

A COMPARATIVE STUDY OF THE INCIDENCE OF *CLADOSPORIUM* CONIDIA IN THE ATMOSPHERE OF FIVE SPANISH CITIES

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SUMMARY: A comparative study was carried out of the incidence of *Cladosporium* in the atmosphere of five sampling sites with differential biogeographic features, all belonging to the Spanish Aerobiology Network (*viz.* Barcelona, Córdoba, Granada, León and Orense). Data provided by the stations were collected between 1993 and 1998, and revealed that *Cladosporium* conidia are present in the atmosphere throughout the year; concentrations may, however, be very low in winter (particularly in cities such as León and Orense). The highest *Cladosporium* concentrations were recorded in Córdoba, particularly in the rainy years (639,123 conidia in 1997) that followed a long drought in the south of the Iberian Peninsula during the years 1993-1995. The highest daily peak was also recorded in Córdoba, with 49,536 conidia/m³ on 17/09/96. In contrast, Barcelona, in 1996, exhibited the lowest concentrations of all the cities, with a total annual count of 90,269 conidia and a daily peak of 2,607 conidia/m³; it was also the city with the most number of days on which conidial counts exceeded the local mean, thus making it the city with the most uniform atmospheric incidence of *Cladosporium*. León and Orense exhibited intermediate conidial concentrations, with a single period of increased concentrations (from April–May to November).

KEY WORDS: *Cladosporium*, Aerobiology, Spanish Aerobiology Network, Spain.

RESUMEN: Se ha realizado un estudio comparativo de la presencia de *Cladosporium* en la atmósfera de 5 estaciones de muestreo pertenecientes a la Red Española de Aerobiología (Barcelona, Córdoba, Granada, León y Orense) que debido a su ubicación poseen diferentes características biogeográficas. Los datos aportados por las diferentes estaciones de muestreo, que pertenecen a los años 1993 a 1998, muestran que los conidios de *Cladosporium* están presentes en la atmósfera durante todo el año, aunque en invierno los valores pueden ser bajos, especialmente en ciudades como Orense o León. Las mayores concentraciones se alcanzaron en la ciudad de Córdoba, especialmente en los años lluviosos (639.123 conidios en 1997) tras la sequía que sufrió el sur de la Península Ibérica durante los años 1993-1995. El pico diario máximo también se alcanzó en Córdoba el día 17/09/96 con un valor de

49.536 conidios/m³. Por el contrario, Barcelona presentó durante el año 1996 los valores más bajos con un total anual contabilizado de 90.269 conidios y un máximo diario de 2.607 conidios/m³; sin embargo, posee el número más alto de días con concentraciones de conidios superiores a la media local, lo que le convierte en la ciudad con una presencia de *Cladosporium* en el aire más constante. León y Orense son las ciudades que poseen unas concentraciones de conidios que podríamos llamar intermedias, con un único periodo de concentraciones más altas entre los meses de Abril-Mayo hasta Noviembre.

PALABRAS CLAVE: *Cladosporium*, Aerobiología, Red Española de Aerobiología, España.

INTRODUCTION

Cladosporium is considered as one of the most cosmopolitan fungi, particularly in temperate regions, where it acts as a secondary invader of dead vegetation; some of its species are also phytopathogenic or saprophytic and colonize a variety of substrates (soil, wool, paper, seeds). This, together with the ease with which its small and multiple conidia can be carried by air over long distances, gives rise to a high incidence of this fungus in air (INFANTE, 1997).

The significance of *Cladosporium* lies basically in its ability to trigger respiratory diseases and allergic processes due to its almost permanent presence in indoor and outdoor air – the genus is considered to be one of the most abundant in the latter medium. Most authors consider *Cladosporium* and *Alternaria* to be the most important fungal aeroallergens (RESANO *et al.*, 1998). This has fostered aerobiological studies throughout the world aimed at determining the incidence, variation and species of this fungus in air.

Cladosporium includes around 40 species; a number of reported additional species may be mere morphological alterations of existing ones, resulting from fungal responses to external stimuli. In fact, each species occurs in many variants, which occasionally hinders taxonomic classification. There are many published references to the atmospheric incidence of *Cladosporium* in

Spain. The following airborne species have been identified in studies using culture medium traps: *C. avellanum*, *C. cladosporioides*, *C. colocasiae*, *C. cucumerinum*, *C. elatum*, *C. herbarum*, *C. macrocarpum*, *C. oxysporum*, *C. sphaerospermum*, *C. spongiosum* and *C. variabile* (HERRERO *et al.*, 1996a; INFANTE & DOMÍNGUEZ, 1988; INFANTE *et al.*, 1988, 1992; MEDIAVILLA *et al.*, 1992, 1996; PAYA & SUÁREZ, 1984).

Cladosporium conidia form simple or branched acropetal chains. However, they vary greatly in size (5-40 x 3-13 µm) and shape (ovoid, lemon-shaped, oblong, spherical), exhibit scars on the bottom, and are unicellular or with 1-3 transverse septa. They can be easily detected with Hirst traps, where they are collected in free or chained form. This, together with the aerobiological interest of this genus, has recently fostered research on the atmospheric incidence of *Cladosporium* in Spain and on the way it is influenced by meteorological parameters. Noteworthy studies include those carried out in Badajoz (GONZALO *et al.*, 1996), Barcelona (BELMONTE *et al.*, 1992), Córdoba (DOMÍNGUEZ *et al.*, 1995; MEDIAVILLA *et al.*, 1997a, b, 1998; GALÁN *et al.*, 1998a, b), Huelva (GONZÁLEZ-MINERO *et al.*, 1993, 1995, 1996), La Laguna (DOMÍNGUEZ & LA SERNA, 1996), León (FERNÁNDEZ-GONZÁLEZ *et al.*, 1993; FERNÁNDEZ *et al.*, 1998), Orense (MÉNDEZ *et al.*, 1997) and Palencia (HERRERO *et al.*, 1996a, b).

Despite the abundant literature on the incidence of *Cladosporium* in the air of certain Spanish cities, few comparative studies have been carried out on aeromycoflora in our country. This is the primary objective of a joint project currently being carried out by several members of the Spanish Aerobiology Network (Red Española de Aerobiología, or REA). Some of the results of this project are reported here.

MATERIAL AND METHODS

The authors used atmospheric conidial counts for five Spanish cities (Barcelona, Córdoba, Granada, León and Orense) belonging to the REA. Conidia were sampled and data processed identically in all cases. Atmospheric conidia were captured using a Hirst-type volumetric inert medium trap (Burkard or Lanzoni) to obtain weekly samplings; the trap was adjusted to aspirate 10 liters of air per minute. Conidia were counted under a light microscope (400x) using two lengthwise counts –except at the Barcelona site, where only one count was taken– per daily preparation. Counts were multiplied by appropriate factors for extrapolation to the whole preparation and expressed as conidia/m³ of air in order to ensure consistency with

REA methodology (DOMÍNGUEZ *et al.*, 1992). This methodology provides counts with very small error due to the high fungal concentrations present in the Spanish atmosphere.

Since the sampling sites studied were geographically disperse, they exhibited differences in terms of height, climate, vegetation, etc. that are summarized in Table 1 and discussed below.

BARCELONA SAMPLING SITE (NE OF THE IBERIAN PENINSULA)

The trap used was located in the NW of the city, approximately 2 km from Sierra de Collserola and 3 km from the sea. Barcelona has a subhumid, maritime, temperate tending towards maritime climate (BOLÓS, 1962) and a typically Mediterranean rain pattern, with long summer droughts and two wet seasons (spring and autumn). The vegetation around the trap included typical urban ornamental plants and ruderals, as well as residual riverside trees such as elms (*Ulmus*) willows (*Salix*), poplars (*Populus*) and horticultural and fruit crops, together with vestiges of natural vegetation such as reeds and glassworts (*Salicornia* sp.). To the north is Sierra de Collserola, largely covered with coastal holm-oak (*Quercus ilex* subsp. *ilex*) woodland

Sites	Sampling station	Station height (m a.s.l.)	Trap height (m a.g.)	Mean temperature (°C)	Annual rainfall (mm)
Barcelona	(41° 24' N, 2° 09' E)	90	25	16.5	595
Córdoba	(37° 50' N, 4° 45' W)	123	15	18	600
Granada	(37° 11' N, 3° 35' W)	685	18	15	400
León	(42° 34' N, 5° 35' W)	860	15	10	550
Orense	(42° 21' N, 7° 51' W)	130	20	14	818

TABLE 1. Location and main climatic features of the sampling sites.

heavily eroded by human action; it also exhibits degradation series, frequently covered with pines (*Pinus halepensis* and *P. pinea*), and dry meadows (BELMONTE *et al.*, 1998). Data used in the study were obtained over the 1995-1998 period.

**CÓRDOBA SAMPLING SITE
(S-SW OF THE IBERIAN PENINSULA)**

The trap was located at the building of the Faculty of Science, on the outskirts of the city, W-SW of the urban area and with no obstructing buildings around it. The city of Córdoba is located in the Guadalquivir valley, on the banks of the river of the same name, and is flanked by Sierra Morena to the NE. It has a Mediterranean climate with certain continental and dry ombroclimatic features, particularly evident during the study period, which included extremely dry years. The trap is under the direct influence of Sierra Morena, which is covered by thermo-Mediterranean vegetation consisting in holm-oaks (*Quercus ilex* subsp. *rotundifolia*) and, to a lesser extent, cork oaks (*Q. suber*); once the climax ceases, the bush stage consists of kermes oak (*Q. coccifera*), lentiscus (*Pistacia lentiscus*) and rockroses (*Cistus*). Extensive areas have been repopulated with *Pinus* to form mixed woodland. The Guadalquivir valley and the other areas with a major influence on the trap are given over to various horticultural, fruit and intensive crops, and also contain ruderal plants and riverside woods. Data from this station were collected from 1993 through 1997.

**GRANADA SAMPLING SITE
(S-SE OF THE IBERIAN PENINSULA)**

The city of Granada lies in the so-called "Intra-Baetic Furrow" and is strongly influenced by its proximity to the Sierra Nevada mountain range. This results in a continental Mediterranean climate with strongly contrasting

inter-seasonal temperatures and rainfall (CAPEL-MOLINA, 1981). The vegetation series consists largely in basophilic Baetic meso-Mediterranean holm-oak woods, which have been replaced with pines (*Pinus* sp.) in many areas (DÍAZ DE LA GUARDIA & ALBA, 1998). Also worth noting is the extensive presence of croplands, coupled with ornamental flora in the urban area. Data from this site were collected from 1994 through 1998.

**LEÓN SAMPLING SITE
(NW IBERIAN PENINSULA)**

The trap was located in the urban area of the city, which lies in the NW of the Spanish plateau, approximately 20 km from a large mountain range that bears a strong influence on its climatic features. This area has a mesothermal Mediterranean, temperate and humid climate with dry summers and rainy winters, and contrasting winter and summer temperatures (ROLDÁN, 1987). Local vegetation consists mainly in Mediterranean riparious geomegaseries and irrigated crops (elms, poplars and willow trees and bushes, as well as Pyrenean oaks (*Quercus pyrenaica*), holm-oaks and replacement stages including abundant moors). Also significant is the presence of croplands and ornamental urban flora (FERNÁNDEZ-GONZÁLEZ *et al.*, 1998). Data from this site were collected in 1994, 1995 and 1998.

**ORENSE SAMPLING SITE
(NW OF THE IBERIAN PENINSULA)**

The city of Orense lies in the depression where the rivers Miño, Loña and Barbaña converge. It is located in a subhumid Mediterranean-to-Atlantic region, on the edge of the subhumid Mediterranean-to-Central European subregion. As a result, rainfall is not uniformly distributed throughout the year and the summer is drier than the other

seasons (ALLUÉ, 1966). The potential vegetation in the area influencing the trap includes Pyrenean oaks and other species such as oak (*Q. robur*), cork oaks or chestnut (*Castanea sativa*). Also in the vicinity of the trap is a coppice of *Frangula alnus*, laurel (*Laurus nobilis*) and *Genista falcata*, highly degraded by the introduction of pines and eucalyptus (*Eucalyptus* sp.) (IGLESIAS *et al.*, 1998). Data from this site were collected over the 1993–1996 period.

RESULTS AND DISCUSSION

Table 2 shows the following data for each sampling station: (a) the sampled years and the total annual number of conidia found at each; (b) for each year, the day on which *Cladosporium* concentration peaked and its value; (c) the mean daily concentration at each sampling point (local concentration) and the number of days on which this mean was exceeded each year at each sampling site; and (d) the average conidial count for the sites as a whole (overall concentration), as well as

the number of days on which this average was exceeded each year at each station.

As shown in Table 2, Córdoba presented the highest conidial concentrations. However, this was the main result of the dramatic increase in figures recorded in the rainy years of 1996 and 1997; the latter exhibited the largest total annual count (639,123 conidia) and the former the daily peak (49,536 conidia/m³, which easily surpassed those recorded at the other sampling stations). In contrast, Barcelona exhibited the lowest total annual concentration (90,269 conidia/m³) and also the lowest peak value (2,607 conidia/m³ on 12 May 1996). These data are obviously consistent with the mean conidial counts at each sampling site (Tab. 2); thus, Córdoba exhibited the highest mean daily concentration (991,29 conidia/m³) and Barcelona the lowest (337,02 conidia/m³).

Table 2 shows that the concentration peaks for the southern cities occurred over two different periods each year; in Córdoba and Granada, these peak days occurred in

Sampling station	Year	Total annual count (conidia)	Peak value (conidia)	Date of the Peak	Annual mean of conidia (local)	Nº of days where mean was exceeded (local)	Annual mean of conidia (overall)	Nº of days where mean was exceeded (overall)
Barcelona	1995	126.165	3.402	12/05/95	337,02	176	605,34	58
	1996	90.269	2.607	12/05/96		94		28
	1997	151.810	2.870	09/06/97		179		108
	1998	123.805	4.365	28/05/98		130		61
Córdoba	1993	293.390	11.765	08/05/93	991,29	70		97
	1994	286.126	15.833	25/05/94		57		79
	1995	141.797	3.646	30/04/95		35		60
	1996	448.674	49.536	17/09/96		87		138
	1997	639.123	27.435	04/10/97		105		154
Granada	1994	284.899	7.803	02/10/94	690,60	81		91
	1998	219.239	3.127	06/06/98		92	110	
León	1994	193.771	5.638	01/10/94	513,78	119	101	
	1995	236.612	9.503	11/07/95		124	111	
	1998	132.205	3.099	06/09/98		80	70	
Orense	1993	113.368	2.702	17/09/93	493,99	79	64	
	1994	201.326	4.990	18/07/94		133	114	
	1995	185.541	8.299	24/06/95		105	89	
	1996	220.987	6.997	07/08/96		115	102	

TABLE 2. Results obtained for each sampling site and year.

the spring and autumn (April–May and September–October in Córdoba, and June and October in Granada). In contrast, the peaks achieved in the northern cities occurred over a single period, namely in May–June in Barcelona, in July–October in León and in June–September in Orense.

A comparison of the mean conidia concentration per sampling site and the average concentration for the five cities as whole (Tab. 2) once again separated the northern and southern cities into two well-defined groups: one including Córdoba and Granada, where the mean concentrations exceeded the city averages; and the other including the other three cities, where concentrations were below the averages (particularly in Barcelona).

In order to facilitate the comparison of sampling stations, a mean curve was plotted from their data (Fig. 1) and used as a temporary model until a larger data set was available. Data were also smoothed using the moving mean for 7 days. Figure 1 also shows the variation of total annual conidial counts corresponding to the mean curve for each sampling site; the plot exposes the above-mentioned differences between sampling points (*viz.* the high concentrations of Córdoba and the low annual mean of Barcelona).

Based on Figure 1, and consistent with previous findings, the sampled cities in the north of the Iberian Peninsula (*viz.* Barcelona, León and Orense) exhibit an annual distribution of *Cladosporium* that differs markedly from those in the south (Córdoba and Granada); the former exhibit a single period of variable length (early summer to late autumn), in which conidial concentrations in the air exceed the means for each city, with clear, and occasionally substantial,

fluctuations; Barcelona exhibits the lowest, but also the most uniform, *Cladosporium* concentrations. The lowest incidence of this genus was recorded in winter; concentrations rose during May and peaked at about 900 conidia/m³ in June–July, after which they gradually fell. León and Orense exhibit a similar annual variation pattern; however, the conidial concentrations and peaks are slightly higher in León. Both cities exhibit virtually zero concentrations during the first few months of the year, and always below those of Barcelona, before rising during April–May (to over 500 conidia/m³) and continuing to increase during July. They then level off at values above the means (with peaks of up to 2,000 conidia/m³) until November, before gradually falling to their lowest levels in December.

In contrast, the atmospheric incidence of *Cladosporium* in Córdoba and Granada is markedly seasonal: most conidia are detected in late spring and autumn. In fact, as shown in Table 2, both cities exhibit very few days on which counts exceed the local mean. In the other three cities, however, roughly one third of the days exceed the mean each year. Córdoba and Granada are also similar in terms of the annual distribution of *Cladosporium* conidia; in both, conidial counts remain low until May, when they suddenly rise to levels as high as 3,500 conidia/m³ that prevail until July, before dropping to around 500 conidia/m³ until September. They then rise sharply once again to levels close to those recorded in the spring. A further decrease occurs in November and counts remain throughout the winter.

CONCLUSIONS

The following conclusions can be drawn from these findings:

1. *Cladosporium* is present throughout the year in the atmosphere of the five sampled cities (Barcelona, Córdoba, Granada, León and Orense). However, conidial concentrations in the atmospheres of León and Orense can be very low in winter. There are substantial inter-annual differences in the total annual counts for each city.

2. Córdoba exhibits the highest conidial concentrations in air. Its annual concentrations and peaks in 1996 and 1997 were both unusually high with respect to the other sites

and years. In contrast, Barcelona exhibits the lowest concentrations. However, it also exhibits the largest number of days on which the local conidial concentration exceeds the mean value, consistent with a long period of high concentrations. This city also presents the most uniform incidence of *Cladosporium*.

3. The southern cities studied (Córdoba and Granada) exhibit very similar annual variation patterns, with two well-defined seasons of increased atmospheric concentrations of *Cladosporium* (spring and autumn).

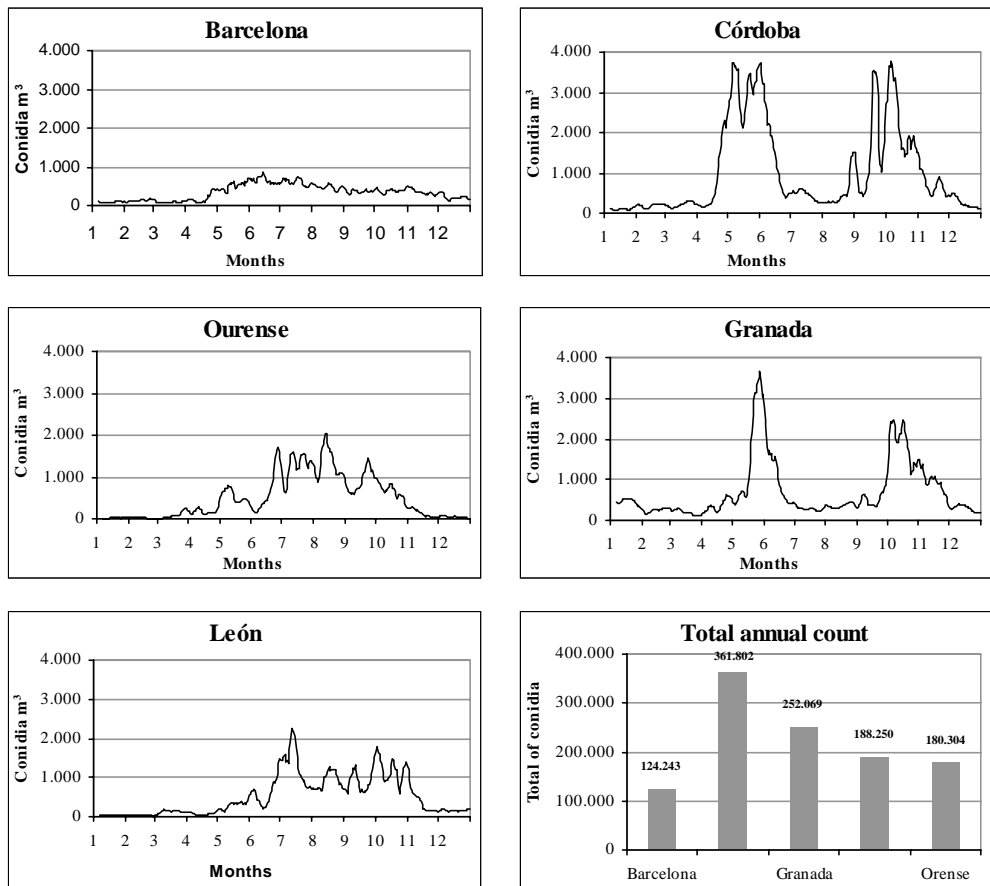


FIGURE 1. Mean curve for each sampling site and total conidial counts derived from it.

This is not the case with the northern cities (Barcelona, León and Orense), which, regardless of the fluctuations observed, appear to exhibit a single period of peak concentrations (between April–May and November).

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