

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, Díaz, 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, Stepalska *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 & Domínguez 1999). Rural areas are of particular interest (Mitakakis & McGee 2000), because large amounts of conidial spores are thought to be released from crops,

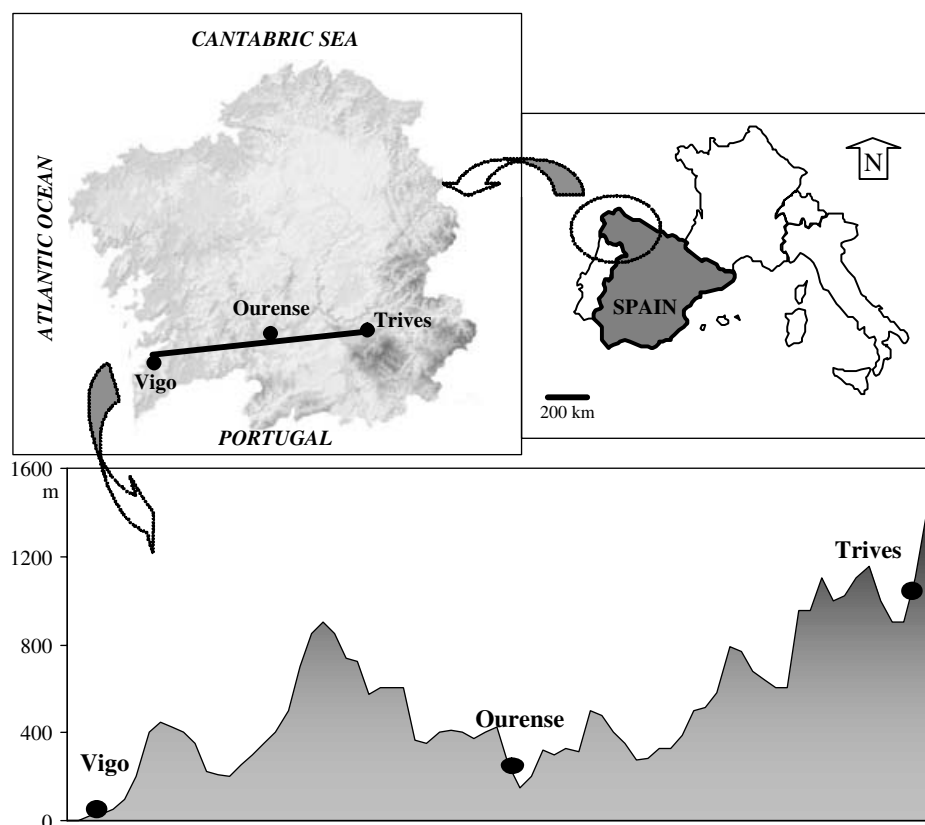


Fig. 1. Sampling locations in north-west Spain.

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 ° and the mean temperature of the minimum temperatures 11 °, and annual precipitation of 1412 mm. Ourense has an ombrothermic dry and warm climate, with an annual mean temperature of 14 °, an average of minimum temperatures of 9.2 °, an average of maximum temperatures of 18.9 ° 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 °, an average of minimum temperatures of 3 °, an average of maximum temperatures of 14.8 ° 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, Carpetan-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*

Table 1. Equations of the different Indices applied.

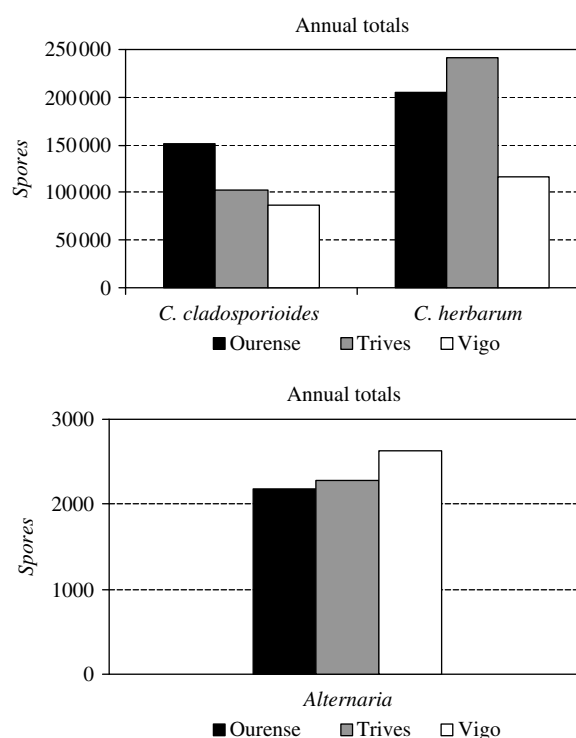
Index		
Continentality	$C.I. = T_{\max} - T_{\min}$	$T_{\max}$ and $T_{\min}$ expresses the maximum and minimum temperatures of the period considered
Thermicity	$T.I. = T + T_{\max} + T_{\min}$	T , $T_{\max}$ and $T_{\min}$ expresses the mean, maximum and minimum temperatures of the period considered respectively
Ombrothermic	$O.I. = 10 P_p T_p^{-1}$	P_p is the sum of the precipitation on days where the mean temperature is above 0°C and T_p is the sum of mean temperatures above 0° for the period considered

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 ($^{\circ}\text{C}$), 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

**Fig. 2.** Total annual count of the three spore types considered.

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 agro-meteorological 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 2. Number of total annual spores counted, Percentage over the total annual spore counted in each location, Maximum value registered (spores m⁻³) and day of the maximum value.

		<i>Cladosporium cladosporioides</i>	<i>Cladosporium herbarum</i>	<i>Alternaria</i> spp.	Total
Vigo	Total annual spores sum	87 148	116 129	2636	205 913
	Percentage	42 %	57 %	1 %	
	Maximum value	5678 spores m ⁻³	6472 spores m ⁻³	90 spores m ⁻³	
	Day	September 15	August 18	August 23	
Ourense	Total annual spores sum	150 518	204 726	2189	346 839
	Percentage	44 %	59 %	1 %	
	Maximum value	6672 spores m ⁻³	4551 spores m ⁻³	63 spores m ⁻³	
	Day	September 16	September 15	September 15	
Trives	Total annual spores sum	103 226	241 334	2279	357 433
	Percentage	30 %	68 %	1 %	
	Maximum value	4756 spores m ⁻³	5617 spores m ⁻³	113 spores m ⁻³	
	Day	September 12	August 13	May 17	

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 *C. 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 *C. 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 counts, ranging from 32–58% (Table 5). Only *Alternaria* in Trives displayed a low r² coefficient value.

Finally, in order to determine more precisely the behaviour of each spore type in different weather conditions and at different altitudes, we calculated the Continentality (CI), Thermicity (TI) and Ombrothermic (OI) Indexes for the three study areas (Table 6). Spore counts were contrasted with these indexes and with altitudinal data for the study areas involved. A correlation analysis was performed with the results obtained, but due to the small number of data no significant results were found; the most important results are shown in the graph (Fig. 6). As the continentality index increased, *C. 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

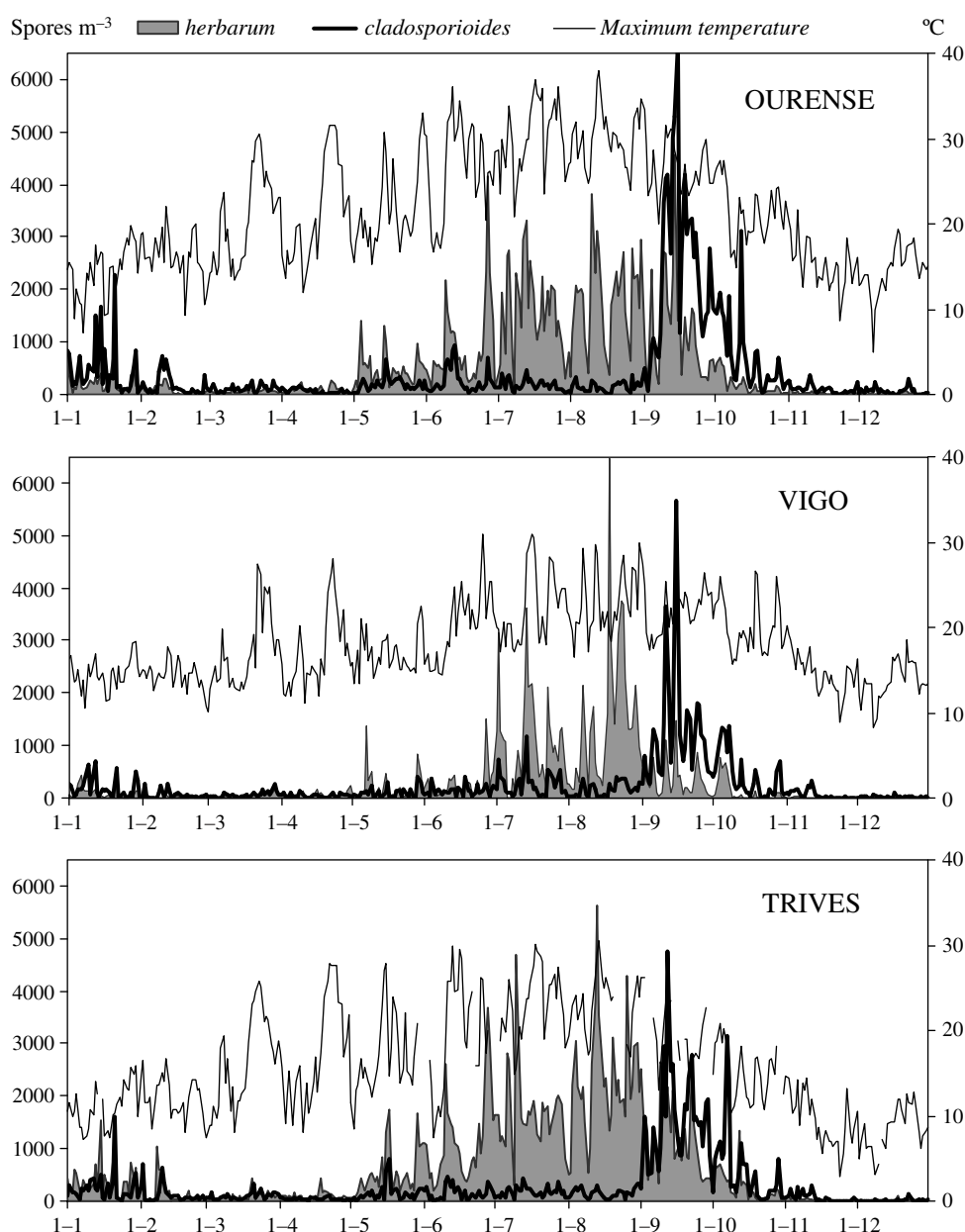


Fig. 3. Daily *Cladosporium* concentrations and maximum temperatures.

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. Scheffé'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

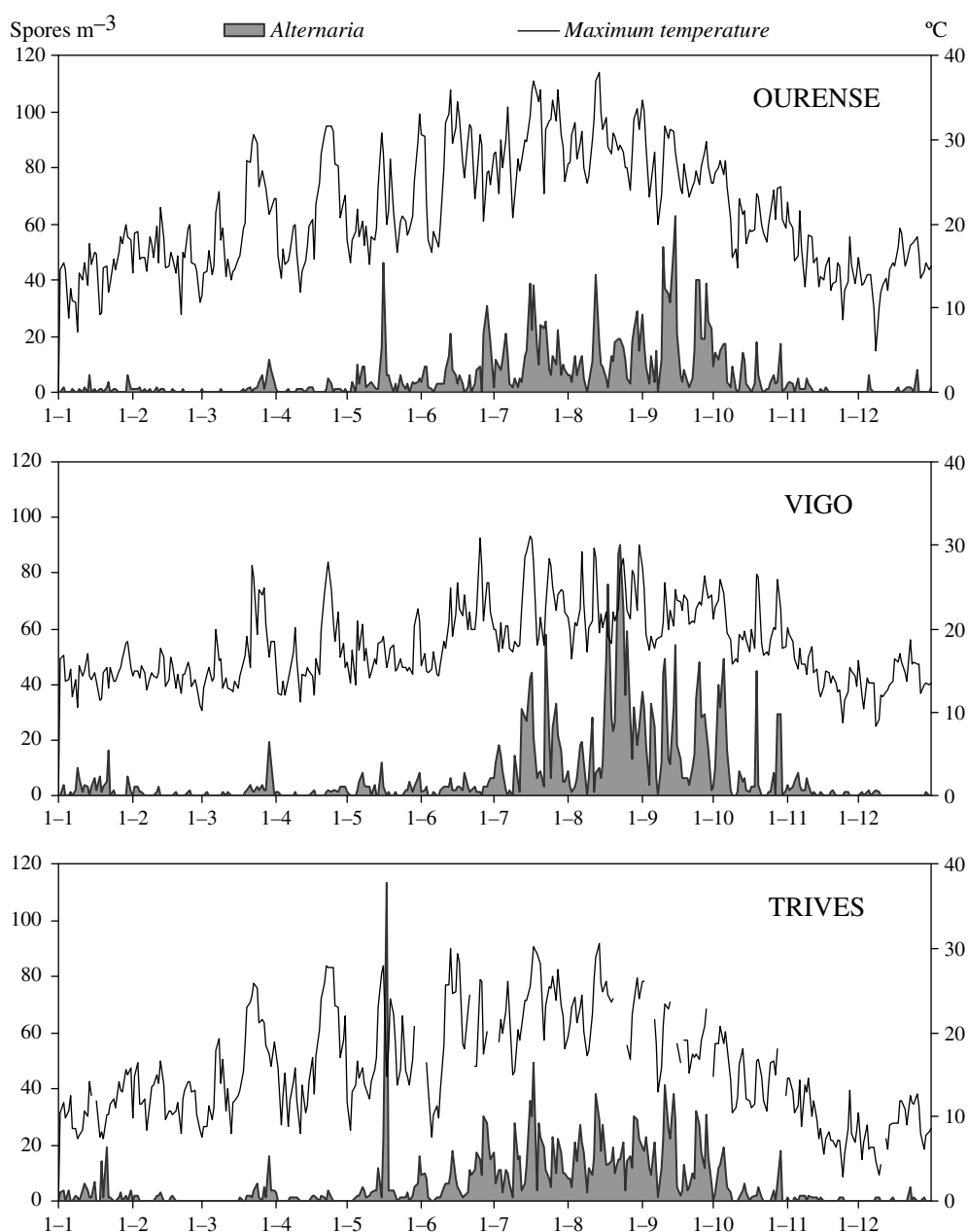


Fig. 4. Daily *Alternaria* spp. concentrations and maximum temperatures.

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 °, 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 ° 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 °

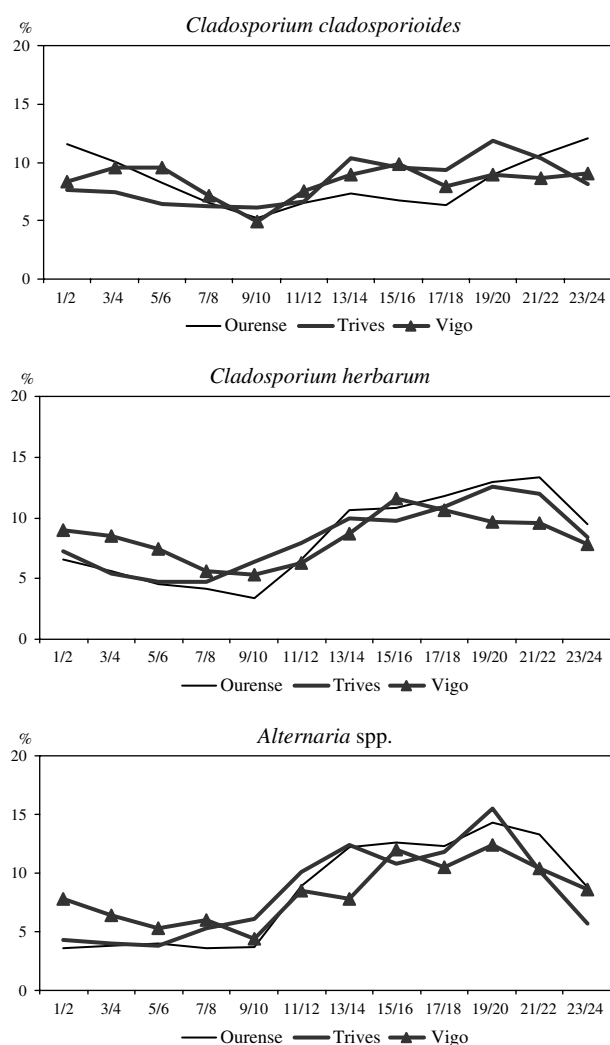


Fig. 5. Bi-hourly average spore concentrations.

with a relative humidity (RH) of 50–60%. According to Hjelmroos (1993), daily rainfall in conjunction with daily mean temperatures of over 15 ° 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 ° 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 °. Hjelmroos (1993) observed optimal development at 22–28 °, growth being rare at 0 °. 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.* 1998, Díaz 1999, Sabariego, Díaz 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

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.

Locality	<i>Cladosporium cladosporioides</i>			<i>Cladosporium herbarum</i>			<i>Alternaria</i> spp.		
	Ourense	Vigo	Trives	Ourense	Vigo	Trives	Ourense	Vigo	Trives
Rainfall	–	–	–0.115*	–0.270***	–0.351***	–0.216***	–0.293***	–0.319***	–0.305***
Rainfall-1	–	–0.124**	–0.122**	–	–0.255**	–0.115*	–0.279***	–0.227***	–0.307***
Humidity	0.118*	–	–	–	–0.230**	–	–0.189**	–0.133*	–
Humidity-1	0.123**	–	–0.164**	–0.294***	–0.240**	–0.202**	–0.340***	–	–
Humidity-2	0.213***	0.133**	–	–	–	–	–0.170**	–	–
Maximum temp	0.286***	0.195***	–	–	–	–	–	–	–
Maximum-1	0.203***	0.562***	0.366***	0.588***	0.581***	0.622***	0.527***	0.640***	0.529***
Maximum-2	0.140**	0.521***	0.302***	0.458***	0.479***	0.560***	0.422***	0.575***	0.474***
Minimum temp	0.126**	0.473***	0.238***	0.418***	0.424***	0.514***	0.303***	0.524***	0.381***
Minimum-1	0.260***	0.593***	0.387***	0.709***	0.486**	0.655***	0.523***	0.644***	0.491***
Minimum-2	0.210***	0.578***	0.380***	0.608***	0.461***	0.657***	0.410***	0.624***	0.452***
Mean temp	0.200***	0.538***	0.314***	0.551***	0.429***	0.615***	0.305***	0.572***	0.421***
Mean-1	0.291***	0.599***	0.398***	0.647***	0.585***	0.673***	0.324***	0.679***	0.558***
Mean-2	0.274***	0.568***	0.350***	0.642***	0.505***	0.631***	0.256***	0.627***	0.501***
Wind calm	0.270***	0.523***	0.285***	0.586***	0.454***	0.583***	0.210**	0.574***	0.427***
Calm-1	–	0.154**	0.210***	–0.284***	–	–	–	0.134*	–
Calm-2	–	0.194***	0.267***	–0.324***	0.141*	–	–	–	–
Wind N-NE	–	0.156**	0.237***	–0.325***	–	–	–	–	0.141*
Wind N-NE-1	–	–	0.243***	0.456***	0.204**	–0.127**	0.232**	–	–
Wind N-NE-2	–	–	0.282***	0.327***	0.145*	–	0.183**	–	–
Wind NE-S	–	–	0.237***	0.255***	–	–0.161**	0.143*	–	–
Wind N-NE-1	0.316***	–	0.286**	–	–	–0.115*	–	0.141**	–
Wind N-NE-2	0.298***	–	0.234***	–	–	–0.112*	–	0.134*	–
Wind S-SW	0.236***	–	0.175**	–	–	–0.176**	–0.148**	0.154**	–
Wind N-NE-1	0.177***	–	–0.199***	–	–0.259**	0.112*	–0.196**	–	0.136*
Wind N-NE-2	0.179**	–	–0.235***	0.120*	–	–	–0.186**	–	–
Wind SW-N	0.277***	0.130**	–0.243***	0.181**	–	–	–0.124*	–	–
Wind N-NE-1	–	–	–	0.426***	–	–	–	–0.231***	–0.176**
Wind N-NE-2	0.107*	–0.128**	–	0.413***	–	–	–	–0.275***	–0.146**
Wind speed	0.140**	–0.139**	0.114*	0.409***	–	–	–	–0.285***	–0.123*
Wind speed-1	0.124**	–0.305***	–0.362***	0.363***	–	–0.144**	–	–0.282***	–0.244***
Wind speed-2	–	–0.412***	–0.414***	0.354***	–0.160*	–0.182**	–0.143*	–0.313***	–0.293***

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

		Vigo	Ourense	Trives
<i>Cladosporium cladosporioides</i>	Vigo	240.16	414.28	283.77
	Ourense	0.001***	–	–
	Trives	0.659	0.024**	–
<i>Cladosporium herbarum</i>	Vigo	320.54	563.56	664.48
	Ourense	0.000***	–	–
	Trives	0.000***	0.239	–
<i>Alternaria</i> spp.	Vigo	7.27	6.02	6.28
	Ourense	0.337	–	–
	Trives	0.501	0.956	–

favoured by relatively strong winds (Hjelmroos 1993), positive correlations were mainly recorded with wind calm (Munuera *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-Bieito & Talón 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

Table 5. Regression model obtained for each spore type and location.

Species	Location	Equation	p	Adjusted r ²	F
<i>Cladosporium cladosporioides</i>	Ourense	$C. clad. = -4338.592 + 0.618 Cladosp-1 + 27.942 Hum-2 + 116.619 \max T$	<0.000	0.580	29.415
	Vigo	$C. clad. = -3889.02 + 0.482 Cladosp-1 + 25.636 Hum-2 + 110.567 \max T$	<0.000	0.435	16.404
	Trives	$C. clad. = -1826.579 + 0.412 Cladosp-1 + 10.033 Hum-2 + 106.782 \max T$	<0.000	0.323	7.062
<i>Cladosporium herbarum</i>	Ourense	$C. herb. = -4612.590 + 0.449 herbarum-1 + 43.635 Hum-1 + 88.934 \max T$	<0.000	0.477	47.313
	Vigo	$C. herb. = -1919.407 + 0.614 herbarum-1 + 15.324 Hum-1 + 48.067 \max T$	<0.000	0.421	30.442
	Trives	$C. herb. = -2549.056 + 0.557 herbarum-1 + 25.770 Hum-1 + 67.935 \max T$	<0.000	0.473	39.602
<i>Alternaria</i> spp.	Ourense	$Altern. = -43.171 + 0.567 Alternaria-1 + 0.375 Hum-1 + 0.875 \max T$	<0.000	0.434	32.009
	Vigo	$Altern. = -57.181 + 0.496 Alternaria-1 + 0.364 Hum-1 + 1.756 \max T$	<0.000	0.400	28.124
	Trives	$Altern. = 20.830 + 0.206 Alternaria-1 - 0.266 Hum-1 + 0.368 \max T$	<0.000	0.166	9.582

Table 6. Values of the different Indices in the three locations.

	Vigo	Ourense	Trives
Continentality Index	29.00	40.40	33.70
Thermicity Index	43.68	45.89	32.44
Ombrothermic Index	2.51	1.57	3.88
Altitude	50	120	760

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 have been discussed earlier. Rainfall and humidity 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 regression lines; r^2 values ranged from 0.40 to 0.48. For *C. cladosporioides* in Ourense, these factors accounted for 58 % of the variance. Lower spore count variability was found in Trives for *C. cladosporioides* and *Alternaria* spp. Several authors (Mediavilla *et al.* 1998, Sabariego *et al.* 2000) report regression coefficients of 27–47 % for the two *Cladosporium* species. With the exception of Trives, similar statistical results were obtained for *Alternaria* type, but in most cases using polynomial regressions (Mediavilla *et al.* 1998, Sabariego *et al.* 2000, Munuera *et al.* 2001).

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

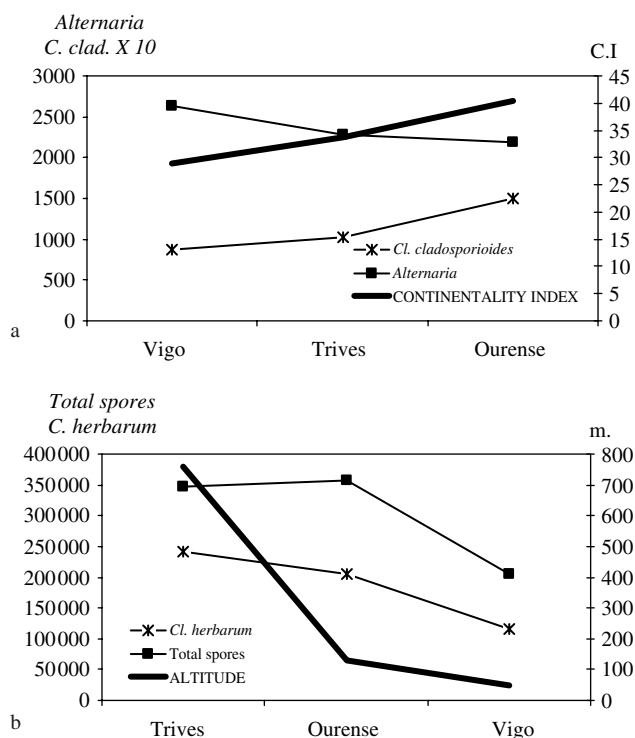


Fig. 6. Comparison between: (a) *Cladosporium cladosporioides* total spores and, *Alternaria* total spores and Continentality Index; (b) relationship between *C. herbarum* total spores, number of total spores and altitude.

maturation, death and disturbance of all plants in the vicinity (including other crops and uncultivated vegetation) and differences in biogeographical or altitudinal conditions prompted differences in the total spore considered counts. The crop harvest coincides with increased spore concentrations. As harvesting aerially disseminates plant waste, spores may be scattered into the wind, enabling dispersal. Below 1000 m a.s.l., the total number of spores tended to increase with altitude. This increase was strongly influenced by the rural 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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