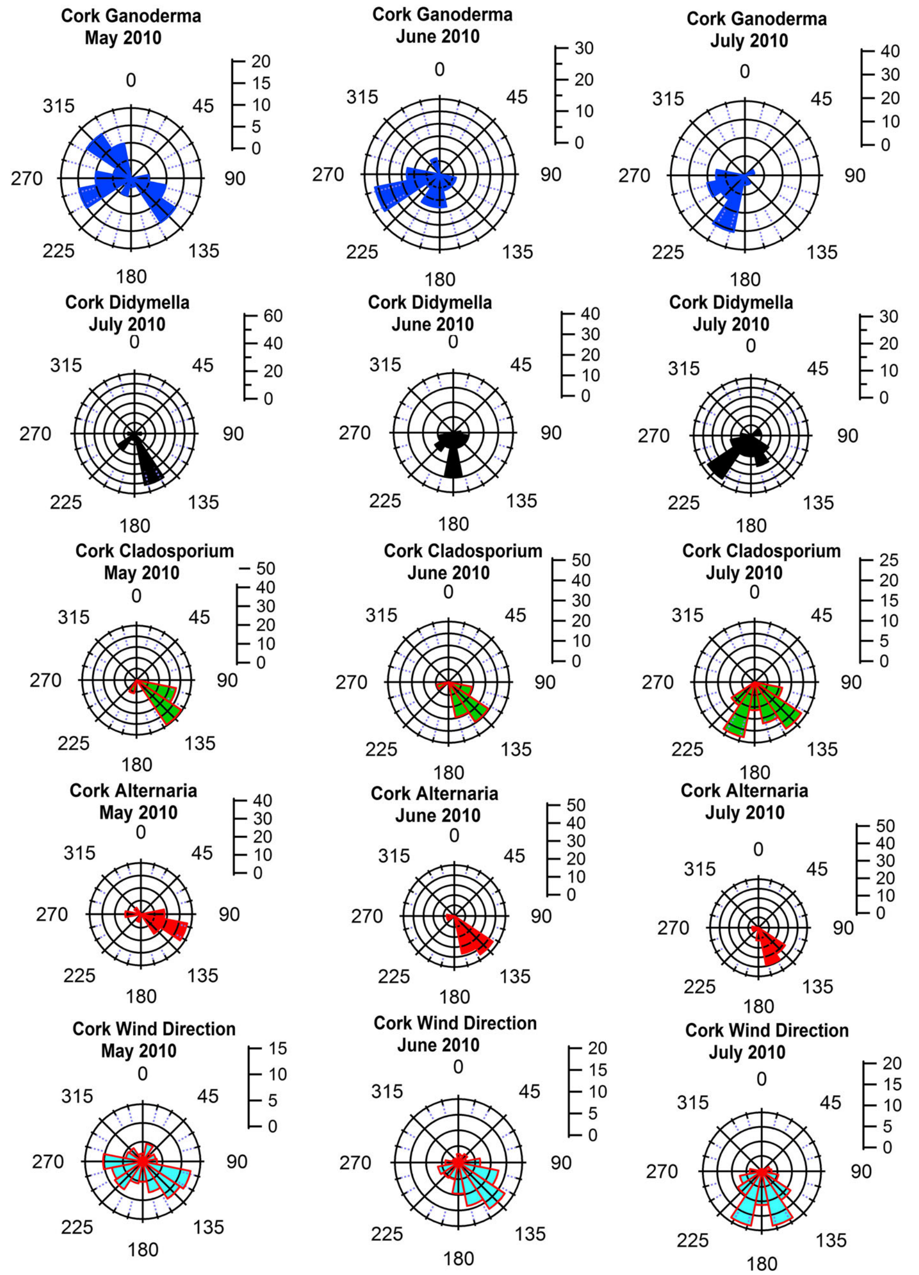

Fig. 7 Fungal spore directional profiles recorded at Worcester from May to July 2010. *Scale bars* represent percentage sampling frequency of fungal spores and wind direction

mechanism seems to be related to "dry" spores as harvesting in general happens during dry weather.

In contrast, both *Didymella* and *Ganoderma* appeared to display quite different directional profiles with southern, south-western and even north-western quadrants showing preponderance for these fungal spores over the duration of the campaign as shown in Fig. 8. These spores, *Ganoderma* and *Didymella*, for the Cork site showed different wind directional profiles for each of the months compared to the other two types (Fig. 8). This finding could be due to the active mechanisms by which *Ganoderma* (e.g. Cui 2009) and *Didymella* are released (e.g. Trapero-Casas et al. 1996). The results showed that both spore types were less related to wind on days with high concentrations, which supports the fact that other mechanisms than wind are the main drivers for release and initial dispersion.

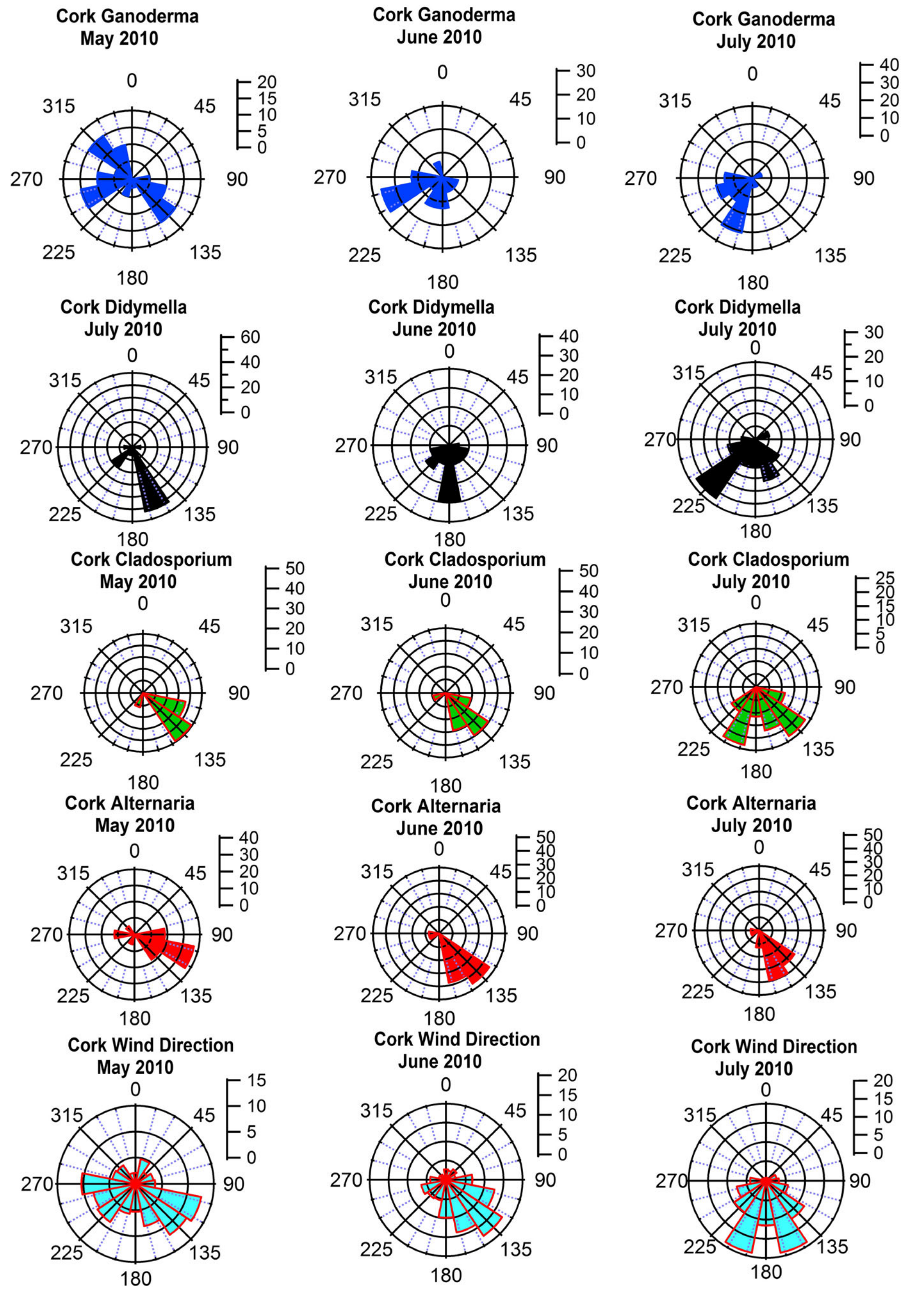

Fig. 8 Fungal spore directional profiles recorded at Cork from May to July 2010. *Scale bars* represent percentage sampling frequency of fungal spores and wind direction

At Worcester, the fungal spore types showed a far more diverse wind direction profile with fungal spores sampled in a number of different quadrants (Fig. 7). Wind was seen to originate primarily from northern directions in both May and June. This corresponded to the behaviour monitored for the fungal spore types with definite spikes in concentration monitored from this bearing. However, other directional bearings also saw significant frequent periods where fungal sores were sampled. Substantial amounts of fungal spores were also measured from eastern and south-western directions. This observation might be related to the fact that one of the dominating land covers near Worcester is small parcels forming a complex agricultural landscape. This means that the potential source areas are much more scattered around Worcester compared to Cork, which in contrast has a large fraction of sea area in the potential source regions. The overall load of large bioaerosols with a considerable deposition velocity is, as a first approximation, linearly related to the amount of sources within 30 km (Skjøth et al. 2010). As sea areas do not contain hosts for most bioaerosols (i.e. land-based vegetation or soil), including those identified from this study, then it must be expected that coastal sites in general will have lower concentrations. This finding agrees well with the previous studies on bioaerosols (e.g. Elbert et al. 2007; Urbano et al. 2011). However, it should be noted that the measured time series employed in the current study is considerably shorter than those used which provide measurement of an overall pollen or spore index. A better understanding of the measured concentrations could therefore be acquired through further investigations that focus on source-receptor studies and the use of atmospheric transport models.

Interestingly, *Didymella* was collected almost exclusively from the north-eastern quadrant in the month of June and suggests that the major source of this spore type is located in this direction (Fig. 7). A different distribution pattern was obtained in the month of July at the Worcester site. All spores showed significant concentrations emanating from south and south-western directions in greater proportions than noted for the previous months. Northern directions were seen again to be important for *Alternaria* and *Didymella* fungal spores.

4 Conclusions

- Due to the time-consuming nature of fungal spore concentration, determination and the inherent difficulties associated with their identifications, little work in the area has been attempted within an Irish context. Thus, for the first time, diurnal profiles have been established for four major fungal spore types related to allergy in Ireland. Meteorological parameters related to their recorded ambient concentrations also were determined statistically for the Irish climate in summer conditions.
- Spore concentrations at the Worcester site were found to be greater than in Cork. Out of the four genera evaluated, *Cladosporium* was seen in highest concentrations.
- Similar diurnal trends were apparent for all of the spore types, both at the Cork and Worcester monitoring stations. Maximum concentrations for *Alternaria* and *Cladosporium* were recorded in the afternoon (16:00 and 12:00–14:00); *Didymella* and *Ganoderma* were the most abundant genera in the air during night-time (04:00–06:00 and 02:00–04:00), respectively.
- There were some clear differences in spore levels detected in the two countries that are apparently time-dependent but also dependent on combinations of several meteorological parameters. Likely reasons for this include the fact that there are more abundant source areas near Worcester, a large difference in precipitation pattern and because Cork is influenced by coastal areas that can be considered a weak source for fungal spores.

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References

Ataygul, E., Celenk, S., Canitez, Y., Bicakci, A., Malyer, H., & Sapan, N. (2007). Allergenic fungal spore concentrations in the atmosphere of Bursa, Turkey. *Journal of Biological & Environmental Sciences, 1*(2), 73–79.

Breitenbach, M., Crameri, R., & Lehrer, S. B. (2002). *Fungal allergy and pathogenicity*. Basel: Krager.

Cavan, G., Alston, E., & Thornes, J. (2004). *Worcestershire climate change impact study, summary report*. Worcester: Worcester County Council.

Corden, J. M., & Millington, W. M. (1994). *Didymella* ascospores in Derby. *Grana, 33*(2), 104–107.

Corden, J. M., Millington, W. M., & Mullins, J. (2003). Long-term trends and regional variation in the aeroallergen *Alternaria* in Cardiff and Derby UK—Are differences in climate and cereal production having an effect? *Aerobiologia, 19*, 191–199.

Craig, R. L., & Levetin, E. (2000). Multi-year study of *Ganoderma* aerobiology. *Aerobiologia, 16*(1), 75–81.

Cui, Y. (2009). Adaptation of the ballitospore discharge mechanism among poroid *Agaricomycetes*. Master thesis, Oxford: Miami University.

Davies, R. R. (1957). A study of air-borne *Cladosporium*. *Transactions of the British Mycological Society, 40*(3), 409–414.

De'ath, G. (2002). Multivariate regression trees: A new technique for modeling species–environment relationship. *Ecology, 83*, 1105–1117.

De'ath, G., & Fabricius, K. E. (2000). Classification and regression trees: A powerful and yet simple technique for ecological data analysis. *Ecology, 81*, 3178–3192.

Douwes, J., Thorne, P., Pearce, N., & Heederik, D. (2003). Bioaerosol health effects and exposure assessment: Progress and prospects. *Annals of Occupational Hygiene, 47*(3), 187–200.

Elbert, W., Taylor, P. E., Andreae, M. O., & Pöschl, U. (2007). Contribution of fungi to primary biogenic aerosols in the atmosphere: Wet and dry discharged spores, carbohydrates, and inorganic ions. *Atmospheric Chemistry and Physics, 7*, 4569–4588.

Frankland, A. W., & Gregory, P. H. (1973). Allergenic and agricultural implications of airborne ascospore concentrations from a fungus, *Didymella exitialis*. *Nature, 245*, 336–337.

Grinn-Gofroń, A., & Strzelczak, A. (2009). Hourly predictive artificial neural network and multivariate regression tree models of *Alternaria* and *Cladosporium* spore concentrations in Szczecin. *International Journal of Biometeorology, 55*, 235–241.

Grinn-Gofroń, A., & Strzelczak, A. (2011). The effects of meteorological factors on the occurrence of *Ganoderma* sp. spores in the air. *International Journal of Biometeorology, 55*, 235–241.

Harries, M. G., Lacey, J., Tee, R. D., Cayley, G. R., & Newman Taylor, A. J. (1985). *Didymella exitialis* and late summer asthma. *The Lancet, 1*(8437), 1063–1066.

Harvey, R. (1967). Air-spora studies at Cardiff. I. *Cladosporium*. *Transactions of the British Mycological Society, 50*(3), 479–495.

Hirst, J. (1952). An automatic volumetric spore trap. *Annals of Applied Biology, 39*, 257–265.

Hollins, P. D., Kettlewell, P. S., Atkinson, M. D., Stephenson, D. B., Corden, J. M., Millington, W. M., et al. (2004). Relationships between airborne fungal spore concentration of *Cladosporium* and the summer climate at two sites in Britain. *International Journal of Biometeorology, 48*(3), 137–141.

Horner, W. E., Helbling, A., Salvaggio, J. E., & Lehrer, S. B. (1995). Fungal allergens. *Clinical Microbiology Reviews, 8*(2), 161–179.

Kasprzyk, I. (2006). Diurnal variations of fungal spores concentration in the town and rural area. *Acta Agrobotanica, 59*, 395–404.

Kasprzyk, I., Grinn-Gofroń, A., Strzelczak, A., & Wolski, T. (2011). Hourly predictive artificial neural network and multivariate regression trees models of *Ganoderma* spore concentrations in Rzeszów and Szczecin (Poland). *Science of the Total Environment, 409*, 949–956.

Lacey, M. E. (1962). The summer air-spora of two contrasting adjacent rural sites. *Journal of General Microbiology, 29*, 485–501.

Lacey, J. (1997). Fungi and Actinomycetes as allergens. In A. B. Kay (Ed.), *Allergy and allergic diseases* (pp. 858–886). Oxford: Blackwell Science.

Lacey, J., & Allitt, U. (1995). *Airborne pollen and spores, a guide to trapping and counting*. Harpenden: The British Aerobiology Federation.

Lacey, M. E., & West, J. (2006). *The air spora: A manual for catching and identifying airborne biological particles*. Dordrecht: Springer.

Langenberg, W. J., Sutton, J. C., & Gillespie, T. J. (1977). Relation to weather variables and periodicities of airborne spores of *Alternaria dauci*. *Phytopathology, 67*, 879–883.

Lewis, S. A., Corden, J. M., Forster, G. E., & Newlands, M. (2000). Combined effects of aerobiological pollutants, chemical pollutants and meteorological conditions on asthma admissions and A & E attendances in Derbyshire UK, 1993–96. *Clinical and Experimental Allergy, 30*(12), 1724–1732.

McDonald, M. S. (1980). Correlation of airborne grass pollen levels with meteorological data. *Grana, 19*(1), 53–56.

McDonald, M. S., & O'Driscoll, B. J. (1980). Aerobiological studies based in Galway. A comparison of pollen and spore counts over two seasons of widely different weather conditions. *Clinical Allergy, 10*, 211–215.

Met Office. (2012). Midlands: Climate. http://www.metoffice.gov.uk/climate/uk/mi/print.html. Accessed 21 May 2013.

Nasser, S. M., & Pulimood, T. B. (2009). Allergens and thunderstorm asthma. *Current allergy and asthma reports, 9*(5), 384–390.

O'Gorman, C. M., & Fuller, H. T. (2008). Prevalence of culturable airborne spores of selected allergenic and pathogenic fungi in outdoor air. *Atmospheric Environment, 42*(18), 4355–4368.

Ostro, B., Lipsett, M., Mann, J., Braxton-Owens, H., & White, M. (2001). Air pollution and exacerbation of asthma in African-American children in Los Angeles. *Epidemiology, 12*(2), 200–208.

Packe, G. E., & Ayres, J. G. (1985). Asthma outbreak during a thunderstorm. *The Lancet, 326*(8448), 199–203.

Pegler, D. N., & Young, T. W. K. (1973). Basidiospore form in the British species of *Ganoderma*. *Kew Bulletin, 28*, 351–364.

Peternel, R., Čulig, J., & Hrga, I. (2004). Atmospheric concentrations of *Cladosporium* spp. and *Alternaria* spp. spores in Zagreb (Croatia) and effects of some meteorological factors. *Annals of Agricultural and Environmental Medicine, 11*, 303–307.

Rice, M. (2011). *Worcestershire demographic report 2011 with South Worcestershire Appendix*. Worcester: Worcestershire County Council.

Richardson, M. J. (1996). The occurrence of airborne *Didymella* spores in Edinburgh. *Mycological Research, 100*, 213–216.

Rodríguez-Rajo, F. J., Iglesias, I., & Jato, V. (2005). Variation assessment of airborne *Alternaria* and *Cladosporium* spores at different bioclimatical conditions. *Mycological Research, 109*(4), 497–507.

Skjøth, C. A., Smith, M., Šikoparija, B., Stach, A., Myszkowska, D., Kasprzyk, I., et al. (2010). A method for producing airborne pollen source inventories: An example of Ambrosia (ragweed) on the Pannonian Plain. *Agricultural and Forest Meteorology, 150*, 1203–1210.

Skjøth, C. A., Sommer, J., Frederiksen, L., & Gosewinkel Karlson, U. (2012). Crop harvest in Denmark and Central Europe contributes to the local load of airborne *Alternaria* spore concentrations in Copenhagen. *Atmospheric Chemistry and Physics, 12*, 11107–11123.

Sreeramulu, T. (1963). Observations on the periodicity in the air-borne spores of *Ganoderma applanatum*. *Mycologia, 55*(4), 371–379.

Stępalska, D., Grinn-Gofroń, A., & Piotrowicz, K. (2012). Occurrence of *Didymella* ascospores in western and southern Poland in 2004–2006. *Aerobiologia, 28*(2), 153–159.

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

Stępalska, D., & Wołek, J. (2009). Intradiurnal periodicity of fungal spore concentrations (*Alternaria*, *Botrytis*, *Cladosporium*, *Didymella*, *Ganoderma*) in Cracow, Poland. *Aerobiologia, 25*, 333–340.

Stephen, E., Raftery, A. E., & Dowding, P. (1990). Forecasting spore concentrations: A time series approach. *International Journal of Biometeorology, 34*, 87–89.

Tarlo, S. M., Bell, B., Srinivasan, J., Dolovich, J., & Hargreave, F. E. (1979). Human sensitization to *Ganoderma* antigen. *Journal of allergy and Clinical Immunology, 64*, 43–49.

Trapero-Casas, A., Navas-Cortés, J. A., & Jiménez-Díaz, R. M. (1996). Airborne ascospores of *Didymella rabiei* as a major primary inoculum for Ascochyta blight epidemics in chikpea crops in southern Spain. *European Journal of Plant Pathology, 102*, 237–245.

Trigo del Mar, M., Toro, F. J., Recio, M., & Cabezudo, B. (2000). A statistical approach to comparing the results from different aerobiological stations. *Grana, 39*, 252–258.

Urbano, R., Palenik, B., Gaston, C. J., & Prather, K. A. (2011). Detection and phylogenetic analysis of coastal bioaerosols using culture dependent and independent techniques. *Biogeosciences, 8*, 301–309.

Vittal, B. P. R., & Krishnamoorthi, K. (1989). Circadian and seasonal periodicities of some mold allergens in the atmosphere of Madras city (India). *Proceedings: Plant Sciences, 99*(3), 247–251.

von Wahl, P.-G., & Kersten, W. (1991). *Fusarium* and *Didymella*—Neglected spores in the air. *Aerobiologia, 7*, 111–117.

Wallace, J. M., & Hobbs, P. V. (1977). *Atmospheric Science. An introductory survey*. London: Academic Press Limited.