

Monitoring of airborne fungi in Madrid (Spain)

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Aeromycological sampling during 2003 and 2004 in the atmosphere of the university campus in Madrid, showed a greater presence of spores during the spring (April–June) and from August to November. Of the groups of spores identified, conidiospores accounted for the greatest percentage (49.5%), followed by teliospores (18%), basidiospores (15.5%) and ascospores (8%). Seventy spore types were identified in 2003 and 83 in 2004, the most abundant of which were: *Cladosporium cladosporioides* (30.2%), *Ustilago* (18%), *Cladosporium herbarum* (14.5%), *Coprinus* (6.9%), *Aspergillaceae* (2.1%), *Lepthosphaeria* (1.7%), *Pleospora* (1.5%) and *Bovista* (1.4%).

Key words: aerobiology, aeromycology, fungi, spore, Madrid, Spain

Introduction

Among the wide variety of biological particles present in the atmosphere, there is a very significant number of fungal spores. Fungi live as saprophytes on organic material or as parasites (mainly plant pathogens), so the majority of fungal spores in the air outdoors come from farms, forest stands and decomposing plant matter.

Because of their volume in the atmosphere and small size, fungal spores play an important role in respiratory allergies and cause a wide range of symptoms, including allergic rhinitis, asthma, chronic bronchitis, etc., (VIJAY et al. 1991, D'AMATO and SPIEKSMÁ 1995, HASNAIN et al. 1998). The main types of allergenic spores are *Alternaria*, *Aspergillus*, *Cladosporium* and *Penicillium*; *Cladosporium* is the most important in northern Europe and *Alternaria* in the Mediterranean area (D'AMATO et al. 1997). Little is known about the allergenic potential of the different types of spores and about the levels of exposure capable of provoking allergic responses when they are encountered in outdoor environments (LEVETIN et al. 1995).

Numerous works have been published on airborne fungal spores worldwide, e.g. Kuwait (HALWAGY 1994), India (CHANDRA and CHANDA 2000; JOTHISH and NAYAR 2004), Australia (MITAKAKIS and GUEST 2001), Chile (IBÁÑEZ et al. 2001) and Poland (STEPALSKA and WOLEK 2005). Among others, in Spain these include works by MEDIAVILLA et al. (1997), BLACK et al. (2000), MUNUERA et al. (2001), LA-SERNA et al. (2002), DOPAZO et al. (2003)

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and SABARIEGO et al. (2004). The main objective of all these works has been to study the diversity, concentration and period of the presence of these spores in the atmosphere, as well as the influence of environmental conditions on their release and dispersion. It is important to highlight that most of these works focus on the study of particular types of spores, and not on the whole spectrum. Previous studies published on aerodynamic flora in the city of Madrid mainly used exposure of Petri dishes with a suitable culture medium to collect the samples (CANTO and JIMÉNEZ 1945, PAYA VICENS 1983, MUÑOZ et al. 1988). Some more recent works have been done using volumetric methodology for sampling periods of one year or less (SUBIZA and JEREZ 1983, SÁENZ and GUTIÉRREZ 2003, DíEZ et al. 2006).

In this work we provide the results of the identification and quantification of airborne spores on the university campus in Madrid in two consecutive years, as our contribution to the knowledge of the diversity and seasonal variation of the spectrum of fungal spores present in the air in Madrid.

Study area

The city of Madrid is located in the centre of the Iberian Peninsula (40° 27' N, 3° 45' O) at an altitude of 637 m above sea level. The sampling station is located on the campus of University City in the northeast of the city (40° 27' N, 3° 45' W).

Phytogeographically, the territory falls within the Carpetan-Iberian-Leonese chorological province, and bioclimatically it is located in the higher meso-Mediterranean belt, with a lower dry ombroclimate. The climate is Mediterranean pluvisesional-oceanic, with an average annual temperature of 13.9 °C and 438 mm of precipitation (RIVAS-MARTÍNEZ et al. 1999).

The study area is characterised by the presence of large parks such as the Dehesa de la Villa, Casa de Campo and Parque del Oeste, all of which are relatively near the University campus, where there is an abundance of gardens with a large number of ornamental trees, hedges and lawns. The natural vegetation is represented by the communities in the meso-supra-Mediterranean Guadarraman-Iberian vegetation series *Junipero oxycedri-Querceto rotundifoliae*.

Materials and methods

The sampling of airborne fungal spores was carried out during 2003 and 2004 using a Hirst trap (Burkard spore trap) placed 8 m above ground level on the roof of the Faculty of Pharmacy at the Complutense University campus. The main body of the spore trap has a 2x14 mm slit for air intake. Inside, there is a rotating drum connected to a timer mechanism, which can run autonomously for a week. This drum is covered with a transparent Melinex polyester tape impregnated with an adhesive substance to which the windborne particles will adhere. The trap is mounted on a wind vane so that the entry slit always faces the prevailing wind direction. On the lower part of the trap, under the main body, there is a vacuum pump which sucks in a volume of air at a rate of 10 L min⁻¹. After exposure, the Melinex tape is divided into seven 48 mm segments, and each one is placed on a microscope slide, using glycerogelatine stained with fuchsine as a medium.

Following the recommendations of the REA (Spanish Aerobiology Network) and IAA (International Association for Aerobiology) (DOMÍNGUEZ et al. 1991, JÄGER 1995), the daily preparations were analysed by optical microscope (400x magnification), with two longitudinal sweeps per slide. A correction factor is used to determine the quantity of spores per cubic metre of air; this is previously calculated according to the system of capture used (volume of air per unit of time, size of the orifice, area of the sample surface, number of rotations (two for spores) and the features of the microscope lens. The data used correspond to the mean daily values expressed as the number of spores per cubic metre of air.

Fungal spore identification was based on morphologic characteristics following NILSSON (1983), SMITH (1990), BARNETT and HUNTER (1998) and VÁNKY (2002). The spore types identified were ordered systematically according to the 9th edition of the »Dictionary of the fungi« by KIRK et al. (2001), which is supported by the latest advances in the knowledge of the ultrastructure, biochemistry and molecular biology of these organisms.

Results

In the aerobiological sampling of the air in Madrid (University City) a total of 2.2×10^5 spores were recorded per year (in 2003, and 2004). There was a great diversity in the type of spores counted; 70 in 2003 (40 types belonging to the division *Deuteromycota*, 20 types to the division *Ascomycota*, 7 types to the division *Basidiomycota*, 2 types to the division *Teliomycota* and a single type to the division *Myxomycota*) and 83 in 2004 (45 deuteromycetes, 26 ascomycetes, 9 basidiomycetes, 2 teliomycetes and 1 myxomycete).

During the sampling period (Fig. 1), the highest concentrations of spores were recorded in spring (April–June) and from August to November. In 2003, October, with a monthly average of 48,447 spores, was the month with the highest levels, followed by June (32,832 spores) and May (24,643 spores). June and May were the months with the highest incidence in 2004 with a monthly total of 47,063 spores and 34,608 spores respectively, followed by October (27,314 spores). The minimum counts were recorded in the winter months, December, January and February, in both 2003 and 2004.

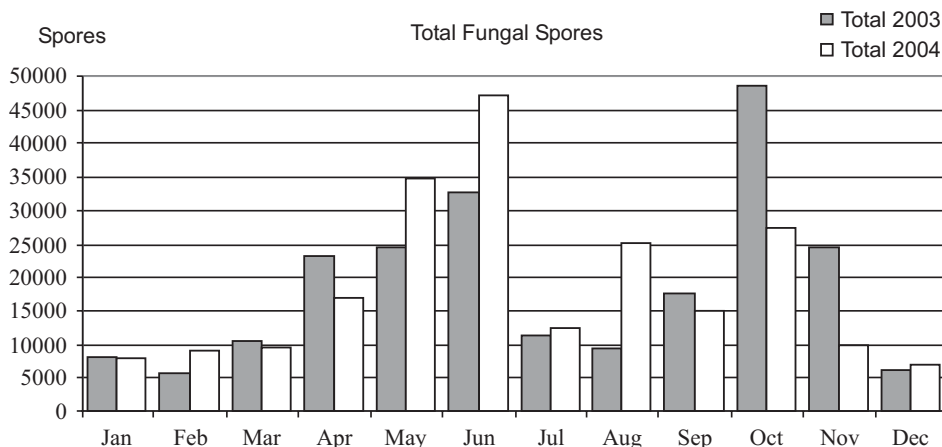


Fig. 1. Monthly variation in total spore concentrations in 2003 and 2004.

Of the groups of spores identified in 2003, conidiospores account for the greatest percentage (46%), followed by basidiospores (20%), teliospores (18%), and ascospores (8%). In 2004 the conidiospore group was also the most abundant (53%); however in 2004, unlike the previous year, teliospores were in second place with 18%, followed by basidiospores (11%) and ascospores (8%) (Fig. 2).

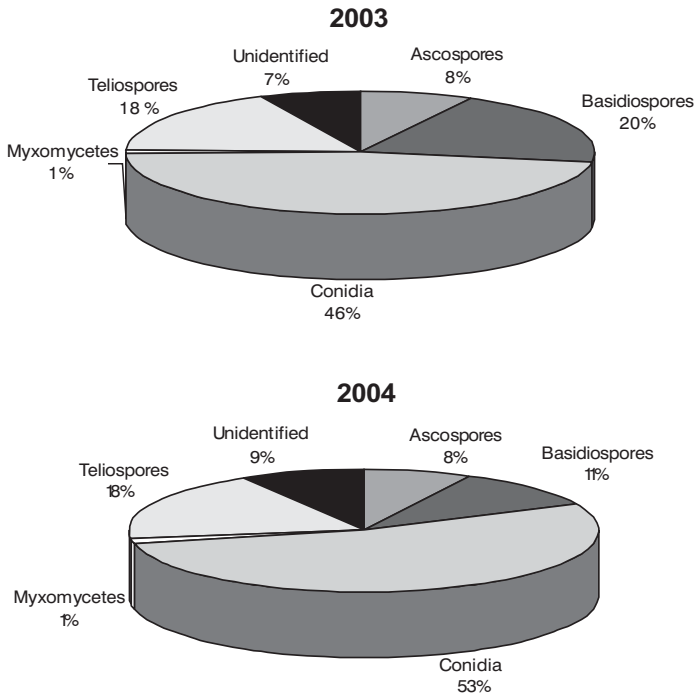


Fig. 2. Relative contribution of different groups of spores analysed during 2003 and 2004.

Ascospores, with a similar presence in the two years analysed (8% of the total) can be found in the atmosphere all year round. There are two periods of maximum incidence, one in spring (March–May) with lower concentrations and another more acute period in August, September and October (Figs. 2–3), with the exception of September 2004, when the monthly concentration collected was very low (fewer than 220 spores). In the two years in the sampling, October had the highest counts, with 3,253 and 4,866 spores respectively. July and December had the lowest levels with monthly totals of less than 700 spores. Within the ascospore group the most frequently occurring taxa were *Leptosphaeria* (22.5%), *Pleospora* (20.2%), *Diatrypaceae* (14.4%), *Chaetomium* (0.9%), *Caloplaca* (0.7%) and *Xylariaceae* (0.6%) (Fig. 3). In 2003 43.2% and in 2004 30.9% of ascospores were unidentified, including spores that have no birth scars or attachment points; they can be multicellular and be divided into two a dozen or more cells by septa.

Basidiospores accounted for 20% in 2003 compared to 11% in 2004 (Fig. 2). In the first year of the sampling a very sharp peak of maximum incidence can be seen in autumn, in October and November (with monthly concentrations of over 13,800 spores), while the rest

of the year they are present in monthly concentrations of less than 4,000 spores. In 2004, as in 2003, basidiospore levels were high in October (6,339 spores), whereas in November the amounts collected were minimal (138 spores). In both the years analysed there was a significant presence of these spores in April, May, June and September. The most frequently occurring basidiospores in the atmosphere of Madrid corresponded to *Coprinus* (46.2%), *Agaricus* (13.4%) *Bovista* (10.9%), *Ganoderma* (5.7%), *Gymnopilus* (2.1%) and *Agrocybe* (1.4%).

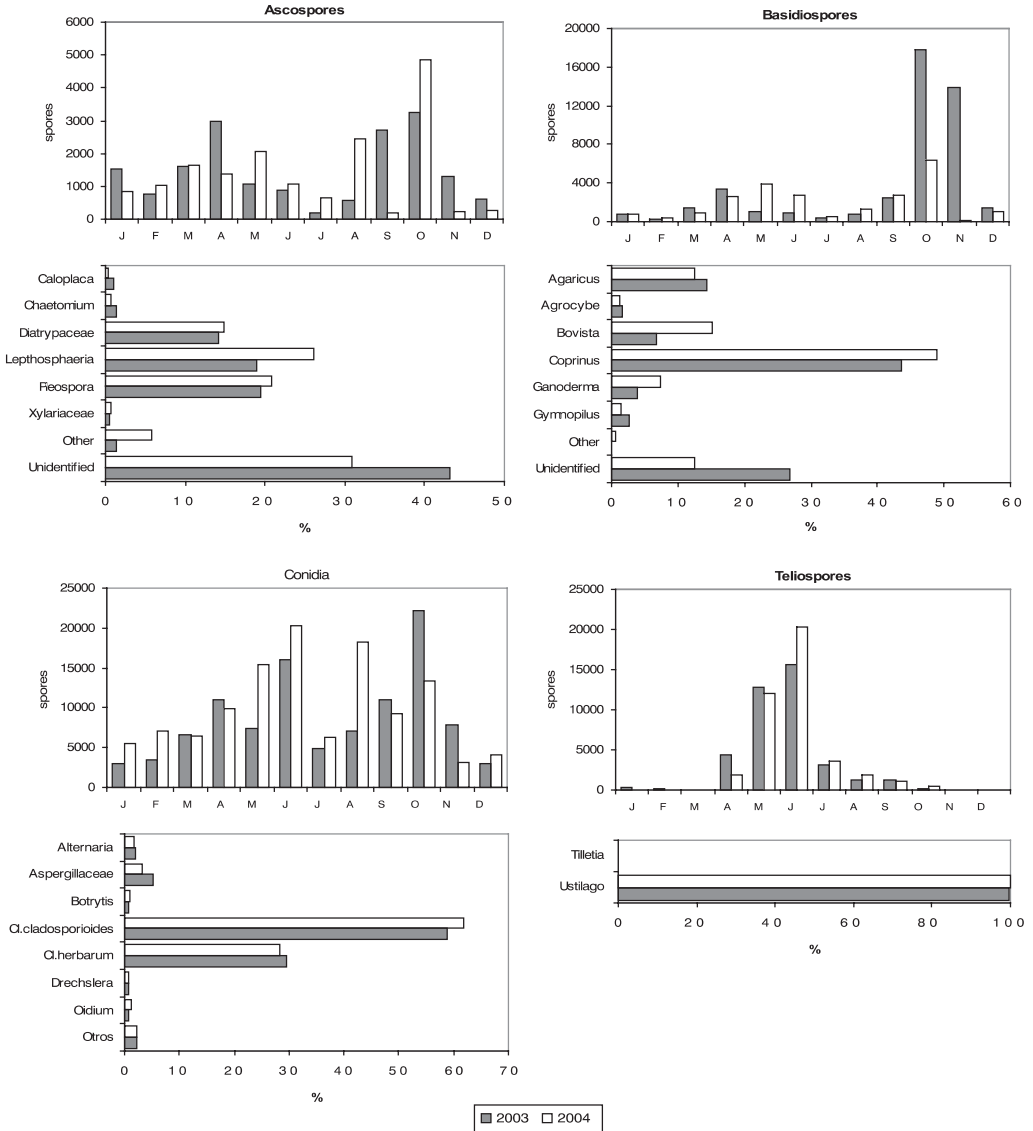


Fig. 3. Monthly variation in spore concentrations of the different groups analysed and percentage of representation of the most significant types.

Conidiospores attained very high levels in the air in Madrid (total monthly concentrations of over 3,000 conidiospores all the months of the year) and accounted for 46% in 2003 and 53% in 2004 (Figs. 2, 3). Their maximum concentrations were recorded from April to June and from August to October. In 2003, October was the month with the highest levels (22,226 conidiospores), while in 2004 the highest levels were detected in June (20,330 conidiospores). The highest concentration of conidiospores in the air was recorded in the winter months of December and January (Fig. 3). The conidiospores of *Cladosporium* were the most abundant; *Cladosporium cladosporioides* accounted for 60.3% and *Cladosporium herbarum* for 29%, out of the total conidiospores counted. The next in importance was *Aspergillaceae* (Incl. *Aspergillus* and *Penicillium*) (4.2%), *Alternaria* (1.8%), *Oidium* (1%), *Botrytis* (0.8%) and *Drechslera* (0.6%).

The teliospore group (Fig. 2) had the same percentage of representation in both the years studied (18%). These spores showed a clearly defined seasonal dynamic; both in 2003 and 2004 they were basically present in the air from April until October, with very acute maximum levels in May and June (with monthly concentrations of over 12,000 spores). This group includes the genera *Tilletia* and *Ustilago*, which had very different percentages of representation: 0.3% and 99.7% respectively (Fig. 3).

Of the total spores recorded, the most abundant spore type was *Cladosporium cladosporioides* (30.2%) with a mean daily concentration of 167 spores m⁻³ (2003) and 200 spores m⁻³ (2004). The maximum value was recorded on 8 August 2004 with 2,955 spores/m³ (Tab. 1). *Ustilago* was the second most frequent type (18%), and its maximum peak was recorded on 2 June 2004 (3,972 spores m⁻³). Next in importance was *Cladosporium herbarum* (14.5%), *Coprinus* (6.9%), *Aspergillaceae* (2.1%), *Lepthosphaeria* (1.7%), *Pleospora* (1.5%) and *Bovista* (1.4%), whose mean annual concentrations, peak days and relative frequency are shown in table 1.

Tab. 1. Data on the characteristics of the most frequently occurring spores in the atmosphere of Madrid.

Year	2003				2004			
	Mean annual concentration	Peak counts	Data	Relative frequency %	Mean annual concentration	Peak counts	Data	Relative frequency %
<i>Cl. cladosporioides</i>	167	2908	16/10	27.3	200	2955	11/08	33.1
<i>Ustilago</i>	107	2274	28/04	17.5	112	3972	02/06	18.5
<i>Cl. herbarum</i>	84	875	01/10	13.8	92	897	11/08	15.2
<i>Coprinus</i>	53	1084	18/10	8.7	31	738	01/06	5.1
<i>Aspergillaceae</i>	15	393	10/02	2.5	10	578	27/04	1.7
<i>Lepthosphaeria</i>	9	157	12/04	1.5	12	249	07/06	2.0
<i>Pleospora</i>	9	385	30/09	1.5	9	416	09/08	1.6
<i>Bovista</i>	8	445	16/10	1.3	10	138	25/10	1.6

Discussion

It can be seen that there is little difference between the total spores collected in 2003 and 2004. In the first year only 381 spores more than in the second year were collected.

Of all the spores collected by our spore trap, conidiospores were the most numerous group; they accounted for 49.5% of the total spores, followed by teliospores (18%), basidiospores (15.5%) and ascospores (8%). These results differ from those described previously by other authors, where conidiospores were much more significantly represented than in Madrid, while the remaining groups had a lower representation. This is the case of Jordan (SHAHEEN 1992): 88% conidiospores, 8.3% teliospores, 2% ascospores and 1.7% basidiospores; and of India (KAKDE et al. 2001): 88.9% conidiospores, 2.1% basidiospores, 1.2% ascospores, 0.3% zygospores and 0.2% oospores.

The conidiospores of *Cladosporium* were the most abundant in the atmosphere of Madrid with an average representation of 44.7% of the total. However, although they were the most frequently occurring in the aerobiological sampling, they accounted for a lower percentage of representation than in other cities such as Santiago (Chile): 70.9% (IBÁÑEZ et al. 2001) or Salamanca (Spain): 81.5% (PÉREZ et al. 2003) and a similar quantity to that described by HERRERO et al. (1995) in Palencia (Spain) and JOTHISH and NAYAR (2004) in Kerala (India), where *Cladosporium* conidiospores had a representation of 46% and 44.7% respectively. In Spain, specifically in the cities of Cordoba, Granada and Leon, these conidiospores are also characterised by their high incidence in the atmosphere (INFANTE et al. 2000). Several works have also confirmed finding large concentrations of spores of the types *Ustilago* (HERRERO et al. 1995), *Coprinus* (CALDERÓN et al. 1995, MITAKAKIS and GUEST 2001) and *Aspergillaceae* (KAKDE et al. 2001) in the air.

The seasonal dynamic of the total spores showed two peaks of maximum concentration, one during spring (April–June) with 26.3% and 36.8% of the yearly total for 2003 and 2004 respectively, and the second from August to November with 44.9% and 34.8%. The existence of two peaks of highest incidence was also demonstrated in a previous work by JOTHISH and NAYAR in India (2004), with a first peak in April and a second peak in September–October; HALWAGY (1994) in the city of Kuwait found two peaks, one in spring (April–May), and another in autumn (October–November). Elsewhere, the annual distribution of spores showed a single concentration peak, as is the case of Krakow (Poland) with maximum counts in June, July and August (STEPALSKA and WOLEK 2005) or Palencia (Spain) where the levels are high from June to October (HERRERO et al. 1995).

The spring peak, when conidiospores (*Cladosporium*) and teliospores (*Ustilago*) were predominant in the air, may be due to favourable temperature and humidity conditions. The second peak (August–November) when we collected above all conidiospores, ascospores and large numbers of basidiospores (mainly *Coprinus*, *Agaricus* and *Bovista*) in August and September, as well as conidiospores and ascospores in October and November, may be due to an increase in the availability of substrate owing to the seasonal processes of decomposition of plant matter (MORALES 2004). In winter there is a decrease in spores due to the low temperatures, which have a negative effect on the content of these particles in the air (ANGULO et al. 1999, MUNUERA et al. 2001), and a high relative humidity which produces an absorption of water by the spores, making them heavier and less transportable by air (GON-

ZALEZ MINERO et al. 1994). In July the low spore records are associated with the large amounts of drying vegetation in the area due to the high temperatures and the absence of rain, as these are unfavourable conditions for the growth of fungus.

The spores accounted for 93% (2003) and 91% (2004) of the total number of biotic particles considered (pollen and spores).

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