Variation assessment of airborne *Alternaria* and *Cladosporium* spores at different bioclimatical conditions

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The study of mould spores is of major importance as many fungi can cause considerable economic losses worldwide acting as plant pathogens or triggering respiratory diseases and allergenic processes in humans. Knowledge of spore production relationships to different altitudes or weather patterns can be applied in a more efficient and reliable use of pesticides or improving diagnosis and treatment of respiratory allergic diseases. In this way monitoring of *Cladosporium cladosporioides*, *C. herbarum* and *Alternaria* spp. airborne spores during 2002 was carried out by means of three LANZONI VPPS 2000 pollen traps located in areas of north-west Spain at various altitudes and with various weather patterns.

High spore counts were recorded in the late summer and early autumn, with a fairly similar hourly spore-count pattern, increasing the concentrations in the late evening (7–10 p.m.). High spore concentrations were detected in inland rural areas in front of coastal ones. As the continentality index increased, *C. cladosporioides* spore concentrations rose and *Alternaria* declined. *C. herbarum* concentrations increased with increasing height above sea level. The weather factor displaying the strongest positive correlation with mean daily spore counts was temperature. The optimal conditions for high airborne spore concentrations were recorded at temperatures ranging from 23–29 °C and RH values of around 80%, followed rapidly by rainfall in Vigo and Ourense and preceded by heavy rain two days prior to recording peak values in Trives.

INTRODUCTION

Because of their adverse effects on crops and as allergens, aerobiological monitoring of mould spores is of major importance. Most fungal species act saprotrophically, colonising all stages of plant growth, or as plant pathogens, prompting considerable economic losses worldwide (Hjelmroos 1993, Infante et al. 1997a, b). Fungal spores can also have a serious detrimental effect on human health, triggering respiratory diseases and allergenic processes (O’Hollaren et al. 1991, Peat et al. 1995, Ricci et al. 1995, Mitakakis & McGee 2000).

Knowledge of plant pathogen spore trends and their relationship with weather variables can be used not only in the early detection of plant infections, thus allowing a more efficient and reliable use of pesticides (Montesinos et al. 1995, Bugiani et al. 1996, Diaz, Iglesias & Jato 1998), but also, combined with clinical data, in the prevention of respiratory allergic diseases, improving diagnosis and treatment (Munuera, Carrion & Navarro 2001). *Cladosporium* and *Alternaria* are the most cosmopolitan fungi involved, particularly in temperate regions, due to their almost permanent presence in outdoor and indoor air (Infante et al. 1997a, b, D’Amato 1981, Ricci et al. 1995). The cross-reactivity of these two allergenic genera may have implications for the treatment of fungal allergies (Jones & Gerson 1971). Numerous surveys of the occurrence of *Cladosporium* spores in different regions of the world show their dominance in comparison with other spores (Infante et al. 1997a, b, Mitakakis et al. 1997, Stepalka et al. 1999). Since *Cladosporium* spores greatly outnumber those of *Alternaria*, this cross-reactivity is of particular significance for *Alternaria*-sensitive patients. The onset of allergenic symptoms in atopic subjects occurs most frequently when the concentration of *Alternaria* spores is equal to or greater than 100 spores m⁻³ (Ballero et al. 1984, Ricci et al. 1995), while the threshold *Cladosporium* count for evoking allergenic symptoms is estimated at 3000 spores m⁻³ (Gravesen 1981). Fungal spore concentrations are governed by the nature and distance of the major sources, prevailing meteorological conditions and local topography (Angulo, Mediavilla & Dominguez 1999). Rural areas are of particular interest (Mitakakis & McGee 2000), because large amounts of conidial spores are thought to be released from crops,
and respiratory symptoms have been recorded coinciding with crop ripening, harvest and storage (Lacey 1971, Hill et al. 1984, O’Hollaren et al. 1991).

There are several previous reports based on the comparative study of airborne fungal spore concentrations between rural-urban or coastal-inland areas (Palmas & Cosentino 1990, Waisel et al. 1997, Mitakakis, Clift & McGee 2001, Corden, Millington & Mullins 2003). This study consider ecologically or geographically different areas, and also aims to chart variations in annual, seasonal and hourly distributions of *Cladosporium cladosporioides, Cladosporium herbarum* and *Alternaria* spp. airborne spores during 2002, in areas of the north-west Spain placed at various altitudes and with various weather patterns. The relationship between spore counts and weather variables such as humidity, temperature, wind speed, hours of sunlight and rainfall was also studied. Finally, the influence of altitude and a several bioclimatic indices on spore concentrations was assessed.

**MATERIAL AND METHODS**

The zones studied are located in the north-west Spain (Fig. 1). As Galicia’s relief and geographical location favour climatic and phytogeographic diversity, one urban city with maritime influence (Vigo), an inland urban city with continental influence (Ourense), and a rural area (Trives) were selected.

The city of Vigo has an ombrothermic (considering rainfall and temperature) dry and warm climate, with annual mean temperature of 14.9 °C, being the mean temperature of the maximum temperatures 18.8 °C and the mean temperature of the minimum temperatures 11 °C, and annual precipitation of 1412 mm. Ourense has an ombrothermic dry and warm climate, with an annual mean temperature of 14 °C, an average of minimum temperatures of 9.2 °C, an average of maximum temperatures of 18.9 °C and an annual total precipitation of 772 mm (Carballeira et al. 1983). Trives has an ombrothermic dry and cold climate, with an annual mean temperature of 8.9 °C, an average of minimum temperatures of 3 °C, an average of maximum temperatures of 14.8 °C and a total annual rainfall of 874 mm.

From a biogeographical point of view, Vigo is located in the Atlantic Province of the Eurosiberian region while Ourense and Trives are located in the Mediterranean region, Carpathian-Iberian-Lionese region (Rivas-Martínez 1987). Climax vegetation consists of *Quercus robur* deciduous forest in Vigo and Ourense, and *Quercus pyrenaica* and *Castanea sativa* in Trives, sometimes together with species such as *Arbutus unedo* or *Laurus nobilis*. Along the river channels, the dominant formations are alder groves with *Alnus glutinosa* accompanied in some areas by *Betula celtiberica* and *Salix atrocinerea*. As a result of human activity, the autochthonous forest has been drastically reduced and substitutive shrubs have spread, giving rise to widespread communities of *Leguminosae* (*Cytisus*
scoparius, C. striatus, Ulex spp., etc.) and Ericaceae. Overall in the western area, for the last forty years fast-growing species like Pinus pinaster, P. radiata and Eucalyptus globulus have been used for reforestation purposes (Rivas-Martínez 1987). The main crops are vines and forage species.

Monitoring was carried out by means three volumetric VPPS 2000 pollen-spore trap now manufactured by Lanzoni (Hirst 1952) situated on the roofs of the City Hall of Vigo, the Science Faculty of Ourense, and the Culture House of Trives. The samplers were placed 25–15 m above ground level. Sampling was carried out continuously from 1 January to 31 December 2002. The Lanzoni sampler is calibrated to handle a flow of 10 l of air per minute, thus matching the human breathing rate. Spores are impacted on a cylindrical drum covered by a melinex film coated with a 2% silicon solution as trapping surface. The drum was changed weekly and the exposed tape was cut into seven pieces, which were mounted on separate glass slides. Spore identification was performed using a Nikon Optiphot II microscope equipped with a 40X/0.95 lens. Spore counts were made using the model proposed by the R.E.A. (Spanish Aerobiology Network), consisting in four continuous longitudinal traverses along the 24 h slide (Dominguez et al. 1992).

Concentrations of the fungal type considered expressed as number of spores m$^{-3}$ of air.

In order to obtain a model reflecting the fluctuation of the spore concentration throughout the day, we followed the aerobiological model proposed by Galán et al. (1991), which selects the days on which the concentrations are greater than the mean of the main spore period and, within such, the days without rainfall. The resulting days were used to calculate the mean concentration every two hours, thereafter expressing the data as percentages.

Spearman’s correlation test was used to find a possible degree of association between daily mean airborne conidial number and the main meteorological factors: rainfall (mm), relative humidity (%), hours of sunshine (h), maximum, minimum and mean temperatures (°C), wind direction (%) and wind speed (m s$^{-1}$). Furthermore, we used the values of the previous day and the two days after. Weather data were supplied by the National Institute of Meteorology, from stations located within 100 m of the sampling points. Linear regression studies were developed, using as spore concentration estimators the meteorological variables with the highest positive correlation coefficients. Scheffé’s test was developed in order to found variance differences in the concentrations of each spore type considered from the three areas studied.

Finally some bioclimatic indexes proposed in agrometeorological studies modified by Rivas Martínez, Sánchez-Mata & Costa (1999) and based on temperature and precipitation (Table 1) have been applied in order to facilitate a better understanding of spore behaviour in air (Valencia-Barrera, Comtois & Fernández-González 2002).

### RESULTS

The highest spore counts were recorded in Ourense, with 357 433 spores belonging to the three types considered. Similar values were registered in Trives with an annual sum of 346 839 spores, while the total spore-count of the three types in Vigo was lower at 205 913 (Fig. 2). For each location, Cladosporium herbarum was the predominant spore type, accounting for 57–68% of the annual total spores considered, C. cladosporioides represented 30–44%, while those of Alternaria spp. never exceeded 1% of the annual spore count (Table 2).

<table>
<thead>
<tr>
<th>Table 1. Equations of the different Indices applied.</th>
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<tr>
<td><strong>Index</strong></td>
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<tr>
<td><strong>Continentality</strong></td>
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<tr>
<td><strong>Thermicity</strong></td>
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<td><strong>Ombrothermic</strong></td>
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**Fig. 2.** Total annual count of the three spore types considered.
On a seasonal basis, the highest *C. herbarum* counts recorded were in summer in all three locations, particularly from June to September, when around 80% of the total count was recorded. Peak levels were observed in August. Concentrations declined considerably in winter, and started to rise from May onwards (Fig. 3). The peak daily mean concentration (6472 spores m$^{-3}$) was recorded in Vigo on 18 August (Table 2).

The highest *Cladosporium cladosporioides* counts observed were at the end of summer in all three locations, particularly in September and October, when 60% of the annual spores counted were recorded. Peak values were found in September. As with *C. herbarum*, counts declined in winter, although they did not start to rise until August (Fig. 3). The peak daily mean concentration (6672 spores m$^{-3}$) was recorded in Ourense on 16 September (Table 2).

Finally, the highest *Alternaria* concentrations were found in July to September in the three study areas (Fig. 4), accounting for 65% of the annual total counted in Ourense and Trives (where an isolated peak was detected in May), and 73% in Vigo. Airborne *Alternaria* counts dropped in winter, and started to rise again in May. *Alternaria* concentrations were lower than those of the other spore types, with a peak daily mean count of 113 spores m$^{-3}$ on 17 May in the Trives area (Table 2).

The curves charting intradiurnal variations in spore concentration show a rising trend in the hours before noon, which was much more marked for *C. herbarum* and *Alternaria*; peak daily counts were recorded in the afternoon and early evening. The lowest levels were detected at dawn and in the early morning (Fig. 5).

An analysis was made of the correlation between spore count patterns and the main weather variables (Table 3). For all spore types and areas, significant positive correlations were obtained between spore counts and maximum, minimum and mean temperatures; a similar correlation was observed in most cases for wind calm. A highly-significant positive correlation was recorded between windspeed and spore counts for the two *Cladosporium* species, whereas a negative correlation was found for *Alternaria*. Finally, there was a highly-significant negative correlation with rainfall and relative humidity, although *Cladosporium cladosporioides* counts did not correlate negatively with relative humidity.

Scheffé’s test was carried out to ascertain whether the entire series of spore types for each city belong to a different population, or by the contrary the varied climatic and altitudinal conditions are the cause of the variation of concentrations and seasonality described (Table 4). Only in the case of *Alternaria* were differences found.

Linear regression analysis was performed to predict spore concentrations, using as independent variables weather parameters with a high correlation coefficient, such as temperature and humidity, and the previous day’s counts. All models accounted for a high percentage of the variability of the results, and significant results were found; the most important results are shown in the graph (Fig. 6). As the continentality index increased, *Cladosporium cladosporioides* spore concentrations rose and *Alternaria* declined (Fig. 6a). The remaining indexes appeared to bear no direct relationship to spore counts. Finally, the *C. herbarum* count increased with increasing height above sea level (Fig. 6b).

**DISCUSSION**

Parasitic or saprophytic species of *Alternaria* and *Cladosporium* have been shown to live on a large range
of plants (Hjelmroos 1993). The three areas studied displayed high spore counts because both genera are widespread in temperate zones (Ebner, Haselwandter & Frank 1989, Hjelmroos 1993, Ricci et al. 1995). Differences in biogeographical conditions and in altitude were related to differences in total spore counts, which were higher in Ourense and Trives. Scheffe’s test shows that only in the case of Alternaria, were variance differences between the three areas studied found, which could be caused by the low number of total spores registered. Populations of the rest of spore types may be considered as homogeneous. Some authors report a considerable decline in spore counts for temperate zones above 1000 m above sea level (Ebner et al. 1989). Below that height, total spore counts, and particularly those of C. herbarum, increased with altitude (Fig. 6b). This increase was strongly related to the rural character of the study areas and by the saprophytic nature of the fungus; for instance, a fourfold increase in spore counts has been reported in association with mown grass (Lacey 1971, Hill et al. 1984, O’Hollaren et al. 1991, Corden et al. 2003). Some authors (e.g. Friesen, De Wolf & Frank 2001) have found that wheat harvesting releases an immense number of fungal spores into the air, many of which are carried long distances in wind currents. Various studies also suggest that spore counts are lower and sensitisation less marked nearer the sea than inland (Peat et al. 1993, Corden et al. 2003). This pattern was clearly displayed by C. cladosporioides: as the continentality index (CI) increased (i.e. the effect of the sea decreased), spore concentrations rose (Fig. 6). The

![Graphs of spore concentrations and maximum temperatures for Ourense, Vigo, and Trives](image)

Fig. 3. Daily Cladosporium concentrations and maximum temperatures.
reverse was true for Alternaria: a comparative study of coastal and landlocked cities in England, covering many years, detected a falling trend in spore counts in coastal areas whereas inland the trend was upwards (Corden et al. 2003).

The genus Cladosporium is the main component of airborne mycobiota in various regions of the world, its predominance over other spores (Mitakakis et al. 1997, Infante et al. 1999a, Stepalska et al. 1999) being greater in more southerly locations (Infante et al. 1999a). Data for annual distribution showed that the C. herbarum type was dominant in summer while the C. cladosporioides type predominated at the start of autumn (Mediavilla et al. 1998). A considerably different annual distribution model is reported for Mediterranean cities, with two well-defined seasons of increased airborne spore concentrations (an additional peak in May/June) (Infante et al. 1999a). The summer dip in counts is thought to be due to temperatures of over 34 °C, that may have a direct effect on spore production or indirectly affect the substrates colonised (Munuera et al. 2001). In the present study, maximum concentrations were observed in September, when temperatures remain below the 34 °C threshold. The growth, sporulation and dispersal of Cladosporium spp. are evidently highly sensitive to changes in the weather. Various weather factors have been shown to favour sporulation, including high temperatures and relative humidity and rainfall prior to spore dissemination (Ebner et al. 1989, Herrero et al. 1996). A number of authors (Shaheen 1992, Herrero et al. 1996) report that the optimum temperature for development of these fungi is 25 °C.

![Figure 4. Daily Alternaria spp. concentrations and maximum temperatures.](image-url)
with a relative humidity (RH) of 50–60%. According to Hjelmroos (1993), daily rainfall in conjunction with daily mean temperatures of over 15 °C may prompt an increase in RH, contributing to higher Cladosporium counts. Here, maximum counts for both Cladosporium types were recorded at temperatures ranging from 23–29 °C and RH values of around 80%, followed rapidly by rainfall in Vigo and Ourense and preceded by heavy rain two days prior to recording peak values in Trives. There appeared to be a slight delay, attributable to spore transport from nearby cropland to the sampler. Several studies focusing on rural areas (Mitakakis et al. 2001) show that spore counts recorded in cropland on a given day correlate with those recorded in town centres one day later.

For Cladosporium, the threshold concentration for evoking allergy symptoms is estimated at 3000 spores m⁻³ air (Gravesen 1981). Spore counts beyond this level were recorded on 17, 13 and 9 days in Ourense, Trives, and Vigo, respectively.

Airborne Alternaria spores are the one of most widespread mould spores found in aerobiological surveys (D’amato 1981), and produce the highest number of positive reactions in patients undergoing allergy testing (Ricci et al. 1995, Tariq et al. 1996) in the late summer and early autumn. The total annual count is reportedly around 2000–2600 spores, concentrations increasing in more southerly, warmer, locations (Infante et al. 1999b). In the southern Spanish city of Córdoba an annual total of 33 000 spores was detected in 1997. Maximum daily mean levels of 63–113 Alternaria spores m⁻³ have been reported when maximum temperatures are in the range 27–30 °C. Hjelmroos (1993) observed optimal development at 22–28 °C, growth being rare at 0 °C. Several authors have reported bursts of Alternaria spores during periods of grass mowing, and during the wheat harvest outside towns (Mitakakis et al. 2001, Corden et al. 2003), especially when the weather has been warm and dry.

Frankland & Davies (1965) suggested a threshold level of 50 Alternaria spores m⁻³, although other researchers have taken the threshold as 100 or more spores m⁻³ (Gravesen 1981); here, these levels were recorded only on one day in Trives.

The three study areas displayed fairly similar hourly spore-count pattern, with an increase in concentrations in the late evening (19.00–22.00 h). Comparable findings are reported by several authors (Ricci et al. 1995, Angulo et al. 1999, Munuera et al. 2001), who have found the lowest counts in the early morning (about 8.00 h), increasing rapidly until 14.00 h, and thereafter more gradually to peak at around 19–20 h, declining from then onwards to the early morning minimum. Maximum concentrations for all three spore types in Vigo were recorded around four hours earlier. Munuera et al. (2001) suggest that the late-afternoon maximum may be linked to a real late-afternoon release of conidia from local sources or to a delay in the capture of spores released earlier and transported to the sampler from rural areas (15–30 km away). There is little cropland surrounding Vigo, so the spore source could be the city centre; thus, since spores are not transported from beyond a certain distance, maximum values were recorded. For that reason, correlations with windspeed were either negative or not significant, while a positive correlation was observed with wind calm. In Ourense and Trives, inland rural areas in which the sampler was surrounded by more cropland, nearby crops may have been the source of most spores; the distance travelled by spore clouds (Mitakakis et al. 1997) would account for the slight delay in capture.

The weather factor displaying the strongest positive correlation with mean daily spore counts was temperature. Similar findings are reported for various locations worldwide (Hjelmroos 1993, Mitakakis et al. 1997, Mediavilla et al. 1.998, Diaz 1999, Sabariego, Diaz de la Guardia & Alba 2000, Munuera et al. 2001). A highly-significant negative correlation was found for rainfall and relative humidity, both of which prompted a decline in airborne spore counts (Mitakakis et al. 1997). Although concentrations are thought to be
Wind speed 0.140** x x Wind SW-N 0.277*** 0.130** x x Wind S-SW 0.236*** – – x x

Maximum temp 0.286*** 0.195*** x x Maximum-1 0.203*** 0.562*** 0.366*** 0.588*** 0.581*** 0.622*** x x

Mean temp 0.200*** 0.473*** 0.238*** 0.418*** 0.424*** 0.514*** x x

Mean-1 0.201*** 0.599*** 0.398*** 0.647*** 0.585*** 0.675*** x x

Wind calm 0.270*** 0.523*** 0.285*** 0.586*** 0.454*** 0.583*** x x

Calm-1 – – 0.154*** 0.210*** – 0.284*** x x

Calm-2 – – 0.194*** 0.267*** – 0.324*** 0.141* x x

Wind N-NE 0.156*** x x Wind N-NE-1 – – 0.237*** – 0.325*** x x

Wind N-NE-2 – – – 0.282*** 0.327*** 0.145* x x

Wind N-NE-S 0.125*** x x Wind N-NE-1 – – 0.237*** – 0.255*** 0.161** x x

Wind N-NE-2 – – – 0.282*** 0.327*** 0.145* x x

Wind N-SW 0.236*** x x Wind N-NE-1 – – – 0.199*** – 0.259*** 0.112* – 0.196** – 0.136* x x

Wind N-NE-2 – – – 0.235*** 0.120* – 0.186** – x x

Wind N-SW-N 0.277*** 0.130*** – 0.243*** 0.181*** – x x

Wind N-NE-1 – – – 0.243*** 0.456*** 0.204** – 0.127** – 0.232** – x x

Wind N-NE-2 – – – 0.282*** 0.327*** 0.145* x x

Wind speed 0.140** – 0.139*** 0.114* 0.409*** – x x

Wind speed-1 0.124** – 0.305*** – 0.362*** 0.363*** – – 0.144** – 0.282** – 0.244** x x

Wind speed-2 – – 0.412*** – 0.414*** 0.354*** – 0.160* – 0.182** – 0.143* – 0.313*** – 0.293** x x

Table 3. Correlation Spearman coefficients (*p < 0.1, **p < 0.05%, ***p < 0.001) between spore concentrations and meteorological parameters. Accumulated values of one and two preceding days are considered.

Table 4. Scheffé coefficients (*p < 0.1, **p < 0.05%, ***p < 0.001) between spore concentrations and different localities.

![Image](image-url)

favoured by relatively strong winds (Hjelmroos 1993), positive correlations were mainly recorded with wind calm (Muniera et al. 2001). Only the two *Cladosporium* species displayed a positive correlation with wind-speed. However, the spore behaviour is a dynamic and complex phenomenon, and meteorological parameters alone do not reflect the global status of the atmosphere. It is difficult to separate out the individual effects of different meteorological parameters, since fungi react simultaneously to a combination of factors (Azcón-Bieto & Tálon 2000). Thus, the use of modified bioclimatic indexes instead of simple meteorological measurements could represent a major step forward (Valencia-Barrera et al. 2002), in that they take account of several weather-related parameters at once. Although the correlation analysis shows that temperature exerts a similar effect on the behaviour of all spore types considered, the Continentality Index provides us additional information, following a strong relationship with *C. cladosporioides* concentrations and an opposite trend with *Alternaria* spp. The areas with a higher thermal oscillation present with higher concentrations of *C. cladosporioides* and lower ones of *Alternaria* spp. This latter spore type requires a warm regime of temperatures to be released into the atmosphere. New studies with a higher number of years need to be conducted with the aim to reach better relationship with Ombrothermic and Thermicity bioclimatic Indexes.

Weather conditions may affect spore production directly, or indirectly, through their effect on the...
substrates colonised by the fungus. For that reason, this study also determined the correlation between spore counts for a given day and the main weather-parameter values for the previous two days. The strongest correlation was generally found with the temperatures of the previous day.

The optimal conditions for high airborne spore concentrations were wind calm associated with high temperatures on the previous day, favouring maturing and release of these spore types. Optimal temperatures appear not to favour spore liberation, their main effect being to prompt the washing-out of spores from the atmosphere. The single exception was *C. cladosporioides* in Ourense, where humidity may have affected airborne spore concentrations.

Finally, the regression analysis performed enabled the development of models to predict airborne concentrations showing the best estimators of data variability to be spore values and the previous days’ humidity and maximum temperatures. Table 5 shows variability to be spore values and the previous days’ parameters. Table 6. Values of the different Indices in the three locations.

<table>
<thead>
<tr>
<th>Indices</th>
<th>Vigo</th>
<th>Ourense</th>
<th>Trives</th>
</tr>
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<tbody>
<tr>
<td>Continenality</td>
<td>29.00</td>
<td>40.40</td>
<td>33.70</td>
</tr>
<tr>
<td>Thermicity</td>
<td>43.68</td>
<td>45.89</td>
<td>32.44</td>
</tr>
<tr>
<td>Ombrothermic</td>
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<td>1.57</td>
<td>3.88</td>
</tr>
<tr>
<td>Altitude</td>
<td>50</td>
<td>120</td>
<td>760</td>
</tr>
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</table>

**Fig. 6.** Comparison between: (a) *Cladosporium cladosporioides* total spores and, *Alternaria* total spores and Continentality Index; (b) relationship between *C. herbarum* total spores, number of total spores and altitude.

**CONCLUSIONS**

In north-west Spain, high spore counts were recorded in the late summer and early autumn. The whole spore season is more likely to be determined by the nature of the study areas. Similarly, spore counts were lower in more coastal areas than inland; as the continentality index rose, so did the total spore count, and particularly that of *Cladosporium cladosporioides*.

Fungal physiology, and therefore spore emission, are complex phenomena. The optimal conditions for high airborne spore concentrations we found were wind
calm associated with high temperatures over the previous days, that favoured the maturing and release of these three spore types. The bioclimatic index may help us to better understand spore behaviour, since fungi react to a combination of weather parameters, rather than to any individual parameter alone.

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