IDENTIFICATION OF FUNGAL SPORES IN THE ATMOSPHERE OF SANTIAGO DE COMPOSTELA (NW SPAIN) IN THE WINTER PERIOD

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SUMMARY: A quantitative and qualitative study of fungal spores in the atmosphere of Santiago de Compostela (NW Spain) was carried out from December 21st, 1997, to March 21st, 1998. The total number of spores collected was 35,987, corresponding to 47 morphological types, including numerous spores that cause respiratory allergies such as *Coprinus* and *Aspergillus-Penicillium*, important plant pathogens such as *Pleospora* and *Paraphaeosphaeria*, as well as spores of clinical and phytopathogenic interest, including *Cladosporium* and *Leptosphaeria*. The maximum value of the total spores was recorded on January 3rd with 1,467 spores/m^3 and the minimum on March 9th with 63 spores/m^3. The influence of the main meteorological factors on spore concentration was studied, with humidity, maximum temperature and hours of sunshine being the most significant parameters for the majority of taxa. Finally, the models of intra-diurnal variation for the most frequent spore types in the city atmosphere are proposed.
KEY WORDS: aerobiology, aeromycology, winter, Santiago, NW of Spain

RESUMEN: Se ha realizado un estudio cuantitativo y cualitativo de las esporas de hongos en la atmósfera de Santiago de Compostela (NO España) desde el 21 de diciembre de 1997 al 21 de marzo de 1998. El número total de esporas recogidas ha sido de 35.987, que corresponden a 47 tipos morfológicos, entre los que se identificaron numerosas esporas causantes de alergias respiratorias como *Coprinus* y *Aspergillus-Penicillium*, importantes patógenos vegetales como *Pleospora* y *Paraphaeosphaeria*, así como esporas que destacan tanto por su interés clínico como fitosanitario, entre ellas, *Cladosporium* y *Leptosphaeria*. El valor máximo del total de esporas se registró el día 3 de enero con 1.467 esporas/m^3 y el mínimo el 9 de marzo con 63 esporas/m^3. Se ha estudiado la influencia de los principales factores meteorológicos en la concentración de esporas, resultando la humedad, la temperatura máxima y las horas de sol, los parámetros más significativos para la mayoría de los taxones. Finalmente, se proponen los modelos de variación intradiurna para los tipos de esporas más frecuentes en la atmósfera de la ciudad.
PALABRAS CLAVE: aerobiología, aeromicología, invierno, Santiago, NO España.

INTRODUCTION

Aerobiological studies enable us to ascertain the concentration of fungal spores in the atmosphere and their application is of phytopathogenic and clinical interest. According to the World Health Organization, allergic rhinitis is an illness that affects 5-25% of the inhabitants of developed countries and its prevalence is on the increase the world over. This inflammatory condition of the upper respiratory tract is caused by a hypersensitivity reaction triggered in the nasal mucus and, in many cases, it is related to the presence of the pollen of different plants and fungal spores (COSENTINO & PALMAS, 1996). Respiratory allergies caused by fungi may affect more than 30% of the allergic human population.

The presence of fungal spores in the atmosphere is common in all areas around the planet, although there are large qualitative and quantitative differences according to the time of year and the geographical area (HALWAGI, 1994). The most frequent conidial types include *Cladosporium* and *Alternaria*, which are both cosmopolitan and highly allergenic (CARETTA, 1992; PÉREZ *et al.*, 1992).

In Galicia (Spain), most of the aerobiological studies that have been systematically carried out by the R.I.A.G. (Galician Aerobiological Research Network) have focussed on atmospheric pollen control (MÉNDEZ, 2000; RODRÍGUEZ, 2000; DOPAZO, 2001), with few references to the mycobiota, being mainly applied to the prevention of crop plagues (DÍAZ, 1999) or routine counting of the most frequent genera (DOMÍNGUEZ MARIÑO, 1997; AIRA & LA-SERNA, 1999; DOPAZO *et al.*, 2002). This paper presents for the first time, in the case of Galicia, the total count of fungal spores identifiable by their morphological characteristics under an optic microscope. The study was carried out in the winter (December 21st, 1997 to March 21st, 1998), since this is the period of greatest diversity.

MATERIAL AND METHODS

The city of Santiago de Compostela (Fig. 1) is situated in the northwestern corner of the Iberian Peninsula and from a biogeographical point of view, is included in the Atlantic Province of the Euro-Siberian Region, having a maritime-temperate climate of high humidity and frequent precipitations in the winter months.

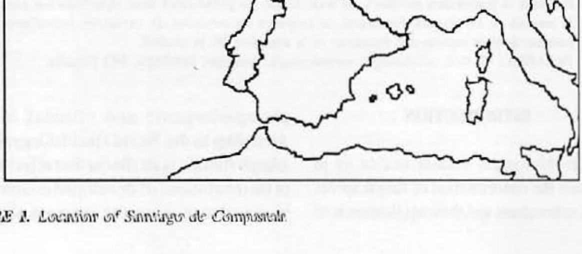

FIGURE 1. Location of Santiago de Compostela

The samples were collected with a Hirst-type trap, Lanzoni VPPS-2000 model, situated in the south campus of the University of Santiago de Compostela at a height of 27 m above ground level and processed following the methodology proposed by the Spanish Aerobiology Network (DOMÍNGUEZ *et al.*, 1991).

The spores were identified using different analytical keys and studies (ELLIS, 1971, 1976; DENNIS, 1978; NILSSON, 1983; ARX, 1987; DOMÍNGUEZ SANTANA, 1992; DOMÍNGUEZ SANTANA & LA-SERNA, 1998), as well as reference plates.

In order to determine the correlations between spore concentrations and meteorological parameters we applied Spearman's test using the *Statistica* computer programme. We also studied the intra-diurnal representation of the most abundant spores (*Coprinus, Cladosporium* and *Aspergillus-Penicillium*) by following the proposals of different authors (GALÁN *et al.*, 1991; DOMÍNGUEZ SANTANA & LA-SERNA, 1998), which resulted in three models. The first model (Model 1 in Figure 6) involved calculating the average of each spore type, always using as the denominator the total number of days of the study's duration (91 days). We then selected the dry days on which the concentration of the corresponding spore had a daily value equal to or higher than the said average. The value represented each hour on the graph corresponds to the sum of the values of that hour expressed as a percentage of the spore type's total during the selected days.

The second and third models (Models 2 and 3 in Figure 6) take into account all of the data of the study period, regardless of whether precipitation was recorded or not, and in both cases percentage graph representation was chosen to make them comparable to the first model. In the second model (2), the graphically expressed value corresponds to the sum of the hourly values, while in the third model (3) it corresponds to the average of each hour. The said average was calculated by dividing the sum of the average of each hour by the number of days on which the corresponding spore was present, thereby obtaining an ideal day. In this case, therefore, the denominator was different for each taxon and each hour.

RESULTS

During the study period we recorded a total of 35,987 spores corresponding to 47 morphological types (Tab. 1), among which *Cladosporium, Coprinus* and *Aspergillus-Penicillium* are the most abundant (Fig. 2). Of the genera identified, 5 were classified as allergenic, 15 are of phytopathogenic interest and 12 share both characteristics (CARETTA, 1992; PÉREZ *et al.*, 1992, among others). The representation of spores considered as a whole varied throughout the study period (Fig. 3), with the maximum values being concentrated in the first fortnight of January and secondary peaks at the beginning of February and the end of March. The running average obtained with values of 5 days shows a gradual increase in the concentration of spores at the end of 1997, which peaked at the beginning of 1998. In relation to the monthly distribution, January was the month with the greatest concentration, with 15,111 spores, followed by February with practically half of this value.

The meteorological data was provided by the University of Santiago's Astronomical Observatory, situated in the surroundings of the pollen trap. During the study period, total precipitation of 521 mm was recorded, with

FUNGAL SPORES	N° days	Max.	Day	Spores/m^3
Alternaria	38	11	28-I-98	82
Arthrinium	7	2	25-XII-97	8
Ascobolus	3	2	20-I-98	4
Aspergillus-Penicillium	84	141	4-II-98	3234
Bipolaris	24	6	10-I-98	40
Botrytis	9	4	6-I-98	16
Cercospora	1	2	4-I-98	2
Cerebella	5	2	24-II-98	7
Chaetoconis	1	10	18-II-98	10
Chaetomium	25	13	17-III-98	56
Cladosporium cladosporioides	90	97	11-III-98	2281
Cladosporium herbarum	91	476	27-XII-97	4050
Coprinus	90	814	31-I-98	7971
Curvularia	12	2	28-XII-97	14
Delitschia	1	3	1-I-98	3
Drechslera	14	2	23-I-98	17
Exerohilum	17	2	3-I-98	21
Fusarium	33	6	5-III-98	70
Fusicladium	3	2	24-I-98	4
Gliomastix	6	1	30-XII-97	6
Heliconia	1	1	11-II-98	1
Heliconyces	20	5	8-I-98	32
Inocybe	2	3	21-XII-97	4
Leptosphaeria	57	20	26-XII-97	245
Lophiostoma	63	21	21-II-98	180
Lycogala	4	6	1-I-98	9
Massarina	50	10	28-XII-97	131
Melanomma	8	3	1-I-98	13
Monodictys	7	6	8-II-98	16
Myxomycetes	36	20	7-III-98	133
Nigrospora	18	15	3-I-98	43
Oidium	3	4	23-XII-97	9
Paraphaeosphaeria	54	13	29-XII-97	152
Periconia	9	11	21-II-98	40
Peronospora	18	7	13-II-98	31
Pestalotiopsis	23	13	16-I-98	45
Pithomyces	12	5	1-I-98	23
Pleospora	66	36	21-II-98	285
Polythrincium	19	9	20-II-98	41
Pseudocercospora	3	1	25-XII-97	3
Sporidesmium	2	1	9-I-98	2
Stemphylium	8	9	10-I-98	16
Tetraploa	2	1	26-I-98	2
Torula	69	19	10-I-98	269
Venturia	36	6	16-I-98	84
Xylariaceae	5	2	28-XII-97	6

TABLE 1. Fungal spores identified.

a daily average of 5.7 mm (Fig. 4). The average humidity was 78% and the average temperature of the averages was 11°C. The average value of hours of sunshine during the winter period was 4 hours 24 minutes (Fig. 5).

The correlations with the main meteorological parameters (rainfall, humidity, temperature and hours of sunshine) were calculated with all the spore types that exceeded values of 70 spores/m^3 during the study period, as well as for the genere *Cladosporium (Cladosporium cladosporioides + Cladosporium herbarum)* and for total spores (Tab. 2), taking into account the total of the data obtained during the study period. The meteorological parameter that influenced the greatest number of spore types was humidity, followed by maximum temperature and hours of sunshine. These three factors explain a high percentage of variability in the data of many taxa and of total identified spores. No significant correlations for *Alternaria, Cladosporium herbarum, Lophiostoma* and *Myxomycetes* were found with any of the said factors.

Precipitation was positive and hours of sunshine negative with a high significance (99%) for four spore types (*Leptosphaeria, Massarina, Paraphaeosphaeria* and *Venturia*), as well as for total spores. Humidity and maximum temperature were highly significant (99%) for five spore types (*Cladosporium cladosporioides, Leptosphaeria, Paraphaeosphaeria, Torula* and *Venturia*), with this last genus producing a highly significant correlation with all of the meteorological parameters except average temperature.

In relation to intra-diurnal variation (Fig. 6), there are no large differences among the three models represented, especially the ones in which the overall data of the study period was taken into account (Models 2 and 3), without excluding the days of rainfall.

The results obtained show that *Cladosporium cladosporioides* spores, in the winter period, were more abundant during the central hours of the day (from 11 am to 6 pm), with a maximum at 6 pm and secondary peaks during the morning. Furthermore, the inflection

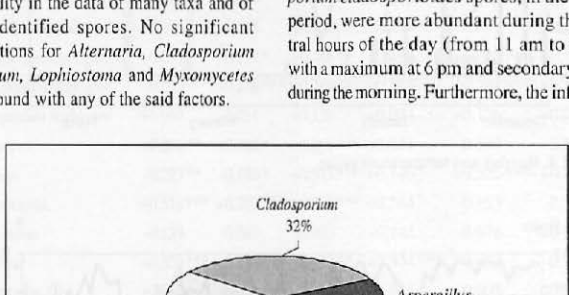

FIGURE 2. Most abundant fungal spores.

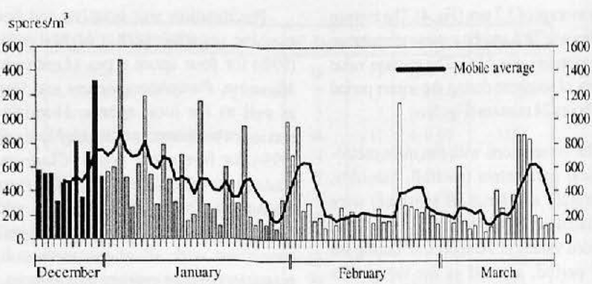

FIGURE 3. Daily fungal spores concentration.

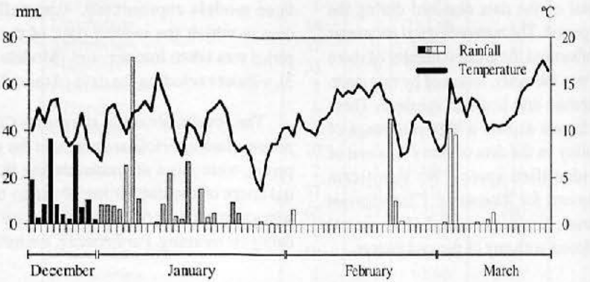

FIGURE 4. Rainfall and temperature values.

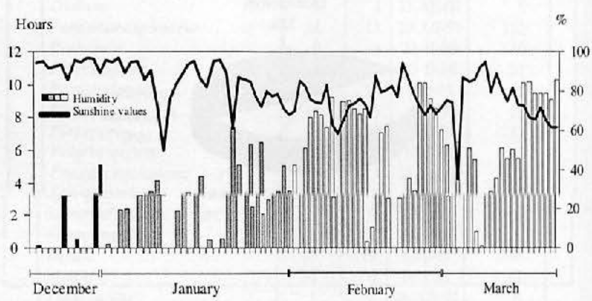

FIGURE 5. Humidity and sunshine values.

points coincide in the three models, being situated between the maximums. The *Cladosporium herbarum* spores are more represented between 1 pm and 10 pm, in addition to a peak at 1 am. Overall, both spore types have a greater representation towards the end of the morning and the beginning of the afternoon, with their maximum value at 6 pm.

The *Aspergillus/Penicillium* group shows an irregular distribution curve throughout the day, with three intervals of greater abundance, between 4 pm and 6 pm, between 11 am and 1 pm and between 7 pm and 11 pm. Finally, *Coprinus* spores, with a very homogeneous representation in the three models, are clearly more abundant from 2 am to 8 am and from 8 pm to midnight.

DISCUSSION

Numerous authors point out that the atmospheric concentration of fungal spores is greater during the spring and summer (GALÁN *et al.*, 1998; BUSTOS *et al.*, 2001), being related in some cases to the temperature increase (EMBERLIN *et al.*, 1995). In this regard, previous studies carried out in Santiago de Compostela (DOMÍNGUEZ MARIÑO, 1997) have also shown that the main period of spore collection took place from June to September, although the greatest variation takes place in the winter period (HERRERO *et al.*, 1996).

Different Spanish cities have reported a higher concentration of spores of *Leptosphaeria*, *Venturia*, *Fusarium* and *Ustilago*

FUNGAL SPORES	Rainfall	Humidity	T° max.	T° min.	T° med.	Sunshine
Alternaria	+0.107	-0.084	+0.090	+0.078	+0.099	-0.157
Aspergillus-Penicillium	-0.076	-0.148	+0.021	-0.214 **	-0.077	+0.145
Cladosporium clad.+herb.	+0.031	-0.177 *	+0.246 **	+0.104	+0.226 **	+0.126
Cladosporium cladospor.	-0.022	-0.278 ***	+0.283 ***	+0.133	+0.283 ***	+0.177 *
Cladosporium herbarum	+0.050	-0.031	+0.139	+0.074	+0.126	+0.018
Coprinus	+0.206 **	+0.188 *	-0.261 ***	+0.041	-0.137	-0.215 **
Fusarium	-0.223 **	-0.198 *	+0.315 ***	+0.140	+0.320 ***	+0.208 **
Leptosphaeria	+0.518 ***	+0.397 ***	-0.362 ***	+0.164	-0.153	-0.517 ***
Lophiostoma	-0.139	-0.047	-0.005	-0.145	-0.076	+0.082
Massarina	+0.336 ***	+0.353 ***	-0.239 **	+0.304 ***	-0.023	-0.294 ***
Mixomycetes	+0.012	+0.027	-0.069	+0.045	-0.005	-0.064
Paraphaeosphaeria	+0.395 ***	+0.416 ***	-0.344 ***	+0.254 **	-0.061	-0.428 ***
Pleospora	+0.286 ***	+0.030	-0.012	+0.305 ***	+0.172 *	-0.216 **
Torula	-0.153	-0.501 ***	+0.445 ***	+0.052	+0.345 ***	+0.275 ***
Uredosporas	-0.046	-0.257 ***	+0.133	+0.050	+0.133	+0.116
Venturia	+0.437 ***	+0.475 ***	-0.255 ***	+0.308 ***	+0.007	-0.452 ***
Total spores	+0.395 ***	+0.277 ***	-0.253 **	+0.163	-0.092	-0.332 ***

TABLE 2. Correlations between spore concentration and metereological parameters. Significance levels: *, 90%; **, 95%; ***, 99%.

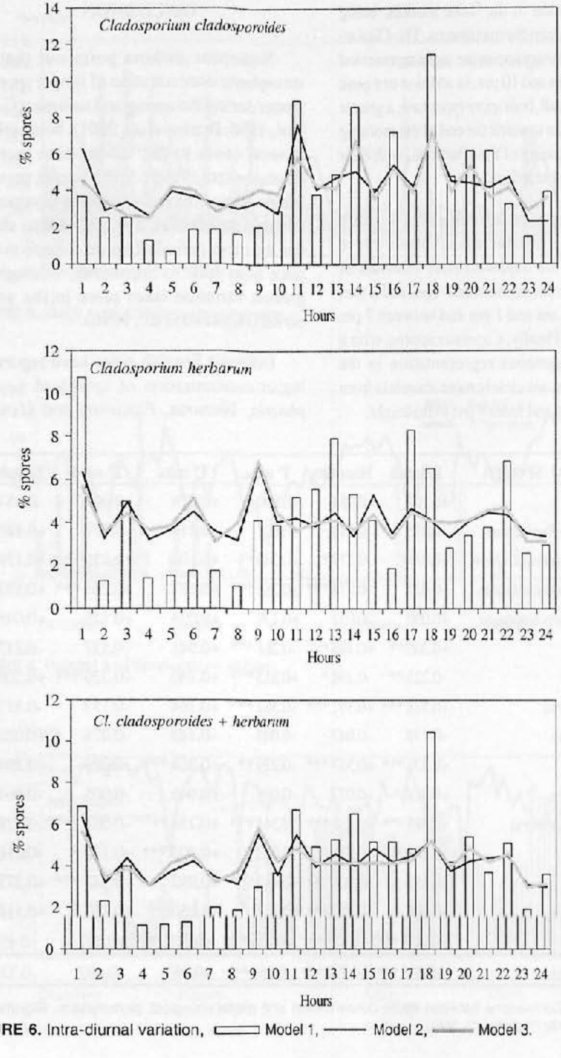

FIGURE 6. Intra-diurnal variation, Model 1, —— Model 2, —— Model 3.

(DÍAZ *et al.*, 1998) during the winter, as well as of *Coprinus* in autumn and of *Aspergillus-Penicillium* during February (DOMÍNGUEZ SANTANA, 1992).

Of the 47 types of fungal spores identified in this study, Coprinaceae basidiospores, which represent 40% of total identified spores, and the *Aspergillus-Penicillium* group (16%) stand out because of their abundance and allergenic importance (GREGORY & HIRST, 1952; LEHRER & HORNER, 1990). Many Coprinaceae stand out due to their high production capacity, such as *Coprinus comatus*, which emits 2.6 x 10^9 spores per day, and due to their high degree of wind dispersion (LEVETIN, 1990); in some cities they are the second-most abundant type after the Deuteromycotina with a representation of 32% in urban-residential areas and 28% in urban-business areas (CALDERÓN *et al.*, 1995).

The *Aspergillus-Penicillium* group includes spores that are difficult to separate

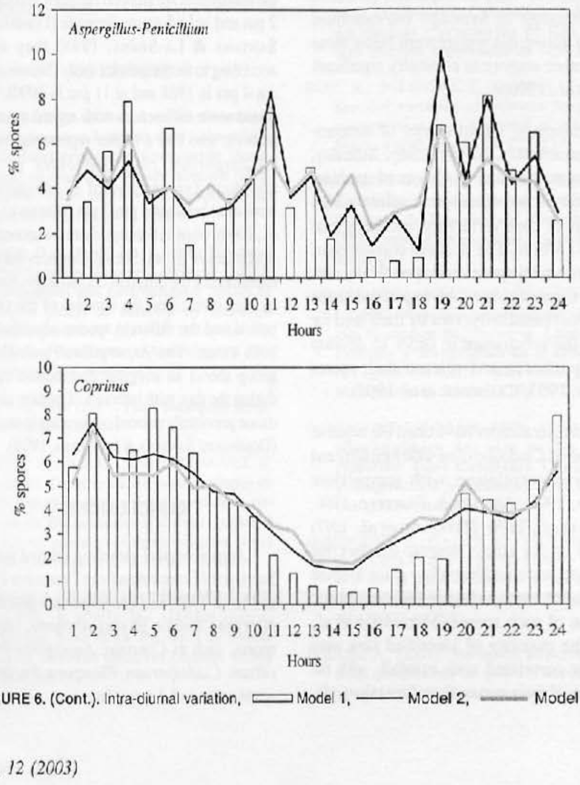

FIGURE 6. (Cont.). Intra-diurnal variation, Model 1, —— Model 2, —— Model 3.

by means of optical microscopy, since aerobiological samples frequently contain small conidia (2 to 7 microns) -rather than conidiophores- of proven allergenic capacity (PALMAS *et al.*, 1997).

The most important spores, from both clinical and pathogenic point of view, included those of *Cladosporium* (32% of total spores) and *Alternaria* (ESCAMILLA *et al.*, 1995; CADMAN *et al.*, 1997; GONZÁLEZ *et al.*, 1994).

Alternaria is extremely abundant in dry and arid areas, where daily maximums of up to 6,000 spores/m^3 may be recorded (CARETTA, 1992), although in Santiago the quantities identified during the winter were below those cited by other authors as clinically significant (FADDA *et al.*, 1990).

In relation to the influence of meteorological parameters, in our study, humidity, maximum temperature and hours of sunshine, in addition to precipitation, explain a high percentage of data variability, including total spores as a whole. The positive correlation of rainfall with total spores and specifically with those of *Coprinus*, may be explained in the case of most basidiomycetes by their need for water in the substratum in order to develop their carpophores and release their spores (HASNAIN, 1993; CALDERÓN *et al.* 1995).

Numerous authors have cited the negative correlation of *Cladosporium* with humidity and its positive correlation with temperature (HASNAIN, 1993; AIRAUDI & FILIPELLO, 1996; HERRERO *et al.*, 1996; MITATAKIS *et al.*, 1997) observed in our study, despite the fact that high temperature and humidity prior to spore dissemination seem to favour the atmospheric dispersion of such spores (MEDIAVILLA *et al.*, 1998). The majority of identified taxa were positively correlated with rainfall, with the dispersion of their spores therefore taking place at the onset of rainfall or directly thereafter, which is not the case of *Fusarium*, as pointed out by WAHL & KERSTEN (1991).

With regard to intra-diurnal variation, the results obtained for *Coprinus* basically agree with those of CALDERÓN *et al.* (1995), since a lower representation in the central hours of the day is observed in both cases. Conversely, *Cladosporium* spores are more abundant towards the end of the morning and during the early hours of the afternoon, with the maximum value being recorded at 6 pm (DOMÍNGUEZ MARIÑO, 1997), while in other Spanish cities the maximums are attained between midnight-2 pm and in La Laguna-Tenerife (DOMÍNGUEZ SANTANA & LA-SERNA, 1998) they vary according to the year under study (between 11 am-4 pm in 1988 and at 11 pm in 1990). We found some differences with regard to other authors, who cite a higher representation of *Cladosporium cladosporioides* in the afternoon-evening and of *Cladosporium herbarum* between 1 pm-3 pm (MEDIAVILLA *et al.*, 1998), while in Santiago there is a secondary maximum at 10 pm. Such differences may be explained by the different climatology characterizing both cities, the duration of the study period and the different species identified in both cases. The *Aspergillus/Penicillium* group shows an irregular distribution curve during the day, with intervals of greater abundance previously pointed out in other lo studies (DOMÍNGUEZ SANTANA & LA-SERNA, 1998).

CONCLUSIONS

Aerobiological sampling carried out in Santiago of Compostela from December 1997 to March 1998, include important potential allergens and/or phytopathogens fungal spores, such as *Coprinus, Aspergillus-Penicillium, Cladosporium, Pleospora, Paraphaeosphaeria* and *Leptosphaeria.*

The maximum value of the total spores was recorded on January and the minimum on March, being the humidity, maximum temperature and hours of sunshine the most significant parameters in their atmospheric dispersion.

With regard to intra-diurnal variation, spores of *Coprinus* basically showed a lower representation in the central hours of the day, *Cladosporium* spores are more abundant towards the end of the morning and the first hours of the afternoon, while the group *Aspergillus/Penicillium* shows an irregular distribution curve.

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