

Fungal spores from Pleosporales in the atmosphere of urban and rural locations in Portugal

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Fungal spores are a significant fraction of the atmospheric bioparticles (bioaerosols) and many species are capable of inducing the production of specific immunoglobulin E (IgE), aggravating the clinical symptoms of allergic respiratory diseases in sensitized individuals. The aim of this work was to evaluate the distribution of potentially allergenic Pleosporales spores in two locations with different urbanization indexes, characterizing its seasonal pattern. The seasonal distribution of several spore types belonging to the Pleosporales (*Alternaria*, *Drechslera*, *Epicoccum*, *Paraphaeosphaeria*, *Pithomyces*, *Pleospora* and *Stemphylium*) in Amares (rural area) and Porto (urban area) was continually studied from January 2005 to December of 2007, using Hirst-type volumetric spore traps. *Alternaria* was the most abundant fungal spore type found in the atmosphere of Amares and Porto. This fungal type, together with *Drechslera*, *Epicoccum*, *Pithomyces* and *Stemphylium*, was mainly present during summer. Nevertheless, *Leptosphaeria*, *Pleospora* and *Venturia* spores were detected during winter and spring, while *Paraphaeosphaeria* spores were also observed during summer and autumn. These different seasonal patterns were responsible for the expansion of the exposure period for the Alt a 1 allergen. The concentration of the studied spore types was higher in the rural area than in the urban one, with exception for *Pleospora* and *Drechslera*. According to the correlations with meteorological factors, the selected fungal spores can be divided into two groups: (i) *Alternaria*, *Drechslera*, *Epicoccum*, *Pithomyces* and *Stemphylium* presented positive correlations with temperature and negative correlations with relative humidity and rainfall; (ii) *Leptosphaeria*, *Paraphaeosphaeria*, *Pleospora* and *Venturia* presented a contrary behavior. Usually, the occurrence of the Alt a 1 allergen has been associated with the presence of airborne *Alternaria* spores; the present work follows the seasonal distribution of other fungal spore species known to contain this molecule. The widespread occurrence of Alt a 1 plays an important role in the incidence and aggravation of allergic disorders.

Introduction

More than 80 mould genera have been shown to induce type I allergies in sensitized patients, whereas allergenic proteins have been identified in 23 fungal genera. Among the most commonly studied fungal species associated with allergic disorders,

Alternaria and *Cladosporium* occur typically outdoors with a maximum of spore concentration in summer and autumn, while *Aspergillus* and *Penicillium* species dominate indoors in winter in humid and cold climates.¹ However, many other less studied species are responsible, as well, for the induction of allergies.

The Order Pleosporales Luttr. ex M.E. Barr, 1983 includes 469 genera corresponding to 1798 species (<http://www.uniprot.org>). This is a large group of Ascomycetes, believed to be monophyletic on the basis of molecular evidence. Among the species included in this Order, several economically important genera of plant pathogens are included, such

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Environmental impact

The present work covers a topic on the distribution of several fungal spores known to induce type I hypersensitive reactions in atopic individuals, in two regions with different urbanization levels from the Northern region of Portugal. In everyday practise of allergy diagnosis, extracts of *Alternaria*, *Asperillus*, *Cladosporium* and *Penicillium* are included in the skin-prick test battery. This work highlights the importance of other fungal taxa (*Drechslera*, *Epicoccum*, *Paraphaeosphaeria*, *Pithomyces*, *Pleospora* and *Stemphylium*) in the total airborne concentration of the Alt a 1 allergen, a widespread allergen among the Pleosporales Order. The publication of the present work may help clinicians and allergy sufferers to recognize and reduce exposure to these relevant aeroallergens, contributing to better care of these allergic patients.

as *Cochliobolus*, *Phaeosphaeria* and *Pyrenophora*, that are parasitic on grasses and cereals. *Pleospora*, *Lewia* and *Leptosphaeria* are common endophytes, saprotrophs or parasites of other herbaceous plants. They and their anamorphs may also have significance as aeroallergens, capable of inducing allergic diseases, such as *Alternaria*, *Drechslera*, *Epicoccum*, *Paraphaeosphaeria*, *Pithomyces*, *Pleospora* and *Stemphylium*, and in the production of mycotoxins.²

It has been demonstrated that culture filtrate extracts from species belonging to Pleosporaceae presented significant amounts of Alt a 1 (*Alternaria alternata*: 0.45 ng mL⁻¹; *Alternaria tenuissima*: 1.93 ng mL⁻¹; *Curvularia lunata*: 0.2 ng mL⁻¹; *Stemphylium botryosum*: 3.22 ng mL⁻¹; *Ulocladium botrytis*: 11.26 ng mL⁻¹) while no significant Alt a 1 concentrations could be detected for species belonging to Dothideales (*Cladosporium herbarum*) or Eurotiales (*Penicillium chrysogenum* and *Aspergillus fumigatus*).³ These results have been recently confirmed in a study using ELISA where spores of 7 fungi, including species of *Alternaria*, *Ulocladium*, *Stemphylium*, *Epicoccum*, *Drechslera*, and *Exserohilum*, strongly inhibited the binding of the polyclonal antibodies. Six other fungi reacted in the ELISA at a lower level, and 11 fungi, including several *Penicillium*, *Aspergillus*, *Fusarium*, and *Cladosporium* species, failed to show inhibition.⁴

The widespread presence of this allergen in several species of the Pleosporales Order could partially explain the cross reactivity detected between the *A. alternata* and *Drechslera*-type, *Stemphylium* sp, *Curvularia* sp, and *Ulocladium* sp extracts.⁵ This highlights the need for monitoring the concentration of less frequent species expressing Alt a1 from the Pleosporaceae family that can play an important role in the outset of allergic respiratory diseases.

The aim of this work was to analyze the content of spores from *Alternaria* and other taxonomically related species of the Pleosporaceae, such as *Drechslera*, *Epicoccum*, *Paraphaeosphaeria*, *Pithomyces*, *Pleospora* and *Stemphylium*, in the atmosphere of Amares (rural area) and Porto (urban area). The knowledge of the seasonal distribution pattern of these taxonomically related airborne fungal spores gives an indication of the airborne concentration and seasonal exposure risks of Alt a 1.

Materials and methods

Daily concentrations of *Alternaria*, *Drechslera*, *Epicoccum*, *Leptosphaeria*, *Paraphaeosphaeria*, *Pithomyces*, *Pleospora*, *Stemphylium* and *Venturia* spores were sampled from January 2005 to December 2007, using two Hirst-type volumetric spore traps (Burkard Manufacturing Co., U.K.; Lanzoni, Italy) that allow a continuous, rather than instant, air sampling with hourly values for airborne concentration. These sampling devices are composed of a built-in vacuum pump that allows the sampling of air at 10 L min⁻¹ (simulates human inhalation; calibrated once a week using an appropriate flowmeter) and has a 2 × 14 mm intake orifice through which the sampled particles (fungal spores and pollen grains) are impacted on transparent plastic tape, covered with a thin film of silicone oil, supported on a clockwork-driven drum (rotating once every 7 days). After exposure, the plastic tape is cut into seven daily segments in the laboratory (48 mm strips representing 24 h of exposure). These segments were then mounted on

microscope slides in glycerol jelly containing basic fuchsin (0.1%) and protected with a cover glass. The number of fungal spores was determined using an optical microscope at a magnification of ×400 along two full lengthwise traverses. Spore counts were then converted to correspond to the daily mean concentration (spores per cubic meter of air sampled per day). Fungal spores were classified by appearance and morphological characteristics (colour, size and shape) and identified, where possible, by comparison with bibliographic material.^{6,7}

In the coastal urban area of Porto, the sampler was located on the roof of the *Faculdade de Ciências* building (41°11' N, 8°39' W; height: 20 m). The district of Porto, in the Douro Litoral Region, has an area of 2395 km² and a population of nearly 1 900 000 inhabitants. Surrounding the sampler, ornamental and non-ornamental trees, shrubs and herbaceous species were present. The west of this region borders the Atlantic Ocean while the south borders the river Douro. According to the reference values for Porto, during the 1961–1990 period, the average maximum temperature was 19.1 °C and the average minimum temperature was 9.9 °C. The average total rainfall level of 1265 mm/year was mainly concentrated from December to February, and roughly absent during summer.

In the inland rural area of Amares, near Braga, the sampler was located on the roof of a farm house (41°38' N, 8°23' W; height: 5 m). The district of Braga, in the Minho Region, has an area of 2673 km² and a population of around 830 000 inhabitants. Around this sampler, greenhouses, forests and crop-producing fields were present. The climate of Braga is affected by maritime air mass influence, being described as rainy with low temperatures in winter, and low humidity and high temperatures in summer. The average annual mean temperature is 14 °C and the average annual minimum and maximum temperatures are 8.5 and 19.5 °C, respectively, and presents a total rainfall level of 1514.9 mm/year.

The two sampling sites differed in habitat characteristics, urbanization index (expressed by the percentage of constructed space), vegetation, weather conditions and sampler height. Therefore, data was standardized to allow comparisons between the different locations using z-scores. The z-scores actual value corresponds to the number of standard deviations the original score is from the mean in that direction.⁸

The Main Sporulation Period (MSP) was defined applying the criteria proposed by Nilsson and Persson. The beginning of the season was defined as the day when 5% of the seasonal cumulative spore counts were reached; the end of the season was defined as the day when 95% of the seasonal cumulative counts were reached. The pre-peak period was defined as the day from the beginning of the MSP until the day when the peak was registered.⁹

The intradiurnal airborne fungal spore concentration was determined, for each year and spore type studied, from the hourly values of atmospheric concentration registered during the main sporulation season, according to the method previously described. So, the days with atmospheric spore concentrations superior to the MSP average and without precipitation were selected. Since the hourly counts vary widely interdaily and interannually, its values were expressed in percentage rather than in absolute numbers.

Correlations were examined between meteorological data (annual mean temperature – Tmean – and annual mean relative humidity – RHmean) and hourly airborne spore concentrations of the selected types by means of the Spearman rank correlation test, with a significance level of 99% and 95%.

To make assumptions about the underlying distribution of the data, the Kolmogorov–Smirnov test was used to verify Normality. To investigate the existence of differences between the numbers of spores in Amares and Porto, the Mann–Whitney U test was used. T-Tests were used to compare the intradiurnal pattern of distribution among the two locations.

Results

Alternaria spores (1%) were the most abundant and frequent analyzed spore type present in the atmosphere of the two locations, being present on average in 76% of the sampled days. In Amares, the annual concentration ranged from 3175 to 3743 spores m⁻³. The average MSP started on 27 April and ended on 30 October (186 days). The threshold of 100 spores m⁻³ was reached twice. In Porto, the annual concentration of *Alternaria* spores ranged from 1882 to 2625 spores m⁻³. The MSP extended from 17 April to 29 October (196 days), the peak being registered on 7 September (71.4 spores m⁻³) (Fig. 1, Table 1).

Drechslera spores (0.02%) were present in the atmosphere of the two locations in 11% of the studied period. In the rural area, the annual concentration of spores ranged from 17 to 76 spores m⁻³. *Drechslera* MSP was recorded from 20 March to 9 November (234 days). In the urban area, the annual concentration of spores ranged from 33 to 70 spores m⁻³. The MSP took place from 20 March to 16 October (210 days) (Fig. 1, Table 1).

Epicoccum spores (0.30%) were the second most frequently analyzed spores, being present in the atmosphere of the two locations for an average 57% of the sampled period. In Amares, during the studied period, the annual concentration ranged from 706 to 1310 spores m⁻³. The *Epicoccum* MSP started on 5 April and ended on 10 November (219 days). In Porto, the annual concentration of spores ranged from 297 to 623 spores m⁻³. The MSP extended from 12 March to 21 November (254 days) (Fig. 1, Table 1).

Leptosphaeria spores (0.16%) were present in the atmosphere of both sampling sites in 30% of the sampled period. In the rural area, the annual concentration ranged from 494 to 1207 spores m⁻³. The MSP of these spores extended from 19 February to 25 November (278 days). In the urban area the annual concentration ranged from 150 to 185 spores m⁻³. The MSP went on from 10 February to 26 November (290 days) (Fig. 1, Table 1).

Paraphaeosphaeria spores (0.10%) were present in the atmosphere of the two locations in 30% of the sampled period. In the countryside, the annual spore concentration ranged from 222 to 423 spores m⁻³. The MSP was extended from 11 February to 5 December (296 days). In the city, the *Paraphaeosphaeria* annual concentration of spores ranged from 147 to 248 spores m⁻³. The MSP in Porto was coincident with the one in Amares, but began one day later and finished one day earlier (295 days) (Fig. 1, Table 1).

Pithomyces spores (0.04%) were present in the atmosphere of the two locations in 15% of the studied period. In Amares, *Pithomyces* annual concentration ranged from 85 to 101 spores m⁻³.

The MSP started on 18 April and ended on 18 November (214 days). In Porto, the annual concentration of spores ranged from 48 to 104 spores m⁻³. The MSP was calculated as being between 2 May and 19 November (201 days) (Fig. 1, Table 1).

Pleospora spores (0.09%) were present in the atmosphere of the two locations in more than 26% studied period. In the rural sampling site, the *Pleospora* annual concentration of spores ranged from 190 to 263 spores m⁻³. The MSP was calculated as being from 25 January to 12 November (291 days). In the urban site, the annual concentration of spores ranged from 146 to 275 spores m⁻³. The MSP ranged from 13 January to 14 November (304 days) (Fig. 1, Table 1).

Stemphylium spores (0.08%) were present in the atmosphere of the two locations in more than 28% of the studied period. In Amares, the *Stemphylium* annual concentration of spores ranged from 150 to 228 spores m⁻³. The MSP started on 2 April and ended on 19 October (200 days). In Porto, the *Stemphylium* annual concentration of spores ranged from 179 to 258 spores m⁻³. The MSP took place between 6 April and 11 October (188 days) (Fig. 1, Table 1).

Venturia spores (0.14%) were present in the atmosphere of the two locations in 34% of the studied period. In the rural sampling area, the annual concentration oscillated from 318 and 754 spores m⁻³. The MSP was calculated from 4 February to 19 November (288 days). In the urban sampling site, the annual concentration fluctuated from 179 to 258 spores m⁻³. The MSP was determined as being between 19 January and 24 November (309 days); this was the longest MSP among the selected spore types (Fig. 1, Table 1).

Another interesting aspect is the existence of a significant association between spore concentrations in both sampled areas, evidenced by the positive correlations obtained. Generally speaking, the spore concentration of *Drechslera* ($p_{2005} = 0.330$, $p_{2006} = 0.371$, $p_{2007} = 0.972$), *Pithomyces* ($p_{2005} = 0.100$, $p_{2006} = 0.751$, $p_{2007} = 0.530$), *Pleospora* ($p_{2005} = 0.461$, $p_{2006} = 0.301$, $p_{2007} = 0.076$) and *Stemphylium* ($p_{2005} = 0.671$, $p_{2006} = 0.320$, $p_{2007} = 0.626$) were not significantly different in the atmosphere of Amares and Porto. *Alternaria* ($p_{2005} = p_{2007} = 0.000$, $p_{2006} = 0.001$), *Epicoccum* ($p_{2005} = p_{2006} = p_{2007} = 0.000$), *Leptosphaeria* ($p_{2005} = p_{2006} = 0.000$, $p_{2007} = 0.021$), *Paraphaeosphaeria* ($p_{2005} = p_{2007} = 0.000$, $p_{2006} = 0.096$) and *Venturia* ($p_{2005} = 0.000$, $p_{2006} = p_{2007} = 0.007$) spore concentrations were significantly different in both locations, being always higher at Amares (Table 1).

In the pre-peak period, *Alternaria* ($r_A = 0.475$, $r_P = 0.440$) was positively correlated with temperature. Similar results were obtained in the MSP ($r_A = 0.363$, $r_P = 0.401$) and in the totality of the year ($r_A = 0.663$, $r_P = 0.559$). During the MSP, positive correlations were found between *Pleospora* spore concentration and rainfall level ($r_A = 0.405$, $r_P = 0.415$). When the whole period was considered, *Stemphylium* spore concentration was positively correlated with temperature ($r_A = 0.399$, $r_P = 0.401$).

The intradiurnal frequency of the selected spore types was calculated. *Paraphaeosphaeria* and *Pleospora* were present in higher concentrations during the morning. *Alternaria*, *Epicoccum* and *Pithomyces* spore concentration was more elevated during the end of the afternoon and evening. *Leptosphaeria* and *Venturia* were more frequent during the night and dawn. *Drechslera* peaks were found during the morning and again during the night. *Stemphylium* presented two peaks during

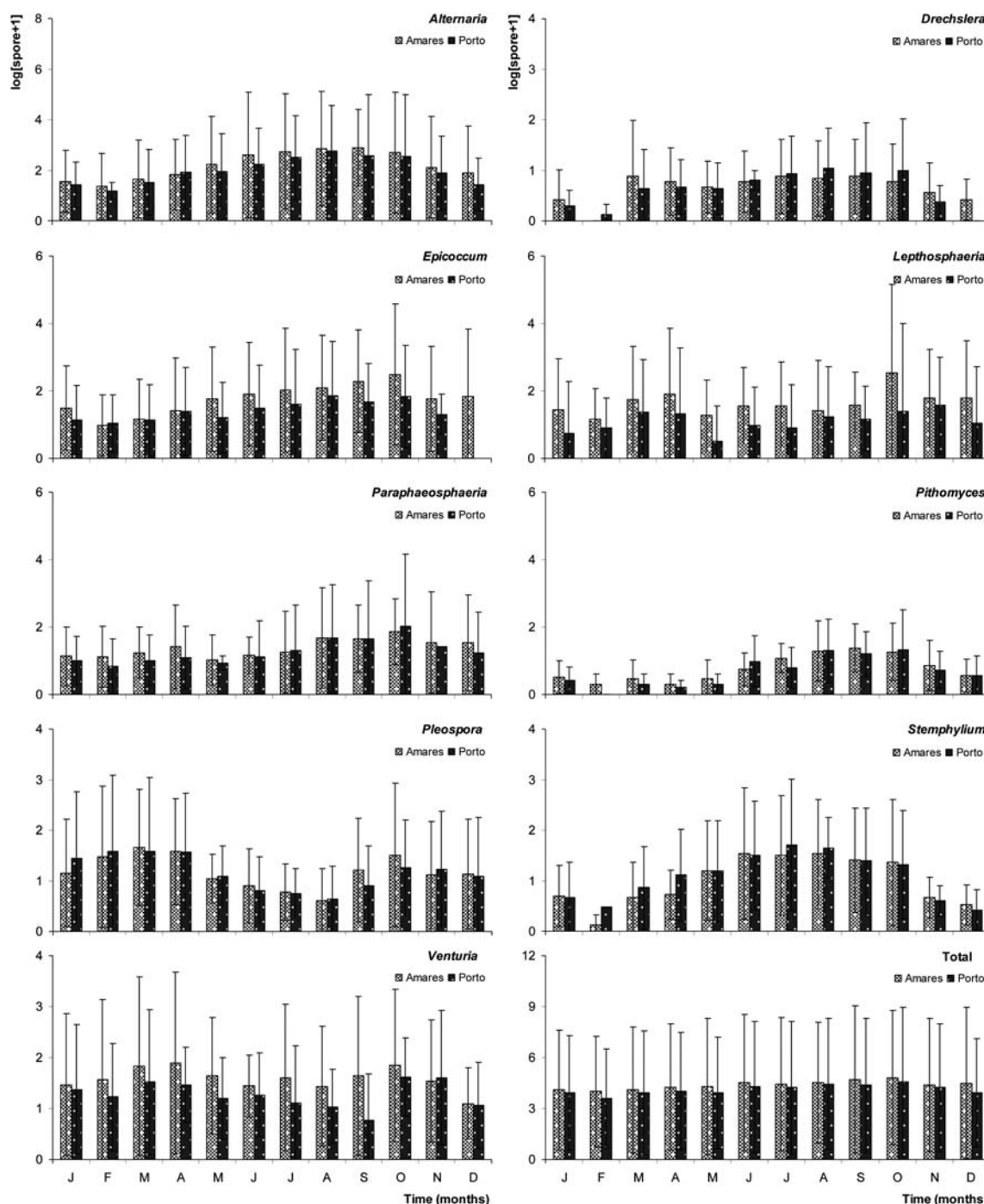


Fig. 1 Mean monthly frequencies of the selected spore types present in the atmosphere of Amares and Porto, from 2005 to 2007. Brackets represent standard deviation values.

the day, a coincidence of the second highest concentration around noon being registered. No significant differences were found between the two sampled sites ($p_{Alt} = 0.963$, $p_{Dre} = p_{Epc} = p_{Par} = p_{Pit} = p_{Plp} = p_{Sim} = p_{Vnt} = 1.000$; $p_{Lpt} = 0.674$), the concentration of *Alternaria* ($r = 0.752$, $p = 0.000$) and *Pleospora* ($r = 0.589$, $p = 0.002$) being associated. In Amares, there was a general tendency to a negative influence of the temperature and positive influence of the relative humidity on the intra-diurnal spore concentration, while in Porto the opposite happened (Table 2).

Discussion

Among the moulds capable of inducing allergic respiratory disorders, *A. alternata* is one of the most frequently encountered species, predominantly occurring in the outdoor environment. The incidence of *A. alternata* sensitization within atopic patients varies between 3.6–39.4%, depending on the climatic zone and the population tested.¹⁰ In the urban area of Porto, the sensitization prevalence to *Alternaria* is 2.5%,¹¹ although data on fungal allergen sensitization in the rural area (Amares) is lacking.

Table 1 Summary of the concentration of the selected genera of Pleosporales, from 2005 to 2007, in the atmosphere of Amares and Porto

				Occurrence				Main spolioation period			Peak	
		Total (spores m ⁻³ day ⁻¹)	Occurrence frequency (%)	Days	Percentage	Daily average (spores m ⁻³ day ⁻¹)	z score	Beginning	End	Length (days)	Value (spores m ⁻³ day ⁻¹)	Date
AMARES												
2005	<i>Alternaria</i> spp.	3742.8	1.15	315	86	10.3 ± 13.3	280.8	5-Apr	26-Oct	204	80.4	30-Jul
	<i>Drechslera</i> spp.	76.4	0.02	60	16	0.2 ± 0.6	134.4	18-Jan	7-Nov	293	6	22-Mar
	<i>Epicoccum</i> spp.	1310.4	0.4	276	76	3.6 ± 4.7	279.5	1-Mar	3-Nov	247	27.8	18-Oct
	<i>Leptosphaeria</i> spp.	688.5	0.21	132	36	1.9 ± 4.8	142.8	13-Jan	11-Nov	302	42.7	22-Apr
	<i>Paraphaeosphaeria</i> spp.	422.6	0.13	163	45	1.2 ± 2.1	200.1	6-Feb	10-Dec	307	18.8	3-Nov
	<i>Pithomyces</i> spp.	101.2	0.03	77	21	0.3 ± 0.6	156.6	17-Feb	16-Nov	272	5	13-Sep
	<i>Pleospora</i> spp.	189.5	0.06	98	27	0.5 ± 1.2	157.7	13-Jan	30-Nov	321	8.9	17-Apr
	<i>Stemphylium</i> spp.	149.8	0.05	99	27	0.4 ± 0.8	186	14-Feb	22-Oct	250	5	27-Jun
	<i>Venturia</i> spp.	753.9	0.23	183	50	2.1 ± 4.0	189	12-Jan	17-Nov	309	27.8	29-Mar
2006	<i>Alternaria</i> spp.	3175.4	0.94	274	75	8.7 ± 14.1	224.7	8-May	11-Oct	156	104.2	22-Jun
	<i>Drechslera</i> spp.	16.9	0.01	13	4	0 ± 0.3	65.7	1-Apr	7-Nov	220	2	23-Aug
	<i>Epicoccum</i> spp.	706.3	0.21	189	52	1.9 ± 3.8	186.8	28-Mar	19-Oct	205	33.7	3-Oct
	<i>Leptosphaeria</i> spp.	1207.3	0.36	117	32	3.3 ± 18.9	63.9	7-Apr	27-Nov	234	321.4	20-Oct
	<i>Paraphaeosphaeria</i> spp.	222.2	0.07	112	31	0.6 ± 1.4	159.4	19-Feb	27-Nov	281	11.9	3-Oct
	<i>Pithomyces</i> spp.	85.3	0.03	41	11	0.2 ± 1.1	79.5	27-Mar	26-Oct	213	15.9	29-Sep
	<i>Pleospora</i> spp.	262.9	0.08	98	27	0.7 ± 1.7	151.8	24-Feb	6-Dec	285	13.9	20-Oct
	<i>Stemphylium</i> spp.	152.8	0.05	83	23	0.4 ± 1.1	142.5	14-Apr	14-Oct	183	8.9	15-Jun
	<i>Venturia</i> spp.	430.5	0.13	147	40	1.2 ± 3.3	128.5	23-Feb	22-Nov	272	49.6	13-Sep
2007	<i>Alternaria</i> spp.	3542.4	1.01	279	79	9.7 ± 13.3	265.6	9-May	24-Nov	199	109.1	4-Oct
	<i>Drechslera</i> spp.	57.5	0.01	49	14	0.2 ± 0.4	135	8-May	14-Nov	190	2	8-Jul
	<i>Epicoccum</i> spp.	1180.5	0.34	234	66	3.2 ± 5.9	199.1	18-May	10-Dec	206	63.5	5-Oct
	<i>Leptosphaeria</i> spp.	494	0.14	83	23	1.4 ± 4.2	117.1	11-Feb	7-Dec	299	37.7	7-Dec
	<i>Paraphaeosphaeria</i> spp.	361.1	0.1	122	34	1.0 ± 2.4	149.6	10-Feb	8-Dec	301	19.8	23-Oct
	<i>Pithomyces</i> spp.	88.3	0.03	58	16	0.2 ± 0.6	137.7	9-Jul	13-Dec	157	4	16-Sep
	<i>Pleospora</i> spp.	205.3	0.06	62	17	0.6 ± 2.7	77.1	8-Jan	1-Oct	266	40.7	7-Feb
	<i>Stemphylium</i> spp.	228.2	0.06	117	33	0.6 ± 1.3	180.6	7-May	21-Oct	167	11.9	3-Oct
	<i>Venturia</i> spp.	318.4	0.09	119	33	0.9 ± 1.9	163.3	9-Feb	20-Nov	284	17.9	23-May
PORTO												
2005	<i>Alternaria</i> spp.	1975.1	1.09	263	72	5.4 ± 8.7	225.2	3-Apr	29-Oct	209	47.6	18-Aug
	<i>Drechslera</i> spp.	54.6	0.03	45	12	0.1 ± 0.4	121.1	9-Mar	25-Oct	230	4	30-Jul
	<i>Epicoccum</i> spp.	545.6	0.03	201	55	1.5 ± 2.3	232.3	16-Feb	15-Nov	272	14.9	30-Jul
	<i>Leptosphaeria</i> spp.	184.5	0.1	63	17	0.5 ± 1.7	111	19-Jan	17-Nov	302	15.9	29-Oct
	<i>Paraphaeosphaeria</i> spp.	157.7	0.09	99	27	0.4 ± 0.9	182.8	18-Jan	8-Dec	324	4	22-Apr
	<i>Pithomyces</i> spp.	86.3	0.05	50	14	0.2 ± 0.9	101.1	1-May	23-Oct	175	9.9	8-Aug
	<i>Pleospora</i> spp.	145.8	0.08	87	24	0.4 ± 0.9	169	9-Jan	25-Nov	320	5	27-Mar
	<i>Stemphylium</i> spp.	178.6	0.1	104	29	0.5 ± 1.0	176.3	23-Mar	20-Oct	211	7.9	4-Jul
	<i>Venturia</i> spp.	321.4	0.18	122	33	0.9 ± 2.1	156.3	8-Jan	29-Nov	325	15.9	24-Jul
2006	<i>Alternaria</i> spp.	1881.8	0.96	255	70	5.2 ± 9.2	202.9	25-Apr	26-Oct	184	81.3	29-Aug
	<i>Drechslera</i> spp.	32.7	0.02	27	7	0.1 ± 0.4	88.8	2-Feb	1-Oct	241	4	28-Jun
	<i>Epicoccum</i> spp.	296.6	0.15	153	42	0.8 ± 1.4	206.6	28-Feb	18-Nov	263	11.9	27-Aug
	<i>Leptosphaeria</i> spp.	180.5	0.09	68	19	0.5 ± 1.7	107.6	25-Feb	2-Dec	280	19.8	15-Aug
	<i>Paraphaeosphaeria</i> spp.	146.8	0.07	87	24	0.4 ± 9.0	1648	2-Mar	17-Nov	260	5	15-Oct
	<i>Pithomyces</i> spp.	47.6	0.02	37	10	0.1 ± 0.4	111	14-Mar	23-Nov	254	3	21-Jun
	<i>Pleospora</i> spp.	274.8	0.14	117	32	0.8 ± 1.7	158	20-Jan	16-Dec	330	14.9	18-Mar
	<i>Stemphylium</i> spp.	191.5	0.1	98	27	0.5 ± 1.2	157.3	14-Apr	3-Oct	172	10.9	17-Jun
	<i>Venturia</i> spp.	238.1	0.12	106	29	0.7 ± 1.5	160.4	19-Jan	26-Nov	311	14.9	5-Nov
2007	<i>Alternaria</i> spp.	2624.8	1.37	249	73	7.2 ± 12.2	213.9	23-Apr	3-Nov	194	85.3	8-Oct
	<i>Drechslera</i> spp.	70.4	0.04	47	14	0.2 ± 0.6	123.7	18-May	23-Oct	158	4	20-Sep
	<i>Epicoccum</i> spp.	623	0.32	183	54	1.7 ± 4.1	151.6	18-Apr	2-Dec	228	51.6	7-Oct
	<i>Leptosphaeria</i> spp.	149.8	0.08	52	14	0.4 ± 2.0	74.3	17-Feb	1-Dec	287	31.7	19-Nov
	<i>Paraphaeosphaeria</i> spp.	248	0.13	96	28	0.7 ± 1.8	138	19-Feb	17-Dec	301	17.9	3-Oct
	<i>Pithomyces</i> spp.	104.2	0.05	68	20	0.3 ± 0.7	145.4	22-Jun	14-Dec	175	6	2-Oct
	<i>Pleospora</i> spp.	217.2	0.11	91	27	0.6 ± 1.5	140.7	12-Jan	2-Oct	263	14.9	6-Feb
	<i>Stemphylium</i> spp.	257.9	0.13	105	31	0.7 ± 1.6	157.3	12-Apr	10-Oct	181	16.9	4-Jul
	<i>Venturia</i> spp.	184.5	0.1	79	22	0.5 ± 1.5	126.5	31-Jan	19-Nov	292	15.9	24-Oct

Alternaria spp. spores constituted more than 1% of the fungal spectrum of Amares and Porto, being present in more than 70% of the sampling period. A recent work performed in Madrid presented a similar abundance of *Alternaria* spores, although this spore was more frequent in the atmosphere of Portugal.¹² In Amares, the *Alternaria* threshold value was surpassed twice

during the period studied. When the seasonal pattern was considered, spore concentrations started to increase in the beginning of the summer, achieved the maximum values during late summer, and remained elevated in early autumn. It has been recently proposed that the *Alternaria* intradiurnal variation was more acute inland than along the coastline due to oceanic

Table 2 Correlation between the intradiurnal fungal spore concentration from selected genera of Pleosporales, and temperature and relative humidity factors, from 2005 and 2007 (Pearson correlation coefficients with a significance level of 99% are presented in bold, regular numbers correspond to a significance level of 95% whereas non-significant correlations are presented in italics)

Fungal spore	Year	AMARES		PORTO	
		Tmean	RHmean	Tmean	RHmean
<i>Alternaria</i>	2005	-0.203	0.063	0.270	-0.289
	2006	-0.413	0.667	0.289	-0.289
	2007	0.139	-0.045	0.228	-0.224
<i>Drechsbra</i>	2005	-0.057	-0.015	0.227	-0.201
	2006	-0.665	0.557	-0.082	0.037
	2007	-0.783	0.687	0.142	-0.155
<i>Epicoccum</i>	2005	-0.588	0.157	0.029	-0.066
	2006	-0.377	0.580	0.445	-0.500
	2007	-0.328	0.367	0.584	-0.533
<i>Leptosphaeria</i>	2005	-0.796	0.678	^a	^a
	2006	^a	^a	0.118	-0.085
	2007	-0.667	0.656	0.446	-0.448
<i>Paraphaeosphaeria</i>	2005	-0.016	0.177	-0.348	0.340
	2006	-0.116	-0.248	0.158	-0.194
	2007	-0.077	0.033	0.364	-0.305
<i>P. Uhomycetes</i>	2005	-0.308	0.359	-0.428	0.438
	2006	0.232	-0.101	0.297	-0.253
	2007	-0.255	0.171	0.304	-0.408
<i>Reospoia</i>	2005	-0.566	0.672	-0.401	0.341
	2006	0.176	-0.380	0.028	-0.204
	2007	-0.267	0.207	-0.293	0.252
<i>Stemphylium</i>	2005	-0.715	0.747	0.314	-0.328
	2006	-0.247	-0.174	0.491	-0.468
	2007	-0.370	0.486	0.509	-0.609
<i>Venturia</i>	2005	-0.435	0.269	-0.566	0.582
	2006	-0.815	0.659	-0.118	0.182
	2007	-0.378	0.440	0.562	-0.338

^a The intradiurnal pattern was not calculated.

influence.¹³ In this study, the contrary happened, since a wider range of values was obtained in Porto than in Amares. These fungi were considered as evening sporulators, since peak daily concentrations were found during the evening in Porto and Amares. Work performed in Spain revealed a similar intradiurnal pattern, where the maximum concentrations were observed between 18:00 and 19:00.¹⁴

Although *Drechslera*-type spores are frequently included in skin test surveys, only a preliminary characterization of the allergens has been reported.¹⁵ *Drechslera* spores were present in the atmosphere of the two locations in 11% of the sampled period. In the atmosphere of Madrid, *Drechslera* spores were more abundant and frequent.¹² *Drechslera* spore concentrations started to increase in June, achieved the maximum values from July to September, and decreased in November. *Drechslera*-type spores presented two peaks during the day, one during the morning and the second during the night. No consistent correlations were obtained between this fungal type and the meteorological factors analysed in any of the considered periods. The *Drechslera*-type group includes *Bipolaris*, *Drechslera*, *Exserohilum*, *Helminthosporium* conidia; these genera are difficult to distinguish using an optic microscope.¹⁶ This fact, together with the sporadic behaviour of this spore type, could explain the lack of correlations, since each spore may be differentially influenced by those meteorological factors.

Epicoccum nigrum, the only species in this genus, is reported frequently in air samplings. To date, studies indicate skin test and RAST reactivity to *E. nigrum* ranging from 20 to 30% in atopic subjects.¹⁵ *Epicoccum* spp. spores were the second most frequent analyzed spores. Spore concentration started to increase in June, reached the maximum values in October, and decreased towards the end of the year. Like *Alternaria*, *Epicoccum* was positively influenced by temperature and negatively by relative humidity and rainfall. In fact, these two fungal spores are commonly considered dry-air spores, being found in higher concentrations during warm, dry weather conditions with high wind speeds.^{17,18} Nevertheless, it has also been found that *Epicoccum* spp. was not correlated with weather variables, but did display a seasonal cycle.¹⁹

Leptosphaeria spores, the most frequent spore during rainy weather and known to produce positive skin-prick test reactions, were present in 30% of the sampling period. The high levels of these spores have been associated with asthma-related emergency department visits in children.²⁰ Ascospores and pycnidiospores were trapped between 9:00 P.M. and 4:00 A.M., when air temperatures were 13–18 °C and relative humidity was greater than 80%. Peak ascospore and pycnidiospore dispersal was associated with rain events, and ascospore dispersal continued for approximately 3 days after such events.²¹

Paraphaeosphaeria spores were present in the atmosphere of the two locations in 30% of the studied period. Like for *Epicoccum* spores, *Paraphaeosphaeria* spores were much more abundant and frequent in the atmosphere of Portugal than in Spain.¹² *Paraphaeosphaeria* spore concentrations started to increase in August, achieved the maximum values in October, and remained elevated in the two following months.

Pithomyces spores were present in the atmosphere of the two locations in 15% of the days. The spores from this fungal type were more abundant and frequent both in the atmospheres of Amares and Porto than in the one of Madrid.¹² In Amares, *Pithomyces* spore concentrations started to increase in July, achieved the maximum values in September, remained elevated in October, and decreased in November. In Porto, this seasonal pattern suffered a one-month delay.

Pleospora spores were present in the atmosphere of the two locations in more than 24% of the studied period. In the atmosphere of Madrid,¹² these spores were more abundant and more frequent. *Pleospora* spore concentrations started to increase in the first months of the year, achieved the maximum values in March, and remained elevated until April. Moreover, in Amares, high spore concentrations were also found in September and October. *Stemphylium* spores were present in the atmosphere of the two locations in more than 28% of the days. This spore type was much more abundant and frequent in the Portuguese locations than in the Spanish one.¹² *Stemphylium* spore concentrations achieved the maximum values in early summer, with elevated values being found until October.

Venturia spores were present in the atmosphere of the two locations in 34% of the sampled period. The fungus *Venturia inaequalis* is the causative agent of apple scab, an important disease of apple orchards throughout the world, being mainly initiated in spring time by ascospores, which are discharged from infected leaves on the ground during periods of rain.²² In fact, in the rural area, the maximum value of this spore type occurred

during spring, while throughout the summer, the cycle of secondary infections continues until the leaves and fruit fall from the tree at the onset of winter, explaining the late peak registered in September in Porto.

When the spore concentration was compared among the two locations, different patterns were observed. During the three years studied, *Alternaria*, *Epicoccum*, *Leptosphaeria*, *Paraphaeosphaeria* and *Venturia* spore concentrations were higher in the rural area than in the urban site, while no significant differences were observed for the remaining spore types. Previous works performed in Europe studied the occurrence of different fungal spore types (namely *Alternaria*, *Epicoccum*, *Pithomyces*, *Stemphylium* and *Drechslera*) in the atmosphere of an urban and rural area, and concluded that the total seasonal sum of all the spores was higher in the countryside than in the city.^{23,24} It has been proposed that rural regions, with lower urbanisation indexes, present higher amounts of organic material than urban areas; this would provide the substrate for these fungi to grow on as parasites or saprophytes, thereby providing the most likely reason why their spores are more common in the air of the rural environments.²³

Alternaria, *Epicoccum*, *Pithomyces* and *Pleospora* presented the same occurrence pattern in other countries.^{19,25–32} Contrarily, different seasonal patterns have been reported for *Drechslera* and *Stemphylium*.^{16,30,31,33}

Conclusion

The current data on the presence of the studied spores in the atmosphere should become a basic tool of any allergologist practitioner. *Alternaria* peak concentration ranges from the early summer to early autumn, coinciding with the maximum values of other species, such as *Epicoccum*, *Pithomyces* and *Stemphylium*, contributing to the increase in the atmospheric availability of Alt a 1. During this period, which coincides with the summer holidays, there is an increase in outdoor activities, contributing to the over-exposition to this aeroallergen, favouring the aggravation of allergic symptoms in sensitized patients. Nevertheless, when the other species included in this study were considered, the occurrence of Pleosporales spores was expanded from January, mainly due to the atmospheric presence of *Drechslera* and *Pleospora*, until December, due to the occurrence of *Paraphaeosphaeria*. The presence of airborne Pleosporales spores throughout the year consequently extends the period in which it is possible to detect the presence of the Alt a 1 aeroallergen.

Nevertheless, in everyday practice, only the data on the occurrence of 2 taxa are usually taken into account: *Alternaria* and *Cladosporium* spp. However, the present work demonstrates the importance of other fungal spore types in the concentration of Alt a 1 allergens.

The availability of the Alt a 1 allergen was higher in the rural area; nevertheless, the prevalence of allergies in urban areas seems to be higher. This fact can be explained by the airway sensitization induced by the modulation of airborne allergens' allergenicity via the air pollutants. But also, the airway mucosal damage and impaired mucociliary clearance induced by air pollution may facilitate the access of inhaled allergens to the cells of the immune system.

Abbreviations

A	Amares
Alt	Alternaria
Dre	Drechslera
Epc	Epicoccum
Lpt	Leptosphaeria
Par	Paraphaeosphaeria
Pit	Pithomyces
Plp	Pleospora
Stm	Stemphylium
Vnt	Venturia
P	Porto

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