



Presence of *Aspergillus* Mich. ex Fr. in several different biotopes in Córdoba (Spain)

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Abstract

The medical, veterinary and saprophytic importance of *Aspergillus* has been acknowledged by many authors. This paper reports on the occurrence of *Aspergillus* species in the air and dust of Córdoba (Spain). Four studies were done: first, on the air of three rooms and the outside area of 14 homes over 2 years; second, outdoor air in the west of the central district, with three samples daily over 1 year; third, the air inside and outside four of the largest silos and commercial grain warehouses in the province, over 1 year; and fourth, the dust of 12 primary schools in Córdoba city, for two consecutive school years. For air-sampling, volumetric and gravimetric methods were used, both with a culture medium and with 20-min exposure. For dust-sampling, a customised vacuum cleaner was used; samples collected were inoculated onto Petri dishes containing culture medium. A total of 38 *Aspergillus* species were identified (we also detected four ascospore states). A comparison of the results obtained at each of the sampling sites was made and an analysis of variance revealed quantitatively significant differences between sampling times.

Keywords: *Aspergillus*; Microfungi; Aerobiology; Indoor air; Outdoor air; Dust fungi

1. Introduction

Molds are the predominant biological particles in the atmospheric environment and especially in indoor ambient air (Comtois, 1990). The sustained interest in studying the occurrence of fungi in indoor and outdoor atmospheric environments is largely due to the great significance of fungi to man (Duce et al., 1986; Larsen and Gravesen, 1991; Ebner et al., 1992; Infante et al., 1992; González et al., 1993). The importance of *Aspergillus* species lies in their ability to grow on a wide variety of substrates and environmental conditions. According to Rippon (1990), *Aspergillus* species are the most frequent fungal species in every natural substrate including soils and decaying vegetation. However, they can also colonize virtually every artificial substrate containing some organic matter and moisture, because the large number of enzymes they produce allow them to feed on a vast variety of substrates.

Many *Aspergillus* species are known to be pathogenic and cause a number of diseases of medical and veterinary relevance (Rippon, 1990; Torres-Rodríguez

and Madrenys-Brunet, 1993). These fungi can also be toxic to man and animals as they produce various mycotoxins (particularly aflatoxins) (Raper and Fennell, 1965; Cole and Kendrick, 1981). They can also induce biodeterioration of a variety of stored commercially available products reducing their value (Cole and Kendrick, 1981; Gallo, 1993).

Aspergillus possesses a high allergenic significance. In fact, some species such as *Aspergillus ruber* or *A. fumigatus*, are reportedly among the most allergenic fungi (Bronswijk et al., 1986; Güneser et al., 1994). Moreover, the presence of *Aspergillus* in the atmosphere of Córdoba is of a high allergologic significance since, based on data reported by the Allergy Service of the University Hospital of this city, 30% of all fungus-sensitive patients are sensitive to *Aspergillus*.

This genus is a worldwide airborne fungus and is one of the more frequent. In fact, *Aspergillus* is the fourth most abundant genus in Copenhagen (Larsen and Gravesen, 1991) and, *A. niger* and *A. fumigatus* the third and seventh, respectively in Adana, Turkey (Güneser et al., 1994). In our city, *Aspergillus* is the third most abundant fungal genus in the air.

Because of the high allergenic incidence of

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Aspergillus in Córdoba, and its high concentration in the environment, our group initiated this work mainly in order to quantify and catalogue species of this genus occurring in various biotopes in our city.

2. Materials and methods

The data used in this work were obtained from several investigations carried out by our group in Córdoba (Southern Spain). The city has a population of ca. 300 000, is located 120 m above sea level, features a Mediterranean to continental climate, is characterized by an average annual rainfall of 674 mm and has an average annual temperature of 18°C (average maximum, 30°C; average minimum, 7°C).

All fungi were isolated on Petri dishes containing 2% agar malt extract culture medium prepared according to Blakeslee's formula (Tuite, 1969). The medium was acidified with 5 ml/l of 25% lactic acid in order to inhibit bacterial growth. After 6–8 days of culturing at 28°C, colonies were counted, isolated and identified. Both the above-mentioned medium and 30% agar Czapek-sucrose (Raper and Fennell, 1965) were used to isolate the *Aspergillus* colonies from axenic cultures. Species were identified according to the following authors: Thom and Church (1926), Gilman (1963), Raper and Fennell (1965), Guarro and Calvo (1978), Samson (1979), and Calvo and Guarro (1981).

The sampling sites and procedure used to capture colony forming units (CFUs) varied with the type of study in progress.

In the first study, we selected 14 homes at different locations in the city, where samples were collected fortnightly in three rooms (kitchen, bedroom and bathroom), as well as outdoors, for 2 years. CFUs were collected by exposing Petri dishes (OPD — Prahl, 1992) ($\Phi = 90$ mm) containing the above-described culture medium for 20 min in each room (Infante et al., 1992).

In the second study, sampling was conducted at the Faculty of Sciences building, (WSW Córdoba), in an area relatively distant from the urban districts. Samples were collected using a Biological Air Sampler (BIAS) from New Brunswick Scientific & Co., which exposed air to Petri dishes ($\Phi = 140$ mm) containing the selected culture medium at a flow-rate of 10 l/min for 20 min. Samples were collected daily at 10:00 h, 12:30 h and 17:00 h for 1 year (Trujillo et al., 1990a).

In a later study, samples were collected indoors and outdoors at four of the largest silos and cereal warehouses in the province of Córdoba. The sampling sites were all in an area of extensive olive tree- and cereal-cultured land far from the city. Samples were collected monthly for 1 year using a volumetric (BIAS) and an OPD method. Both involved the use of the selected culture medium on Petri dishes ($\Phi = 140$ mm) and an exposure for 20 min (Mediavilla et al., 1992).

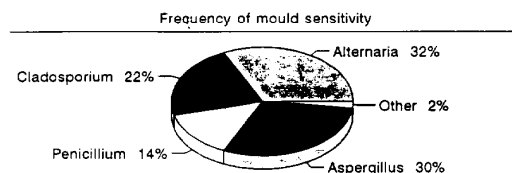


Fig. 1. Incidence of the most common moulds inducing allergic sensitization in Córdoba.

Finally, dust samples were collected at 12 primary schools in Córdoba. Samples were collected fortnightly over two consecutive school years. Dust was collected from the floor of one classroom (always the same in each school) by means of a portable vacuum cleaner until an appreciable amount was obtained. The instrument was sterilized after each sampling in order to avoid contamination. A 10-mg quantity of each sample was subsequently added to a Petri dish ($\Phi = 140$ mm) containing the culture medium (Angulo et al., 1993).

3. Results and discussion

The chief purpose of the present study was to identify the different species and to assess the annual variation of the incidence of the genus *Aspergillus* in air and house dust in Córdoba. To this aim, we have grouped the most interesting results obtained for this taxon in the four above-mentioned studies. Due to their heterogeneity and to the fact that they were gathered in the course of different investigations, the data are not presented in absolute terms, but as percentages, in order to facilitate comparisons.

In Córdoba, *Aspergillus* is an important allergen; 30% of all fungus-sensitive patients are sensitive to *Aspergillus*, which is the second most frequent genus responsible for allergies in our city (Fig. 1; data reported by the Allergy Service of the University Hospital).

The genus *Aspergillus* is a major component of the

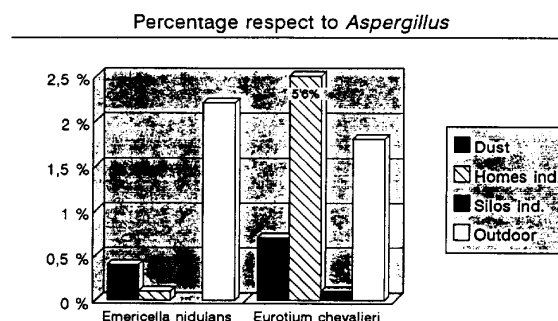


Fig. 2. Proportion of *Emericella nidulans* and *Eurotium chevalieri* among *Aspergillus* species in each type of habitat studied.

Table 1
Presence (*) of *Aspergillus* species in each type of habitat

<i>Aspergillus</i> spp.	Indoors		Outdoors	Indoor + outdoor air	Dust
	Homes	Silos			
<i>A. alliaceus</i>			*	*	*
<i>A. asperescens</i>	*		*	*	
<i>A. aureolatus</i>			*	*	
<i>A. brunneo-uniseriatus</i>			*	*	
<i>A. candidus</i>	*	*	*	*	*
<i>A. carneus</i>	*	*	*	*	*
<i>A. eburneo-cremeus</i>					*
<i>A. flavipes</i>			*	*	*
<i>A. flavo-furcatis</i>			*	*	
<i>A. flavus</i>	*		*	*	*
<i>A. fumigatus</i>	*	*	*	*	*
<i>A. heteromorphus</i>	*	*	*	*	
<i>A. janus</i>	*		*	*	
<i>A. japonicus</i>			*	*	*
<i>A. melleus</i>		*		*	*
<i>A. minioluteum</i>			*	*	
<i>A. niger</i>	*	*	*	*	*
<i>A. niveus</i>	*	*	*	*	*
<i>A. ochraceus</i>	*	*	*	*	*
<i>A. oryzae</i>	*	*	*	*	*
<i>A. parasiticus</i>	*		*	*	*
<i>A. petrakii</i>		*	*	*	*
<i>A. proliferans</i>	*		*	*	
<i>A. restrictus</i>	*		*	*	
<i>A. sclerotiorum</i>			*	*	
<i>A. silvaticus</i>			*	*	*
<i>A. speluneus</i>					*
<i>A. subsesilis</i>	*			*	
<i>A. sulphureus</i>		*		*	
<i>A. sydowi</i>	*	*	*	*	*
<i>A. tamarii</i>	*	*	*	*	
<i>A. terreus</i>	*	*	*	*	*
<i>A. terricola</i>			*	*	
<i>A. tubingensis</i>	*		*	*	
<i>A. ustus</i>	*		*	*	*
<i>A. versicolor</i>	*		*	*	*
<i>A. violaceus</i>	*		*	*	
<i>A. wentii</i>	*	*	*	*	
<i>Emericella nidulans</i>	*		*	*	*
<i>Emericella variegata</i>	*			*	
<i>Eurotium chevalieri</i>	*	*	*	*	*
<i>Sartorya fumigata</i>					*

aeromycoflora of many cities. In fact, it is the third most abundant fungal genus in Córdoba and one of the most frequently occurring as well. This is consistent with the observations of several authors in other Spanish cities: *Aspergillus* is the fourth most abundant genus in Zaragoza (Duce et al., 1986) and the fifth in Barcelona (Calvo et al., 1980). In addition, Larsen and Gravesen (1991) found *Aspergillus* to be the fourth most common fungal genus in the atmosphere of Copenhagen in a 10-year study.

We identified 38 *Aspergillus* species overall (Table 1),

i.e. many more than in the other cities previously studied. Calvo et al. (1980) cited 12 species in the atmosphere of Barcelona (Spain), Misra and Jamil (1991) cited 15 species in the flour mills indoors in Lucknow (India), seven species were found in Dutch houses and non-industrial working environments (Reenen-Hoekstra et al., 1991) and six species of *Aspergillus* were cited by Savino and Caretta (1992) in a rice mill in Pavia (Italy).

Of the 38 species identified by us, 36 were found to occur in the air and 21 in house dust (the type of habitat

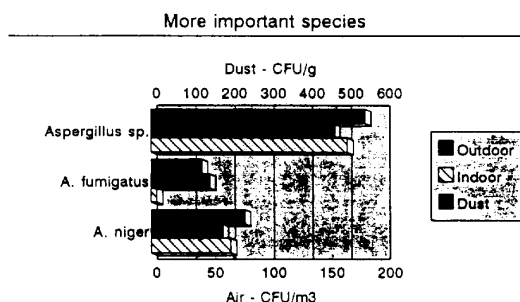


Fig. 3. Average counts of *Aspergillus fumigatus*, *A. niger* and *Aspergillus* species as a whole obtained for air (indoor and outdoor) and dust samples.

in which each species was found is stated in Table 1). It should be noted that despite the much shorter sampling period, dust samples were found to contain two species not occurring in the air, *A. eburneo-cremeus* and *A. speluneus*. On the other hand, 17 *Aspergillus* species were isolated only in the air (never in dust); among them *A. subssesilis* and *A. sulphureus* were only isolated from indoor air (Table 1).

As shown in Table 1, four *Aspergillus* teleomorphic states was isolated several times. The most frequently encountered of such species were *Eurotium chevalieri* (the most cosmopolitan) and *Emericella nidulans*; on the other hand, *Sartorya fumigata* was only detected in house dust, probably because it occurs very frequently in the soil (Moubasher and Moustafa, 1970; Abdel-Hafez, 1982a,b). In any case, as can be seen in Fig. 2, these species accounted for a very low proportion of the genus *Aspergillus*. *Eurotium chevalieri* was the single species occurring at a significantly high concentration in indoor air of homes (5.6%). On the other hand, *Emericella nidulans* was more frequently detected outdoors (2.2%), but scarcely present in house dust.

Fig. 3 shows the average counts of *Aspergillus* genus

and its two most significant species (*A. fumigatus* and *A. niger*) isolated from dust and air, indoors and outdoors. The higher incidence of this genus indoors is probably a result of its saprophytic ability. This allows them to colonize foods, wet leather, paper, cotton, fabrics and many other substrates, which makes them important biological deterioration agents to be taken into account when investigating indoor environments. For this reason, *Aspergillus* and *Penicillium* have been termed 'indoor fungi' by many authors (Pasanen, 1992). On the other hand, the presence of *Cladosporium* and *Alternaria* indoors is more likely to be due to outdoor conidia entering buildings (Lung and Kramer, 1972; Solomon, 1975; Duce et al., 1986; Muñoz et al., 1988). Duce et al. (1986) also found this genus to predominate indoors in Zaragoza. There are, however, some contradictory results in this respect, since Muñoz et al. (1988) and Ebner et al. (1992) found *Aspergillus* populations outdoors to exceed those indoors in Madrid and Innsbruck, respectively — at least at certain times of the year.

Aspergillus species accounted for 19% of the dust mycoflora and made up the second most abundant genus (Angulo et al., 1993). We believe such a high incidence is a result of the presence of some xerophilic species usually occurring indoors (e.g. *A. niger*). The dominance of *A. niger*, *A. fumigatus* and other *Aspergillus* species is quite significant as it assures predigestion of materials present in house dust, which makes nutrient supplies indispensable for dust mite development (Chirila et al., 1981; Bessot and Pauli, 1985). This in turn makes the genus *Aspergillus* doubly significant in allergological terms as it can induce reactions by itself and through the mites it helps to develop.

Based on the seasonal variation of *Aspergillus* (Fig. 4), the genus occurred more frequently in the indoor-air during the winter and autumn, and outdoors during the autumn; on the other hand, *Aspergillus* concentrations in dust peaked in the summer. Larsen and Gravesen (1991), who studied the atmosphere of Copenhagen for 10 years, also found this taxon to occur most frequently

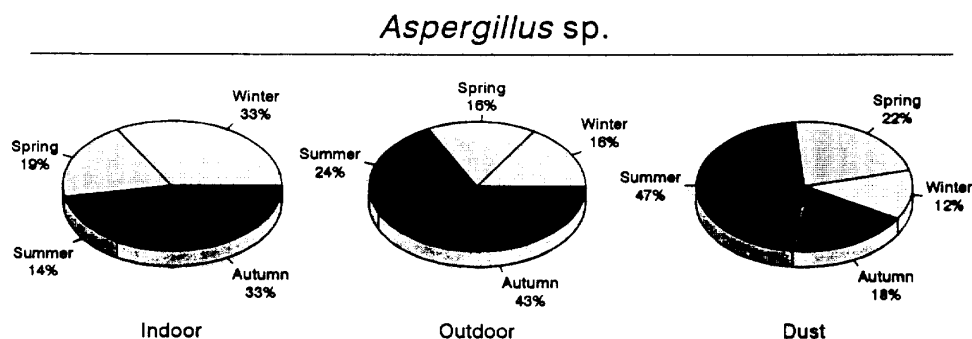


Fig. 4. Seasonal incidence of *Aspergillus* in dust and air (average values for indoor and outdoor samples).

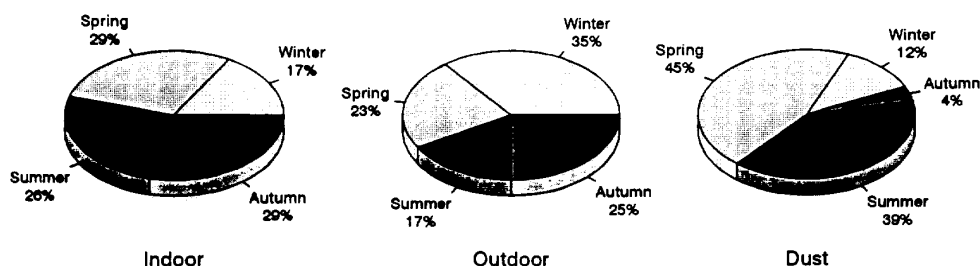
Aspergillus fumigatus

Fig. 5. Seasonal incidence of *Aspergillus fumigatus* in dust and air (average values for indoor and outdoor samples).

in the air in autumn; though Pasanen (1992) reported that *Aspergillus* is dominant in Europe and North America in the winter. However, the genus concentrations in the atmosphere of Barcelona were found to be highest in the spring (Calvo et al., 1980).

Aspergillus fumigatus was isolated throughout the year from the air and dust (Fig. 5). In the air, no clear prevalence of this taxa was observed in any season and this contradicts the findings of Calvo et al. (1978) in the atmospheres of three coastal Spanish cities (Barcelona, Tarragona and Valencia), where the species was predominant in the summer and autumn.

On the other hand, the *A. fumigatus* concentrations in dust (Fig. 5) were clearly higher in spring and summer. The reason is far from obvious as this fungal species requires both a high temperature and a substrate with a high water activity to proliferate (Mullins et al., 1976).

Aspergillus niger behaved rather differently. Fig. 6 shows the seasonal pattern of *A. niger*; in the air (indoor and outdoor), it is more abundant during the autumn, and in dust, the major incidence is found during the summer.

We carried out an analysis of variance in order to determine whether the differences found between counts of colonies collected at different times of day were statistically significant. The analysis involved the 24 *Aspergillus* species collected in study no. 2 above, during which samples were obtained daily by means of the BIAS trap at 10:00 h, 12:30 h and 17:00 h for 1 year (Trujillo et al., 1990b). As shown in Table 2, six of the species occurred at significantly different concentrations ($P < 0.1$ for *A. oryzae* and $P < 0.001$ for the other five) depending on the sampling time; all six were found to occur at greater concentrations in the air during the morning when the temperature was lower and moisture higher than in the afternoon. The incidence of *A. fumigatus* and *A. ochraceus* was highest at 12:30 h, whereas those of the other species peaked at 10:00 h — none occurred preferentially in the afternoon, a fact consistent with reported data for *Aspergillus* in Barcelona (Calvo et al., 1980), as well as for Tarragona, and Valencia (Calvo et al., 1978).

Finally, we believe that the microecological conditions of each indoor habitat considerably influence the

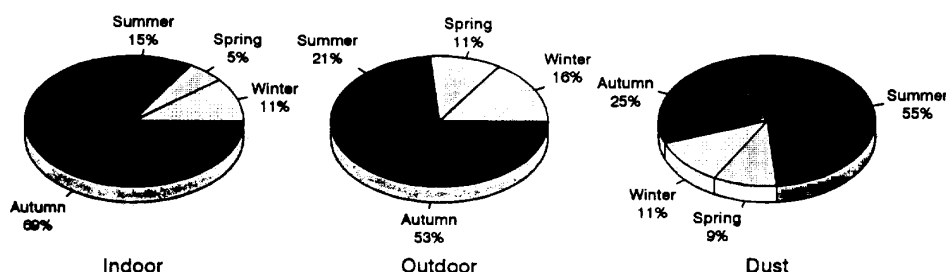
Aspergillus niger

Fig. 6. Seasonal incidence of *Aspergillus niger* in dust and air (average of indoor and outdoor values).

Table 2

Analysis of variance showing the significance of differences in species concentrations according to the time of day (species with non-significant variations are excluded)

Taxa	F-value	10:00 h	12:30 h	17:00 h
<i>A. candidus</i>	7.148***	x		
<i>A. fumigatus</i>	11.946***		x	
<i>A. niger</i>	8.141***	x		
<i>A. ochraceus</i>	9.216***		x	
<i>A. oryzae</i>	4.321*	x		
<i>A. terreus</i>	10.508***	x		

*95% significance.

***99.9% significance.

incidence of the species encountered. Thus, in many cases, external conditions are not crucial as regards fungal incidence indoors. Therefore, one should always investigate the peculiar features of the habitat concerned in studying fungal incidence indoors. By way of example, *Aspergillus versicolor* was detected in very large amounts (1260 colonies) between January and March as a result of the non-uniform, isolated appearance of this taxon in one of the sampled homes (no. 9). This house was very damp, with wall marks, as a result of being on the top floor, under a poorly insulated roof, and with a lack of room ventilation. While the home in question was found to contain 2586 colonies — probably more since counting was impossible in many cases — in the 2 years studied, the air outside the same sampling site contained only 57 colonies. On the other hand, only 1–33 *A. versicolor* colonies were found indoors and 3–57 outdoors in the other homes.

4. Conclusions

Aspergillus is a fungus whose presence in the air and dust of Córdoba seems important in the light of the results obtained, since numerous species are present some of which have high levels of incidence. Moreover, the sensitization of the allergic population of Córdoba to this fungus can be considered high.

While present throughout the year, *Aspergillus* prevails in the air in the autumn and in dust in the summer. This seasonal pattern is probably due to the high concentrations of *A. niger*, which is indeed a seasonal species and reaches peak values in such seasons — at least in the habitats studied.

The presence of *Aspergillus candidus*, *A. fumigatus*, *A. niger*, *A. ochraceus*, *A. oryzae* and *A. terreus* is greater in the air during the morning.

Thus the microecological conditions in each indoor habitat significantly influence the occurrence and incidence of fungal species.

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