

The effects of meteorological factors on airborne fungal spore concentration in two areas differing in urbanisation level

M. Oliveira · H. Ribeiro · J. L. Delgado · I. Abreu

Received: 3 September 2008 / Revised: 5 October 2008 / Accepted: 6 October 2008
© ISB 2008

Abstract Although fungal spores are an ever-present component of the atmosphere throughout the year, their concentration oscillates widely. This work aims to establish correlations between fungal spore concentrations in Porto and Amares and meteorological data. The seasonal distribution of fungal spores was studied continuously (2005–2007) using volumetric spore traps. To determine the effect of meteorological factors (temperature, relative humidity and rainfall) on spore concentration, the Spearman rank correlation test was used. In both locations, the most abundant fungal spores were *Cladosporium*, *Agaricus*, *Agrocybe*, *Alternaria* and *Aspergillus/Penicillium*, the highest concentrations being found during summer and autumn. In the present study, with the exception of *Coprinus* and *Pleospora*, spore concentrations were higher in the rural area than in the urban location. Among the selected spore types, spring-autumn spores (*Coprinus*, *Didymella*, *Leptosphaeria* and *Pleospora*) exhibited negative correlations with temperature and positive correlations both with relative humidity and rainfall level. On the contrary, late spring-early summer (Smuts) and summer spores (*Alternaria*, *Cladosporium*, *Epicoccum*, *Ganoderma*, *Stemphylium* and *Ustilago*) exhibited positive correlations with temperature and negative

correlations both with relative humidity and rainfall level. Rust, a frequent spore type during summer, had a positive correlation with temperature. *Aspergillus/Penicillium*, showed no correlation with the meteorological factors analysed. This knowledge can be useful for agriculture, allowing more efficient and reliable application of pesticides, and for human health, by improving the diagnosis and treatment of respiratory allergic disease.

Keywords Meteorological factor · Portugal · Rural area · Spore concentration · Urban area

Introduction

Fungal spores are an ever-present component of the atmosphere and are present in almost all seasons of the year (Burch and Levetin 2002; Troutt and Levetin 2001). Nevertheless, sporulation and the dispersion of spores are closely related to variations in meteorological conditions (Angulo-Romero et al. 1999; Sabariego et al. 2000).

Knowledge of the relationships between spore production and different environmental growth conditions, such as meteorological factors, can be used to effect more efficient and reliable application of pesticides, or to improve diagnosis and treatment of respiratory allergic diseases (Rodríguez-Rajo et al. 2005).

Cladosporium, *Alternaria*, *Epicoccum*, and *Dreschlera* spores tend to be found in higher concentrations during warm, dry weather conditions with high wind speeds (Burch and Levetin 2002; Troutt and Levetin 2001). However, ascospores and basidiospores tend to be more frequent during wet or humid conditions. Precipitation is required for the release of many ascospores, their concentrations usually increasing during and after rainstorms; nevertheless,

M. Oliveira · H. Ribeiro · I. Abreu (✉)
Grupo de Ambiente, Sociedade e Educação do Centro de
Geologia & Departamento de Botânica, Faculdade de Ciências,
Edifício FC4,
Rua do Campo Alegre s/n,
4169 Porto, Portugal
e-mail: ianoranh@fc.up.pt

J. L. Delgado
Serviço e Laboratório de Imunologia, Faculdade de Medicina,
Universidade do Porto,
Porto, Portugal

excessive rain tends to wash spores out of the atmosphere (Burch and Levetin 2002; Troutt and Levetin 2001).

The study of fungal spores is of critical importance since many of these bioparticles can induce considerable economic losses, acting as plant pathogens or triggering respiratory diseases and allergenic processes in humans. Most aeromycological studies are conducted in urban environments, with very little data from rural settings. Consequently, it is difficult to compare the presence and concentration of airborne fungal spores in the atmospheres of both urban and rural areas (Kasprzyk and Worek 2006). Nonetheless, previous studies have reported higher spore concentrations in inland rural areas than in coastal areas (Rodríguez-Rajo et al. 2005). In the present study, fungal spore concentrations in Porto (coastal urban area) and Amares (inland rural area) were correlated with meteorological data from 2005 to 2007. The purpose of this study was to analyse the content of several spore types in the air of two locations differing in urbanisation level, and to determine how different weather conditions affected spore concentrations in both locations.

Materials and methods

Daily spore concentrations were sampled between 1 January 2005 and 31 December 2007, using two 7-day First-type volumetric spore traps (Burkard Manufacturing, Rickmansworth, UK) with a flow rate of 10 L min⁻¹. Spores were trapped onto a Melinex adhesive tape, and then cut into daily segments. The slides with adhesive segments were covered with fucsin-stained glycerol jelly and with a cover glass. The daily mean concentration of the number of

fungal spores was determined using an optical microscope at a magnification of $\times 400$ along two full lengthwise traverses. Spore counts were then converted to spores per cubic metre of air sampled per day (spores m⁻³ day⁻¹). Fungal spores were classified by appearance and morphological characteristics (colour, size and shape) and identified, where possible, by comparison with bibliographic material (Lacey and West 2006; Nilsson 1983).

In the coastal urban area (Porto), the sampler was located at *Faculdade de Ciências* (41°11' N, 8°39' W; height: 20 m) (Fig. 1). Porto district, in the Douro Litoral Region, has an area of 2,395 km² and a population of nearly 1,900,000 inhabitants. Ornamental and non-ornamental trees, shrubs and herbaceous species surrounded the sampler. The west of this city borders the Atlantic Ocean while the south borders the river Douro. According to the reference values for Porto, from 1961 to 1990, the average maximum temperature was 19.1°C, and the average minimum temperature was 9.9°C (Meteorologia 1992). The total rainfall level during the reference period was 1,265 mm, concentrated mainly from December to February and largely absent during summer (Meteorologia 1992).

In the inland rural area (Amares; near Braga), the sampler was located on a farm (41°38'N, 8°23'W; height: 5 m) (Fig. 1). The Braga District, in the 'Vinhos Verdes' Demarcated Region, has an area of 2,673 km² and a population of around 830,000 inhabitants. This sampler was surrounded by greenhouses, forests and crop-producing fields.

During this study, the most abundant fungal spore types were divided in two sets. *Alternaria*, *Cladosporium*, *Epicoccum*, *Drechslera*, *Ganoderma* and *Stemphylium* were

Fig. 1 Sampler location in the two studied areas. In the coastal urban area of Porto, the sampler is surrounded by several buildings of different heights, and the surrounding flora is constituted by ornamental and non-ornamental trees, shrubs and herbaceous species. In the inland rural area of Amares, only a few small houses and greenhouses are present in the proximity of the sampler and the surrounding flora is constituted mainly by pine and eucalyptus forests, grape and kiwi vines and crop fields



considered as dry-air spores, while *Coprinus*, *Didymella*, *Leptosphaeria*, *Pleospora*, and *Ustilago* were selected as wet-air spores. Moreover, *Aspergillus/Penicillium* spores were counted due to their role in human health. Rust (including species such as *Puccinia* and *Uromyces*) and Smuts spores (including species such as *Urocystis* and *Tilletia*) were chosen for their phytopathological importance.

The airborne spore concentration observed in both regions were standardised using a Z-scores transformation (the raw value to be standardised is subtracted from the sample mean and divided by the sample standard deviation) to allow comparisons between the two different locations.

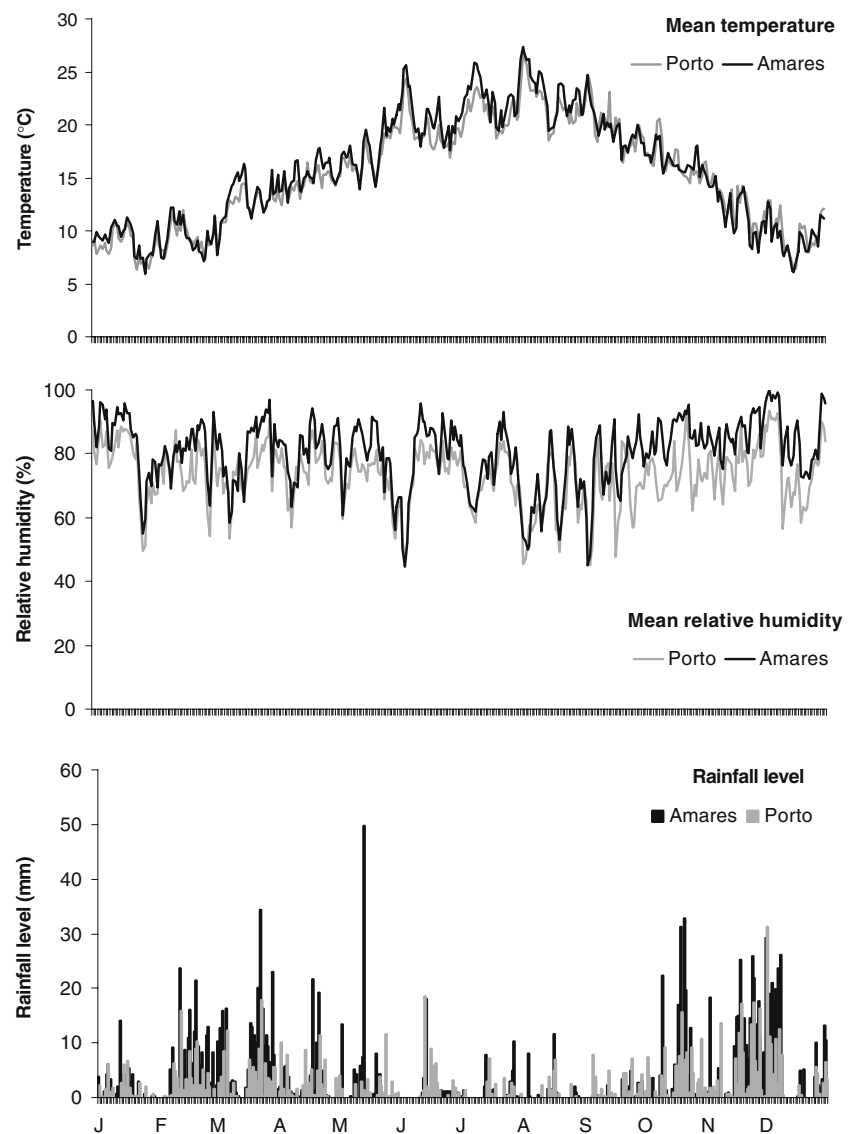
Meteorological data were correlated with the airborne spore concentrations of the selected types by means of the Spearman rank correlation test, with a significance level of 99% and 95%. Afterwards, a multivariate hierarchical cluster analysis was carried out on the correlation coef-

ficients obtained in both locations in order to identify possible homogeneous groups of fungal spores having similar relationships to meteorological parameters. To perform this analysis, the correlation coefficient values were recoded as 1 when significantly positive, -1 when significantly negative, and 0 when they were not significant. The number of clusters was determined using the Square Euclidean Distance as a distance measure, and the linking method used was the Ward method.

Results

In Porto and Amares, the lowest temperatures were recorded in the first months of the year, with the maximum temperatures being reached in August (Fig. 2). During the study period, the annual average, maximum and minimum temper-

Fig. 2 Weather conditions registered in Porto and Amares (daily average values from 2005 to 2007). Both locations had similar temperatures and relative humidity values. Rainfall level was the most distinctive meteorological factor analysed, Amares being rainier than Porto



atures, were 15.4°C, 20.2°C and 11.1°C, respectively, in Porto, and 15.7°C, 22.0°C and 10.3°C in Amares (Table 1).

In both regions, the highest relative humidity values were recorded in the first month of the year, with minimum values being reached in the summer months (Fig. 2). In the urban area, the annual average relative humidity was 73.5%, the average maximum was 91.7% and the average minimum was 52.2%. In the rural area, the annual average relative humidity was 80.2%, the average maximum was 95.9% and the average minimum was 57.4% (Table 1).

Rainfall in Porto and Amares was concentrated mainly in the spring and autumn months (Fig. 2). The average value in the urban area was 883.3 mm, while it was 1,514.5 mm in the rural area. The maximum rainfall levels were registered in 2006 (Porto: 1,291.6 mm; Amares: 2,675.0 mm), while the minimum values were registered in 2007 in both locations (Porto: 661.0 mm; Amares: 856.6 mm) (Table 1).

A total of 50 fungal spore types were identified in the atmosphere, with the majority being present throughout the year (Table 2). In Porto, a total of 568,244 spores were recorded, reaching a maximum value of 8,509 spores/m⁻³ on 8 October 2007 (Table 3). The most abundant fungal spores were *Cladosporium* (60%), *Agaricus* (6%), *Ganoderma* (3%), *Agrocybe* (1%), *Alternaria* (1%), *Smuts* (1%), *Rusts* (1%) and *Aspergillus/Penicillium* (1%) (Fig. 3). In Amares, a total of 1,013,901 spores were recorded, reaching a maximum value of 8,761 spores/m⁻³ on 8 December 2007 (Table 3). The most abundant fungal spores were *Cladosporium* (64%), followed by *Agaricus* (4%), *Agrocybe* (1%), *Alternaria* (1%) and *Aspergillus/Penicillium* (1%) (Fig. 3).

Among the spore types analysed, it was possible to distinguish spores present throughout the year in variable concentrations (*Alternaria*, *Cladosporium*, *Coprinus*, *Rusts* and *Smuts*) and spores with sporadic occurrence (*Aspergillus/Penicillium*) or present in annual concentrations below 1,000 spores (*Didymella*, *Drechslera*, *Leptosphaeria*, *Pleo-*

Table 2 Fungal spore types found in the atmosphere of Porto and Amares from 2005 to 2007

<i>Aerobasidium</i>	<i>Agaricus</i>	<i>Agrocybe</i>
<i>Albugo</i>	<i>Alternaria</i> ^a	<i>Aspergillus/Penicillium</i> ^a
<i>Boletus</i>	<i>Botrytis</i>	
<i>Cercospora</i>	<i>Cladosporium</i> ^a	<i>Coprinus</i> ^a
<i>Corynespora</i>	<i>Curvularia</i>	
<i>Didymella</i> ^a	<i>Diplocadiella</i>	<i>Drechslera</i> ^a
<i>Entomophthora</i>	<i>Epicoccum</i> ^a	<i>Erysiphe</i>
<i>Fusarium</i>		
<i>Ganoderma</i> ^a	<i>Geoglossum</i>	
<i>Helicomyces</i>		
<i>Leptosphaeria</i> ^a		
<i>Nectria</i>	<i>Nigrospora</i>	
<i>Oidium</i>		
<i>Paraphaeosphaeria</i>	<i>Periconia</i>	<i>Peziza</i>
<i>Phoma</i>	<i>Pithomyces</i>	<i>Pleospora</i> ^a
<i>Polythrincium</i>	<i>Puccinia</i>	
<i>Rhizopus</i>	<i>Russula</i>	<i>Rusts</i> ^a
<i>Smuts</i> ^a	<i>Spegazzinia</i>	<i>Sporobolomyces</i>
<i>Sporomiella</i>	<i>Stachybotis</i>	<i>Stemphylium</i> ^a
<i>Tilletia</i>	<i>Torula</i>	
<i>Uromyces</i>	<i>Ustilago</i> ^a	
<i>Venturia</i>		
<i>Xylaria</i>		

^a Fungal spore types considered in this study

spora, *Stemphylium* and *Ustilago*). *Epicoccum* and *Ganoderma* had different patterns according to location, being frequent in Porto, but sporadic in Amares. On a seasonal basis, in both locations, the selected spore types presented higher airborne concentrations during late summer and early autumn (September and October) while the lowest concentrations were registered in the winter months (January–February) (Figs. 4, 5).

Coprinus, *Didymella*, *Leptosphaeria* and *Pleospora* presented two peaks during the year, one during spring and other during autumn (Figs. 4, 5). *Coprinus* spore

Table 1 Meteorological factors registered in the city of Porto and the rural area of Amares (2005–2007)

	T _{mean} ^a		T _{max} ^a		T _{min} ^a		RH _{mean} ^b		RH _{max} ^b		RH _{min} ^b		R _{total} ^c	
	Porto	Amares	Porto	Amares	Porto	Amares	Porto	Amares	Porto	Amares	Porto	Amares	Porto	Amares
2005	15.0± 5.5	15.7± 6.0	19.8± 5.9	21.9± 7.0	10.9± 5.6	10.5± 5.8	72.5± 15.4	76.5± 18.4	90.5± 12.4	93.9± 12.5	52.3± 16.0	54.1± 20.9	698.7	1,011.8
2006	16.0± 5.5	15.8± 6.2	20.9± 6.2	21.8± 7.2	11.6± 5.4	10.8± 5.8	74.4± 14.4	82.1± 16.1	92.7± 10.6	96.1± 10.3	52.6± 15.5	60.6± 22.1	1,291.6	2,675.0
2007	15.1± 4.6	15.2± 5.2	20.6± 5.1	21.9± 5.8	11.2± 4.6	9.2± 5.4	73.0± 13.5	82.6± 13.3	91.7± 11.2	97.7± 7.1	51.0± 15.0	58.7± 19.4	661.0	856.6
Average	15.4± 5.3	15.7± 5.8	20.2± 5.8	22.0± 6.7	11.1± 5.3	10.3± 5.7	73.5± 14.5	80.2± 16.3	91.7± 11.5	95.9± 10.3	52.2± 15.6	57.4± 20.9	883.8± 353.7	1,514.5± 1,008.0

^a Temperature (in °C)

^b Relative humidity (in %)

^c Rainfall (in mm)

Table 3 Spore distribution in the atmosphere of Porto and Amares from 2005 to 2007. Values are in spores/m⁻³

Spore type	Year	Total ^a		Average		Maximum		Peak date ^a		Z score		Value above threshold ^b	
		Porto	Amares	Porto	Amares	Porto	Amares	Porto	Amares	Porto	Amares	Porto	Amares
<i>Alternaria</i>	2005	1,975	3,743	5±9	10±13	48	80	18 August	30 July	225	281	0	0
	2006	1,882	3,175	5±9	9±14	81	104	29 August	22 June	203	225	0	1
	2007	2,625	3,542	7±12	10±13	85	109	8 October	4 October	214	266	0	1
<i>Aspergillus/Penicillium</i>	2005	2,416	4,981	7±17	14±29	152	303	1 November	22 November	141	171	nd ^c	nd
	2006	1,697	3,704	5±13	10±22	122	242	9 August	2 October	126	167	nd	nd
	2007	1,054	2,419	3±8	7±13	87	98	4 June	17 April	127	180	nd	nd
<i>Cladosporium</i>	2005	111,536	216,988	306±458	594±679	2,972	4,682	31 July	4 June	243	319	0	4
	2006	99,716	188,296	273±494	516±833	3,958	5,885	21 August	29-Sep	201	225	1	8
	2007	127,054	229,226	348±707	628±966	8,297	7,567	8 October	4 October	179	237	4	13
<i>Coprinus</i>	2005	1,999	2,685	5±8	7±12	55	106	24 October	22 October	241	223	nd	nd
	2006	1,466	2,589	4±5	7±13	35	133	9 March	7 October	282	202	nd	nd
	2007	351	725	1±2	2±4	10	42	8 January	7 December	204	182	nd	nd
<i>Didymella</i>	2005	88	156	0±1	0±1	9	7	30 December	6 July	101	147	nd	nd
	2006	213	235	1±1	1±2	9	12	15 November	13 September	147	138	nd	nd
	2007	146	325	0±2	1±3	20	26	1 May	27 April	93	103	nd	nd
<i>Drechslera</i>	2005	55	76	0±0	0±1	4	6	30 July	22-Mar	121	134	nd	nd
	2006	33	17	0±0	0±0	4	2	28 June		89	66	nd	nd
	2007	70	58	0±1	0±0	4	2	20 September	7 October	124	135	nd	nd
<i>Epicoccum</i>	2005	546	1,310	1±2	4±5	15	28	30 July	18 October	232	279	nd	nd
	2006	297	706	1±1	2±4	12	34	27 August	3 October	207	187	nd	nd
	2007	623	1,180	2±4	3±6	52	63	7 October	5 October	152	199	nd	nd
<i>Ganoderma</i>	2005	7,807	633	21±35	2±3	190	21	9 September	30 August	220	235	nd	nd
	2006	6,630	553	18±28	2±2	180	16	11 September	27 August	233	234	nd	nd
	2007	4,934	370	14±19	1±2	121	11	14 September	7 September	255	219	nd	nd
<i>Leptosphaeria</i>	2005	185	688	1±2	2±5	16	43	29 October	4 April	111	143	nd	nd
	2006	181	1,207	0±2	3±19	20	321	15 August	20 October	108	64	nd	nd
	2007	150	494	0±2	1±4	32	38	19 November	7 December	74	117	nd	nd
<i>Pleospora</i>	2005	146	189	0±1	1±1	5	9	27 March	17 April	169	158	nd	nd
	2006	275	263	1±2	1±2	15	14	18 March	20 October	158	152	nd	nd
	2007	217	205	1±2	1±3	15	41	6 February	7 February	141	77	nd	nd
Rusts	2005	1961	2,597	5±9	7±12	97	105	8 November	7 November	211	223	nd	nd
	2006	2,745	3,436	8±12	9±14	88	153	17 October	10 October	233	251	nd	nd
	2007	1,141	1,555	3±3	4±8	20	127	19 September	5 October	328	199	nd	nd
Smuts	2005	2,575	2,436	7±17	7±8	200	50	8 August	4 April	150	293	nd	nd
	2006	2,553	2,162	7±17	6±11	157	73	30 May	10 May	147	201	nd	nd
	2007	1,664	1,603	5±10	4±9	113	87	12 May	9 May	167	170	nd	nd
<i>Stemphylium</i>	2005	179	150	0±1	0±1	8	5	4 July	16 October	176	186	nd	nd
	2006	191	153	1±1	0±1	11	9	17 June	15 June	157	142	nd	nd
	2007	258	228	1±1	1±1	17	12	4 July	3 October	157	181	nd	nd

Table 3 (continued)

Spore type	Year	Total ^a	Average		Maximum		Peak date ^a		Porto	Amares	Z score		Value above threshold ^b	
			Porto	Amares	Porto	Amares	Porto	Amares			Porto	Amares	Porto	Amares
<i>Ustilago</i>	2005	251	338	1±2	19	16	8 May		13 August		159	188	nd	nd
	2006	173	221	0±1	15	12	6 February	19 June	29 June		122	159	nd	nd
	2007	97	132	0±1	12	22	15 July		9 October		105	97	nd	nd
Total	2005	180,488	325,223	494±533	891±775	3,248	4,755	31 July	4 June		338	419	nd	nd
	2006	195,874	336,926	537±593	923±1,069	4,153	7,119	21 August	29 September		329	314	nd	nd
	2007	191,882	351,752	525±784	964±1,123	8,509	8,761	8 October	8 December		244	312	nd	nd

^a When spore concentrations presented more than two peaks, dates are not shown^b *Alternaria* threshold value: 100 spores/m³; *Cladosporium* threshold value: 3,000 spores/m³^c Not determined

concentration was in the range of 351–1,999 spores/m⁻³ in Porto, and 725–2,685 spores/m⁻³ in Amares; *Didymella* spore concentration in the urban area varied from 88 to 213 spores/m⁻³ and from 156 to 325 spores/m⁻³ in the rural area; *Leptospheria* spore concentration oscillated from 150 to 185 spores/m⁻³ in Porto, and from 494 to 1,207 spores/m⁻³ in Amares; and *Pleospora* spore concentration ranged from 146 to 275 spores/m⁻³ in the urban area, and from 189 to 263 spores/m⁻³ in the rural area (Table 3).

Smuts spores were most frequent during late spring and summer (Figs. 4, 5). Spore concentration ranged from 1,664 to 2,575 spores/m⁻³ in Porto, and from 1,603 to 2,436 spores/m⁻³ in Amares, with a peak in May (Table 3).

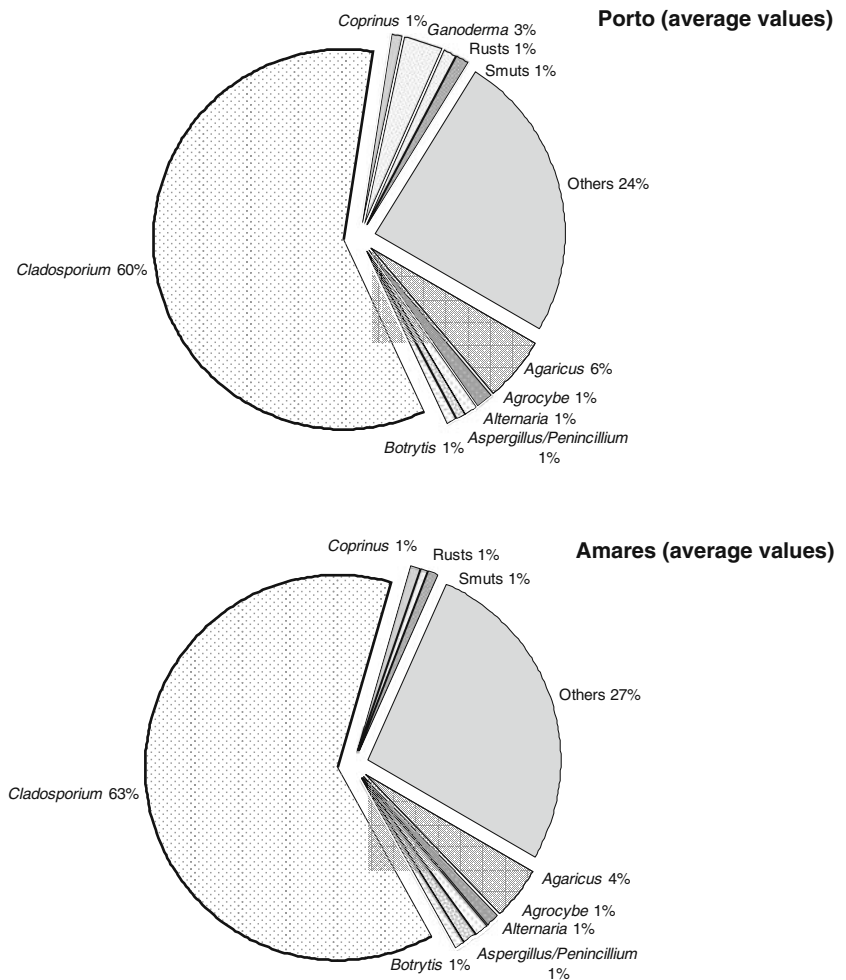
During summer, the most frequent spores were *Alternaria* and *Cladosporium* (Figs. 4, 5). *Alternaria* spore concentration ranged from 1,882 to 2,625 spores/m⁻³ in Porto, and from 3,175 to 3,743 spores/m⁻³ in Amares, the highest values being registered from June to October. In the rural area, the *Alternaria* threshold (100 spores/m⁻³) was surpassed once in 2006 and 2007. *Cladosporium* spore concentration in the urban area varied from 99,716 to 127,054 spores/m⁻³, and in the rural area from 188,296 to 229,226 spores/m⁻³, the highest values being registered from June to October. In Porto, the *Cladosporium* threshold (3,000 spores/m⁻³) was exceeded 30 times, five in Porto and 25 in Amares (Table 3).

In late summer and early autumn, *Epicoccum*, *Ganoderma*, *Stemphylium* and *Ustilago* spores were abundant in the atmosphere (Figs. 4, 5). *Epicoccum* spore concentration ranged from 297 to 623 spores/m⁻³ in Porto, and 706 to 1,310 spores/m⁻³ in Amares, and maximum value dates were registered in July and October. *Ganoderma* spore concentration in the urban area varied from 4,934 to 7,807 spores/m⁻³ and from 370 to 633 spores/m⁻³ in the rural area, peak dates being registered from August to September. *Stemphylium* spore concentration oscillated from 179 to 258 spores/m⁻³ in the city, and from 150 to 228 spores/m⁻³ in the country, maximum value dates being registered in June, July and October. *Ustilago* concentration ranged from 97 to 251 spores/m⁻³ in Porto, and 132 to 338 spores/m⁻³ in Amares, maximum dates being registered in February, May, June and July in the urban area and in June, August and October in the rural location (Table 3).

The maximum value of Rusts as well as the maximum monthly values of *Aspergillus/Penicillium* were registered during autumn (Figs. 4, 5). Rusts spore concentration varied from 1,141 to 2,745 spores/m⁻³ in Porto, and from 1,555 to 3,436 spores/m⁻³ in Amares while *Aspergillus/Penicillium* spore concentration oscillated from 1,054 to 2,416 spores/m⁻³ in the urban area, and from 2,419 to 4,981 spores/m⁻³ in the rural area, peak dates being registered in April, June, August, October and November (Table 3).

Finally, *Drechslera* spore concentration ranged from 33 to 70 spores/m⁻³ in Porto, and from 17 to 76 spores/m⁻³

Fig. 3 Average annual distribution of the main spore types present in the atmosphere of Porto (urban area) and Amares (rural area), from 2005 to 2007. Only spore types constituting more than 1% of the airborne fungal spectrum are represented



in Amares, maximum value dates being registered in March and June–October.

The standardised values of each airborne spore concentration observed in both regions showed that higher concentrations of *Alternaria*, *Aspergillus/Penicillium*, *Cladosporium* and Smuts were found in the rural area during the 3 years of the study, while *Coprinus* and *Pleospora* had higher concentrations in the urban area. However, *Didymella*, *Leptosphaeria*, *Drechslera*, *Epicoccum*, *Stemphylium*, Rust and *Ustilago* spores did not exhibit a consistent behaviour (Table 3).

According to the correlation coefficients obtained and the cluster analysis performed, it was possible to establish four different groups: (1) *Aspergillus/Penicillium*, *Ustilago* and Rusts spores lacking any correlation or consistent correlation with the meteorological factors analysed; (2) *Alternaria*, *Cladosporium*, *Epicoccum* and Smuts spores exhibiting positive correlations with temperature and negative correlations with relative humidity and rainfall level; (3) *Coprinus*, *Didymella*, *Leptosphaeria* and *Pleospora* spores with negative or inconsistent correlations with temperature and positive correlations with relative humidity

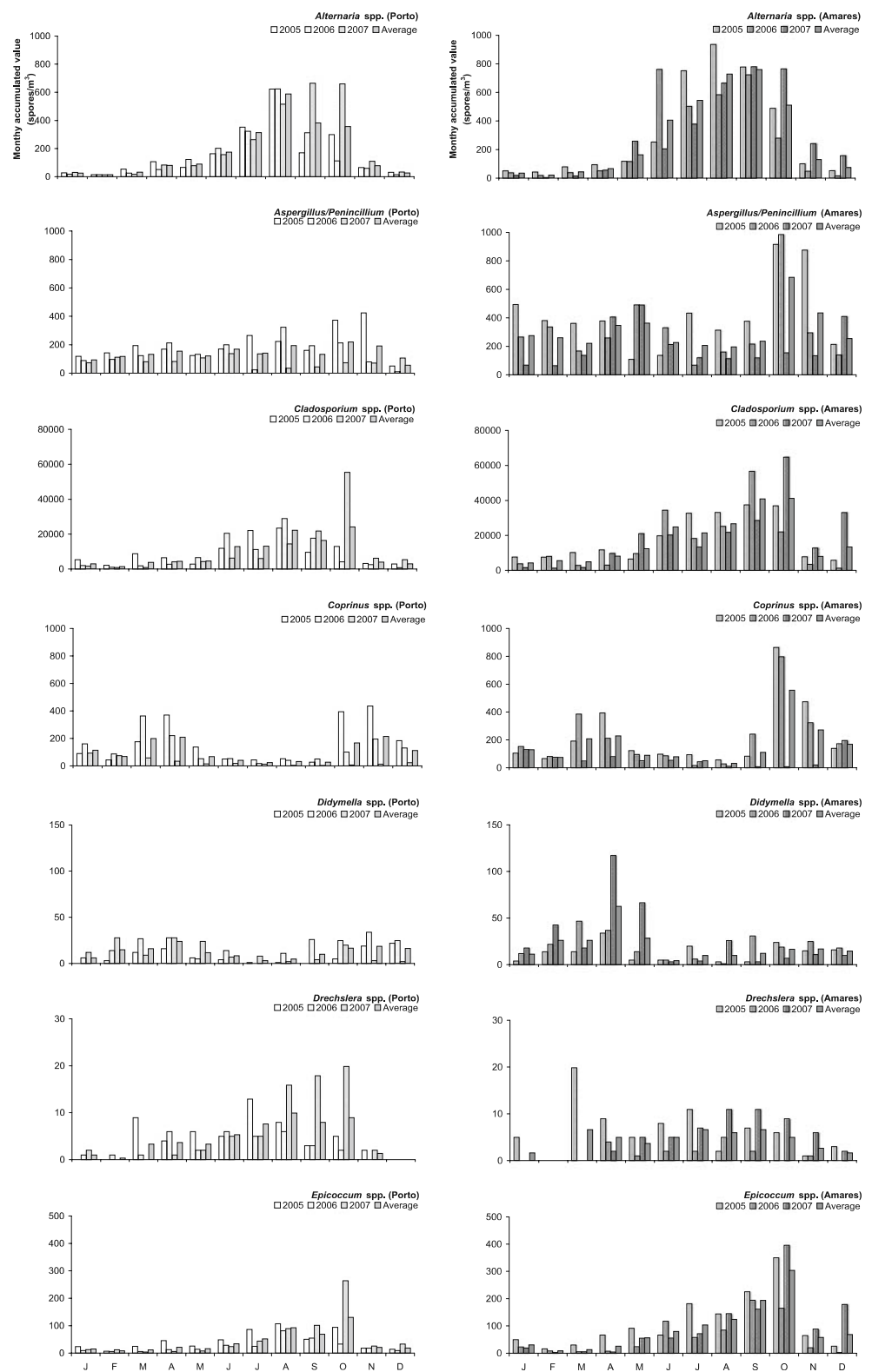
and rainfall level; (4) *Drechslera*, *Ganoderma* and *Stemphylium* spores, which presented positive correlations with temperature and inconsistent or no correlation with relative humidity or rainfall level (Table 4, Fig. 6).

Discussion

During 2005, Amares suffered two heat waves, the first from 30 May to 11 June, and the second from 13 to 25 June (Meteorologia 1992). No data was available for the other study years, which might explain the reduced values of *Aspergillus/Penicillium* and total spores observed in May and June in the rural area not matching the same behaviour as in the urban area. Also, in July, *Alternaria*, *Cladosporium* and total spore concentration in Amares was higher in 2005 than in other years, and did not correspond to the same pattern as in Porto, which may also have been influenced by the high temperatures registered during the preceding months.

Only 2006 had a rainfall level comparable to the reference value, although in the other years approximately

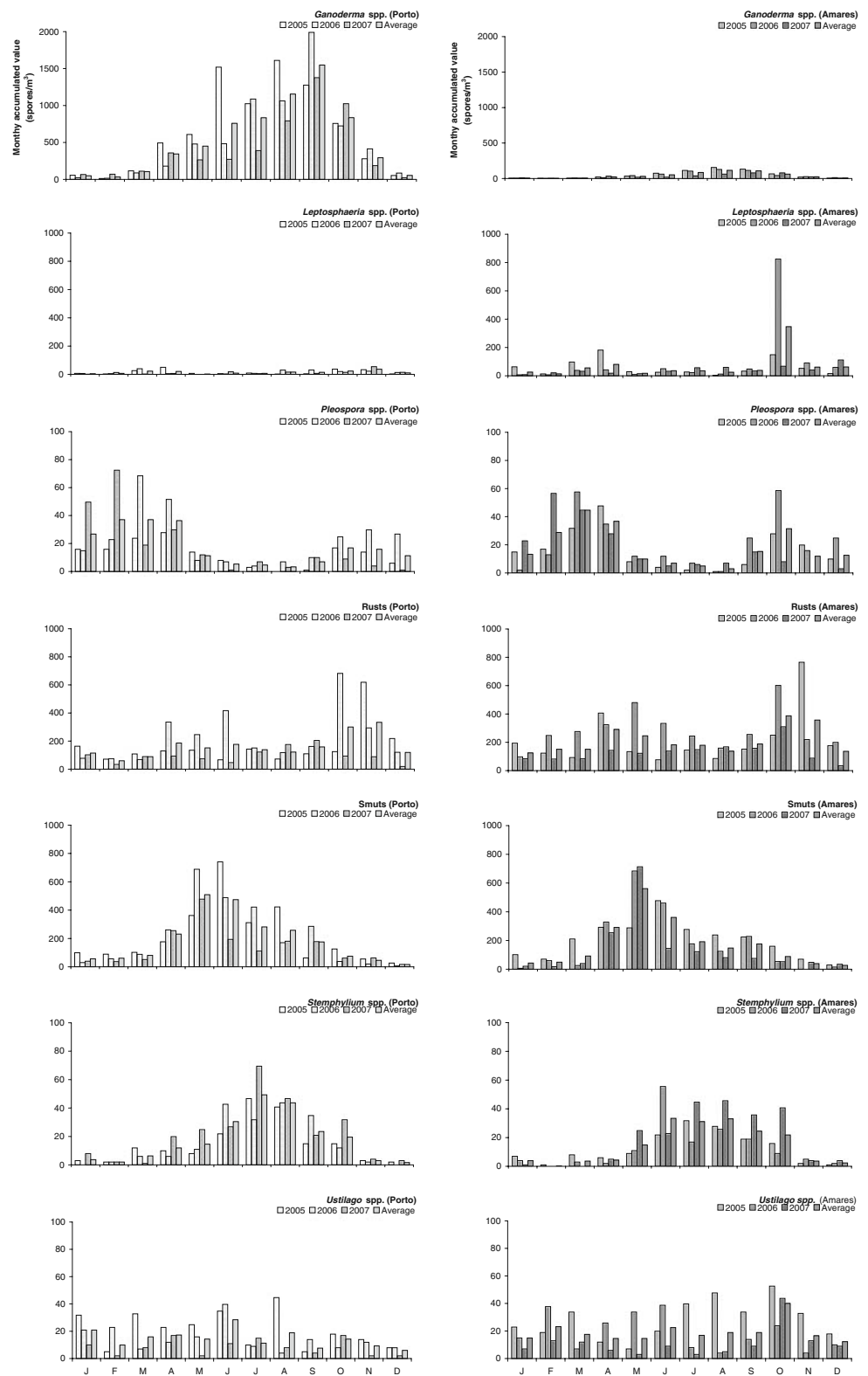
Fig. 4 Monthly seasonal variation of *Alternaria*, *Aspergillus/Penicillium*, *Cladosporium*, *Coprinus*, *Didymella*, *Drechslera* and *Epicoccum* in the atmosphere of Porto (urban area) and Amares (rural area) from 2005 to 2007. Higher airborne concentrations were found during late summer and early autumn while the lowest concentrations were registered in winter months



half of the average annual value was registered. Again, in Amares, the reference rainfall value was surpassed only in 2006. During this year, high values of *Didymella*, *Leptosphaeria*, *Pleospora*, Rusts and Smuts and low values of

Alternaria, *Cladosporium*, *Drechslera*, *Epicoccum* and *Ustilago* were observed in Amares, particularly in October and November — the months with the heaviest rainfall levels of the year.

Fig. 5 Monthly seasonal variation of *Ganoderma*, *Leptospheria*, *Pleospora*, Rusts, Smuts, *Stemphylium* and *Ustilago* in the atmosphere of Porto (urban area) and Amares (rural area) from 2005 to 2007. Higher airborne concentrations were found during late summer and early autumn while the lowest concentrations were registered in winter months



The seasonal behaviour of *Alternaria* spores registered in this work has also been observed by several other authors (Bruno et al. 2007; Corden and Millington 2001; Mitakakis and Guest 2001; Myszkowska et al. 2002; Oliveira et al.

2005). However, in Murcia and Córdoba (Spain), these spores presented as two peaks as a consequence of the summer drop in concentration (Angulo-Romero et al. 1999; Giner et al. 2001). As shown here, *Alternaria* spores had a

Table 4 Correlations between fungal spore concentrations and meteorological factors in Porto and Amares (2005–2007)

Spore type	Year	T _{mean}		T _{max}		T _{min}		RH _{mean}		RH _{max}		RH _{min}		P _{total}	
		Porto	Amares	Porto	Amares	Porto	Amares	Porto	Amares	Porto	Amares	Porto	Amares	Porto	Amares
<i>Alternaria</i>	2005	0.546**	0.755**	0.554**	0.761**	0.504**	0.673**	-0.182**	-0.282**	-0.114*	-0.162**	-0.153**	-0.274**	-0.250**	-0.302**
	2006	0.582**	0.705**	0.590**	0.709**	0.531**	0.628**	-0.361**	-0.256**	-0.189**	-	-0.367**	-0.276**	-0.285**	-0.340**
	2007	0.601**	0.567**	0.640**	0.624**	0.524**	0.381**	-0.349**	-0.324**	-0.284**	-	-0.288**	-0.298**	-0.312**	-0.320**
<i>Aspergillus/Penicillium</i>	2005	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2007	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Cladosporium</i>	2005	0.353**	0.608**	0.340**	0.585**	0.359**	0.587**	-	-0.109*	-	-	-	-0.124*	-0.106*	-
	2006	0.472**	0.633**	0.456**	0.619**	0.454**	0.602**	-0.336**	-0.170**	-0.187**	-	-0.303**	-0.191**	-0.271**	-0.210**
	2007	0.473**	0.364**	0.520**	0.409**	0.403**	0.217**	-0.319**	-0.125*	-0.255**	-	-0.276**	-0.124	-0.264**	-0.194**
<i>Coprinus</i>	2005	-0.257**	-0.205**	-0.283**	-0.269**	-0.203**	-	0.318**	0.487**	0.293**	0.296**	0.281**	0.483**	0.292**	0.424**
	2006	-0.451**	-0.348**	-0.423**	-0.401**	-0.424**	-0.228**	0.348**	0.550**	0.241**	0.284**	0.277**	0.543**	0.251**	0.441**
	2007	-0.326**	-0.252**	-0.362**	-0.289**	-0.294**	-0.145**	0.290**	0.356**	0.287**	0.189**	0.218**	0.290**	0.307**	0.276**
<i>Didymella</i>	2005	-	-0.107*	-0.185**	-0.211**	-	-	0.292**	0.402**	0.138**	0.281**	0.297**	0.376**	0.459**	0.462**
	2006	-0.125*	-0.212**	-0.182**	-0.289**	-	-	0.224**	0.432**	-	0.193**	0.209**	0.416**	0.456**	0.506**
	2007	-0.109*	-0.121*	-0.192**	-0.214**	-	-	0.282**	0.362**	0.229**	0.187**	0.232**	0.377**	0.371**	0.319**
<i>Drechslera</i>	2005	0.192**	0.148**	0.193**	0.140**	0.183**	0.123*	-	-	-	-	-	-	-	-
	2006	0.103*	0.147**	-	0.144**	-	0.127*	-	-	-	-	-	-	-	-
	2007	0.262**	0.239**	0.294**	0.274**	0.231**	0.153**	-	-0.166**	-	-	-	-0.175**	-0.169**	-0.160**
<i>Epicoccum</i>	2005	0.328**	0.512**	0.333**	0.516**	0.307**	0.462**	-	-	-	-	-	-0.113*	-0.159**	-0.151**
	2006	0.329**	0.476**	0.340**	0.475**	0.303**	0.427**	-0.184**	-	-	-	-0.171**	-0.121*	-0.199**	-0.173**
	2007	0.390**	0.249**	0.423**	0.341**	0.338**	-	-0.207**	-0.147**	-0.199**	-	-0.182**	-0.168**	-0.274**	-0.231**
<i>Ganoderma</i>	2005	0.478**	0.646**	0.426**	0.659**	0.507**	0.587**	0.288**	0.234**	0.234**	0.109*	0.268**	-0.103*	-	-0.130*
	2006	0.479**	0.555**	0.457**	0.541**	0.462**	0.496**	-0.133*	-0.117*	-	-	-0.113*	-0.138**	-	-0.227**
	2007	0.535**	0.371**	0.548**	0.436**	0.469**	0.241**	-	-0.123*	-	-	-	-0.137**	-0.168**	-0.207**
<i>Leptosphaeria</i>	2005	-	-0.172**	-0.133*	-0.241**	-	-	0.293**	0.467**	0.156**	0.326*	0.277**	0.447**	0.393**	0.426**
	2006	-	-	-	-0.151**	-	-	0.137**	0.448**	-	0.261**	-	0.464**	0.332**	0.447**
	2007	-	-	-0.126*	-0.179**	-	-	0.136**	0.275**	-	-	0.118*	0.294**	0.225**	0.234**
<i>Pleospora</i>	2005	-0.181**	-0.155**	-0.233**	-0.249**	-0.110*	-	0.240**	0.324**	0.131*	0.175**	0.272**	0.330**	0.389**	0.405**
	2006	-0.207**	-0.162**	-0.257**	-0.260**	-0.127*	-	0.221**	0.470**	-	0.227**	0.168**	0.454**	0.444**	0.505**
	2007	-0.229**	-	-0.304**	-0.111**	-0.161**	0.109*	0.218**	0.225**	0.168**	-	0.178**	0.238**	0.348**	0.269**
Rusts	2005	-0.280**	-0.250**	-0.266**	-0.250**	-0.268**	-0.219**	-	-	-	-	-	-	0.125*	0.175**
	2006	0.216**	0.164**	0.205**	0.156**	0.207**	0.183**	-	-	-	-	-	-	-	-
	2007	0.281**	0.340**	0.286**	0.334**	0.245**	0.272**	-0.234**	-0.119*	-0.224**	-	-0.194**	-	-0.111*	-
Smuts	2005	0.349**	0.454**	0.351**	0.449**	0.344**	0.380**	-0.146**	-0.228**	-0.175**	-0.124*	-0.105*	-0.221**	-0.174**	-0.238**
	2006	0.376**	0.485**	0.337**	0.520**	0.372**	0.390**	-0.275**	-0.329**	-0.236**	-	-0.214**	-0.349**	-0.300**	-0.341**
	2007	0.300**	0.319**	0.278**	0.292**	0.264**	0.278**	-0.137**	-0.104*	-	-	-0.124*	-	-0.121*	-0.156**
<i>Stemphylium</i>	2005	0.386**	0.391**	0.369**	0.364**	0.380**	0.381**	-	-	-	-	-	-	-0.121*	-
	2006	0.386**	0.325**	0.385**	0.320**	0.379**	0.283**	-0.187**	-	-	-	-0.188*	-	-0.171**	-
	2007	0.374**	0.497**	0.342**	0.449**	0.338**	0.459*	-	-0.156**	-	-	-	-0.113*	-	-

Table 4 (continued)

Spore type	Year	T _{mean}		T _{max}		T _{min}		RH _{mean}		RH _{max}		RH _{min}		P _{total}	
		Porto	Amares	Porto	Amares	Porto	Amares	Porto	Amares	Porto	Amares	Porto	Amares	Porto	Amares
<i>Ustilago</i>	2005	-	0.133*	-	0.180**	-	-	-0.208**	-0.314**	-0.158**	-0.263**	-0.199**	-0.301**	-0.233**	-0.264**
	2006	-	-	-	0.103*	-	-	-	-0.220**	-	-	-	-0.234**	-0.124*	-0.248**
	2007	-	-	-	-	-	-	-0.167**	-	-	-	-0.186**	-0.111*	-	-

* $P < 0.05$; ** $P < 0.01$; only significant correlations are presented

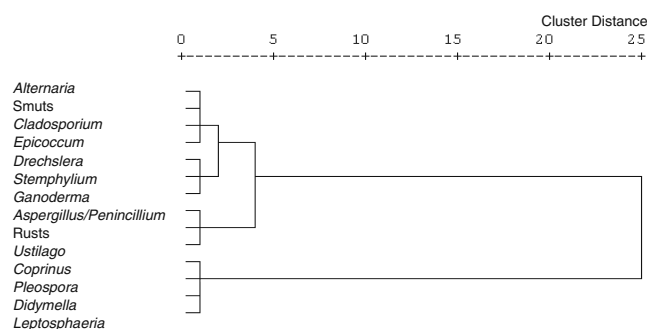


Fig. 6 Dendrogram of fungal spores studied derived by hierarchical cluster analysis from the dissimilarity matrix of the correlation coefficients between spore atmospheric concentrations and meteorological factors (mean maximum and minimum temperature, relative humidity, and total precipitation). Distances between accessions are shown by the length of the horizontal connecting segments, with their values given by a bar at the top of the figure

positive correlation with temperature and negative correlations with relative humidity and rainfall level. Similar results were obtained in a previous study that demonstrated positive correlations between *Alternaria* spore concentration and temperature (maximum and minimum) and sunshine hours, and a negative correlation with relative humidity (Fernández et al. 1998).

Aspergillus/Penicillium spore concentration was rather constant throughout the study years, with a slight increase in middle autumn. Using different sampling techniques, the highest concentrations of *Aspergillus* spores were found in October or during winter (Mullins et al. 1984; Rosas et al. 1990). A previous work on *Penicillium* spore concentrations in a tropical region reported a constant spore concentration between the dry and rainy season, while no differences were observed in locations of different urbanisation index (Rosas et al. 1993). In this study no correlations were found between *Aspergillus/Penicillium* spore concentrations and the meteorological factors analysed. This result was consistent with that of a previous study where this spore type did not present any correlation with temperature (Li and Kendrick 1995). This lack of association between spore occurrence and the analysed meteorological data could be explained by the sporadic behaviour of these spores.

Worldwide, including several regions with different weather conditions, *Cladosporium* spores are reported to be the most abundant fungal spore found in the atmosphere (Damialis and Gioulekas 2006; Gillum and Levettin 2008; Hasnain et al. 2005; Henriquez et al. 2001; Herrero et al. 2006; Mitakakis and Guest 2001; Myszkowska et al. 2002; Oliveira et al. 2005; Pepeljnjak and Segvi 2003). Previous works reported results similar to those observed in our study, where increases in *Cladosporium* concentration were registered in April – May; a maximum was reached during autumn, with the lowest concentrations being found in January and February (Henriquez et al. 2001; Herrero et al.

2006; Oliveira et al. 2005). Nevertheless, in Australia, this spore type presented a peak spanning spring, summer and autumn (Mitakakis and Guest 2001). In the present work, *Cladosporium* spores presented a positive correlation with temperature and negative correlations with relative humidity and rainfall level. Previous studies also confirmed the positive influence of temperature and the negative influence of relative humidity and rainfall on the concentration of this spore type (Katial et al. 1997; Molina et al. 1998).

The present work demonstrates that *Epicoccum* spores showed a positive correlation with temperature and negative correlations with relative humidity and rainfall level. However, it has been reported that *Epicoccum* spores do not have a significant relationship with temperature (Li and Kendrick 1995).

Ganoderma spore concentration peaked from August to September, having a positive correlation with temperature. Rust maximum values were registered during autumn; these spores also had a positive correlation with temperature (in 2006 and 2007). Smut spores were most frequent during late spring and summer, and were positively correlated with temperature and negatively correlated with relative humidity and rainfall level. *Ustilago*, a sporadic behaviour-spore, presented similar concentrations throughout the year, being negatively correlated with relative humidity and rainfall. In Badajoz (Spain), the basidiospores, including *Coprinus*, *Ganoderma*, Rusts, Smuts and *Ustilago*, were present in the atmosphere mainly during autumn. The maximum concentrations were found in November, with the lowest values being found during summer (Gonzalo et al. 1997). Negative correlations were obtained with thermal factors while positive correlations were registered with relative humidity. Nevertheless, no correlation with rainfall was observed (Gonzalo et al. 1997).

Similarly to the results presented in the current work, it has been previously reported that the total number of fungal spores and the frequency of occurrence of some types are higher in rural than in urban areas (Guinea et al. 2006). However, only a few studies have compared spore concentrations in the two environments. An exception is *Aspergillus/Penicillium* spores, for which more isolates were found in urban than in the rural areas (Guinea et al. 2006; Rosas et al. 1993). In the case of Porto and Amares, the *Aspergillus/Penicillium* spore concentration was also higher in the rural area. These differences may be associated with several factors, such as the distribution of forests, occurrence of crops, weeds or even livestock that may play a role as intermediary host for mould development, increasing spore concentration. Besides that, farming operations create mechanical disturbances allowing fungal spore release.

Another interesting factor was to compare data from the z scores of transformation with data from the correlation with meteorological factors. *Alternaria*, *Aspergilaceae*, *Cladospo-*

rium, *Smuts*, *Coprinus* and *Pleospora* had both consistent correlations and distribution among the two regions. In contrast, *Didymella*, *Drechslera*, *Epicoccum*, *Leptosphaeria*, *Stemphylium*, Rust and *Ustilago* were inconsistent in the two sets of data. This fact highlights the importance of joint studies of Aerobiology and Meteorology.

Conclusions

On a seasonal basis, in Porto (urban coastal area) and in Amares (inland rural area), the selected spore types had higher airborne concentrations during late summer and early autumn while the lowest concentrations were registered in the winter months.

In the present study, spore concentrations of the selected types were compared in both locations, it being possible to conclude that *Alternaria*, *Aspergillus/Penicillium*, *Cladosporium* and Smuts were found in higher concentrations in the rural area, while *Coprinus* and *Pleospora* exhibited higher concentrations in the urban area.

As a summary and in general terms, it is possible to affirm that, among the selected spore types, spring–autumn spores (*Coprinus*, *Didymella*, *Leptosphaeria* and *Pleospora*) showed a negative correlation with temperature and positive correlations with both relative humidity and rainfall level. In contrast, late spring–early summer (Smuts) and summer spores (*Alternaria*, *Cladosporium*, *Epicoccum*, *Ganoderma*, *Stemphylium* and *Ustilago*) exhibited positive correlations with temperature and negative correlations both with relative humidity and rainfall level. One exception is Rust, which, besides being frequent during summer, had a positive correlation only with temperature. Furthermore, *Aspergillus/Penicillium* showed no correlation with any of the meteorological factors analysed.

Acknowledgements The authors are grateful to Prof. Dr. Manuel de Barros, from the *Instituto Geofísico da Universidade do Porto*, and Eng. Guerner-Moreira, from the *Direcção Regional de Agricultura e Pescas do Norte - Divisão de Protecção e Controlo Fitossanitário*, for the meteorological data provided. This work was partially supported by *Fundação Calouste Gulbenkian* (project: 77161) and a grant from the *Fundação para a Ciência e Tecnologia* (SFRH/BD/18765/2004).

References

- Angulo-Romero J, Mediavilla-Molina A, Domínguez-Vilches E (1999) Conidia of *Alternaria* in the atmosphere of the city of Cordoba, Spain in relation to meteorological parameters. *Int Arch Allergy Immunol* 43:45–49
- Bruno A, Pace L, Tomassetti B, Coppola E, Verdecchia M, Pacioni G, Visconti G (2007) Estimation of fungal spore concentrations associated to meteorological variables. *Aerobiologia* 23:221–228. doi:10.1007/s10453-007-9066-y

- Burch M, Levetin E (2002) Effects of meteorological conditions on spore plumes. *Int Arch Allergy Immunol* 46:107–117
- Corden J, Millington W (2001) The long-term trends and seasonal variation of the aeroallergen *Alternaria* in Derby. *UK Aerobiologia* 17:127–136. doi:10.1023/A:1010876917512
- Damialis A, Gioulekas D (2006) Airborne allergenic fungal spores and meteorological factors in Greece: forecasting possibilities. *Grana* 45:122–129. doi:10.1080/00173130600601005
- Fernández D, Valencia R, Molnár T, Vega A, Sagüés E (1998) Daily and seasonal variations of *Alternaria* and *Cladosporium* airborne spores in León (North-West, Spain). *Aerobiologia* 14:215–220. doi:10.1007/BF02694209
- Gillum S, Levetin E (2008) The air spora close to a compost facility in Northeast Oklahoma: Part I—spore trap sampling. *Aerobiologia* 24:3–12. doi:10.1007/s10453-007-9074-y
- Giner M, Garcia J, Camacho C (2001) Airborne *Alternaria* spores in SE Spain (1993–98): Occurrence patterns, relationship with weather variables and prediction models. *Grana* 40:111–118. doi:10.1080/00173130152625842
- Gonzalo M, Paredes M, Muñoz A, Tormo R, Silva I (1997) Dinámica de dispersión de basidiosporas en la atmósfera de Badajoz. *Rev Esp Alergol Inmunol Clin* 12:294–300
- Guinea J, Peláez T, Alcalá L, Bouza E (2006) Outdoor environmental levels of *Aspergillus* spp. conidia over a wide geographical area. *Med Mycol* 44:349–356. doi:10.1080/13693780500488939
- Hasnain Sm, Fatima K, Al-Frayh A, Al-Sedairy St (2005) One-year pollen and spore calendars of Saudi Arabia: Al-Khobar, Abha and Hofuf. *Aerobiologia* 21:241–247. doi:10.1007/s10453-005-9000-0
- Henriquez V, Villegas G, Nolla J (2001) Airborne fungi monitoring in Santiago, Chile. *Aerobiologia* 17:137–142. doi:10.1023/A:1010833101583
- Herrero A, Ruiz S, Bustillo M, Morales P (2006) Study of airborne fungal spores in Madrid, Spain. *Aerobiologia* 22:133–140. doi:10.1007/s10453-006-9025-z
- Kasprzyk I, Worek M (2006) Airborne fungal spores in urban and rural environments in Poland. *Aerobiologia* 22:169–176. doi:10.1007/s10453-006-9029-8
- Katial R, Zhang Y, Jones R, Dyer P (1997) Atmospheric mold spore counts in relation to meteorological parameters. *Int J Biometeorol* 41:17–22. doi:10.1007/s004840050048
- Lacey M, West J (2006) *The air spora*. Springer, Dordrecht
- Li D-W, Kendrick B (1995) A year-round study on functional relationships of airborne fungi with meteorological factors. *Int J Biometeorol* 39:74–80. doi:10.1007/BF01212584
- Meteorologia (1992) Normais climatológicas 1961–1990 (Serra do Pilar) (Meteorologia, I.d. (ed) Instituto de Meteorologia, Lisboa, pp 1–3
- Mitakakis T, Guest D (2001) A fungal spore calendar for the atmosphere of Melbourne, Australia, for the year 1993. *Aerobiologia* 17:171–176fs. doi:10.1023/A:1011028412526
- Molina A, Angulo Romero J, Garcia-Pantaleon I, Comtois P, Vilches E (1998) Preliminary statistical modeling of the presence of two conidial types of *Cladosporium* in the atmosphere of Cordoba, Spain. *Aerobiologia* 14:229–234. doi:10.1007/BF02694211
- Mullins J, Hutcheson P, Slavin R (1984) *Aspergillus fumigatus* spore concentration in outside air: Cardiff and St Louis compared. *Clin Exp Allergy* 14:351–354. doi:10.1111/j.1365-2222.1984.tb02215.x
- Myszkowska D, Stepalska D, Obtulowicz K, Porebski G (2002) The relationship between airborne pollen and fungal spore concentrations and seasonal pollen allergy symptoms in Cracow in 1997–1999. *Aerobiologia* 18:153–161. doi:10.1023/A:1020603717191
- Nilsson S (1983) *Atlas of airborne fungal spores in Europe*. Springer, Heidelberg
- Oliveira M, Ribeiro H, Abreu I (2005) Annual variation of fungal spores in the atmosphere of Porto: 2003. *Ann Agric Environ Med* 12:309–315
- Pepeljnjak S, Segvi M (2003) Occurrence of fungi in air and on plants in vegetation of different climatic regions in Croatia. *Aerobiologia* 19:11–19. doi:10.1023/A:1022693032075
- Rodríguez-Rajo F, Iglesias I, Jato V (2005) Variation assessment of airborne *Alternaria* and *Cladosporium* spores at different bioclimatical conditions. *Mycol Res* 109:497–507a. doi:10.1017/S0953756204001777
- Rosas I, Escamilla B, Calderon C, Mosiño P (1990) The daily variations of airborne fungal spores in Mexico City. *Aerobiologia* 6:153–158. doi:10.1007/BF02539108
- Rosas I, Calderon C, Ulloa M, Lacey J (1993) Abundance of airborne penicillium CFU in relation to urbanization in Mexico City. *Appl Environ Microbiol* 58:2648–2652
- Sabariego S, Diaz De La Guardia C, Alba F (2000) The effect of meteorological factors on the daily variation of airborne fungal spores in Granada (southern Spain). *Int J Biometeorol* 44:1–5. doi:10.1007/s004840050131
- Troutt C, Levetin E (2001) Correlation of spring spore concentrations and meteorological conditions in Tulsa, Oklahoma. *Int J Biometeorol* 45:64–74. doi:10.1007/s004840100087