

Aerobiological study of fungal spores from Palencia (Spain)

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Abstract

A study of the concentration of fungal spores has been carried out in the atmosphere of Palencia town (NW Spain) during 1992. The volumetric method of filtration has been used. Half of the daily filter sample has been cultivated in Czapekdox-agar or Sabouraud-agar for the identification of fungal colonies, and the other half has been examined by optical microscopy. Several colonies belonging to 26 genera have been identified. Deuteromycetes (54%) and Zygomycetes (28%) are assembled in four genera, and Bacteria and Actinomycetes (18%) in three genera. The greatest concentrations occur for *Aspergillus* (23%), *Mucor* (25%), followed by *Penicillium* (16%). The greatest diversity and abundance of fungal spores are found in September–December. The viable colonies are more abundant in Czapekdox-agar culture, whereas Bacteria were more frequently found in Sabouraud medium.

Keywords: Aerobiology; Aeromycology; Spores; Fungi; Meteorological factors

1. Introduction

The aim of this work has been to study the influence of meteorological factors in the dispersion and concentration of spores in the atmosphere of Palencia, as well as the identification of spores and other particles (bacteria) most readily identifiable under the optical microscope, and therefore requiring different culture media for their identification.

Fungi are biological organisms with a great capacity to colonize all kinds of substrate and to develop in extreme environmental conditions, soils, plants and animal remains (Comtois, 1990).

Many are pathogenic to humans, causing asthmatic and allergenic problems, due to differential deposition in the respiratory system (Myngind, 1982). *Aspergillus* and *Penicillium* spores are some of the most important allergens on a global scale (Roses-Codinach et al., 1992).

In recent years, several studies have been carried out on aerobiological particles (fungal spores and bacteria) in the atmosphere of different regions of Spain (Infante and Domínguez, 1988; Roses-Codinach et al., 1992; González Minero et al., 1993). Therefore, it was considered interesting to establish a comparison between meteorological parameters (rain, wind velocity and direction, humidity, temperature) in addition to the flora and fauna of the sampled area to study their relationship in the capture of the particles and the implications for respiratory ailments.

Palencia is located at latitude 42°0'N and 4°32'W, in north-west Spain. Mean annual precipitation is 351.5 mm and mean temperature is 11.7°C.

Bioclimatically, Palencia is in the lower supra-Mediterranean stage, with a growing season of 8 months (Rivas Martínez, 1987).

2. Materials and methods

The sampling was completed in 1992, in a station placed in Palencia (Spain). The active filtration method,

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described by Suárez-Cervera and Seoane-Camba (1983), was used. The system has a filter chamber with a weathercock device connected to a vacuum pump and an air volume counter. The filter is located in the filter chamber and is made of cellulose ester, 5- μ m pore size and 7 cm in diameter. These filters become transparent when immersion oil is added, and they can then be examined with optical microscopy.

The samples were gathered daily, after 24 h of exposure and filtering about 4 m³ of air. Half of the filter was dipped in immersion oil to quantify the total number of fungal particles; the other half was cut in two pieces, one of these pieces was incubated in Czapekdox medium and the other in Sabouraud medium at 24°C.

Five days after the sampling, the colonies were counted and 7 days later they were identified. For the identification, the usual atlas and aids in this type of works were employed (Grant Smith, 1984, 1986; Fassatová, 1986; Barnett and Hunter, 1987).

The filter chamber was cleaned daily with wet cotton soaked with alcohol, in order to eliminate contaminants.

The method used in this study allowed us to observe directly, by optical microscopy, one half of the filter, in order to evaluate the number of spores per m³ of air and, from the other half, to identify the types of viable spores growing on both culture media.

The number of colony forming units per unit volume is not discussed because they do not correspond exactly to the number of spores counted by optical microscopy, due to the fact that many of the spores seen were not viable (Hurtado et al., 1989).

Two statistic analyses have been made with the total number of spores and the viable colony forming units: a correlation analysis and a multiple regression. The correlation analysis was made between the number of colonies grown daily and the daily value of the meteorological variables (mean temperature, relative humidity, precipitation and wind velocity). The number of colonies recorded was 2787, and a confidence interval of 95% was used.

A multiple regression was made with the daily spore concentration values against the value of the meteorological variables employed in the correlation analysis. Samples were taken for 230 days and the confidence interval was 95%. A one-factor variance analysis (one-way) was made with the daily values of spore concentration and the number of colonies grown, against the qualitative parameter of wind direction, giving values of 1 to 4 depending of the quadrant, and 5 for calm conditions. The number of colonies recorded was 2787 with a 95% confidence interval, and two multiple range tests (Bonferroni's test and Duncan's test, in O'Neill and Wetherill, 1971) showed significant differences.

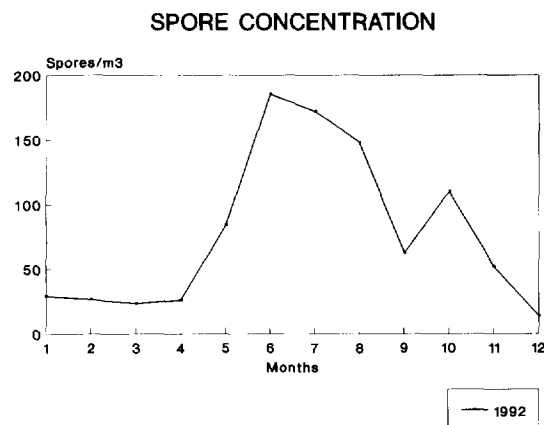


Fig. 1. Monthly concentration of spores found in the atmosphere of Palencia during 1992.

The meteorological data were gathered from the Meteorological Center for the Duero basin, at its station in Autilla-Palencia (Fig. 1).

3. Results

In this study we have observed the continuous presence of spores in the air of Palencia throughout the year sampled, but there are important seasonal variations (Fig. 2). The maximum concentrations are produced during the spring–summer period, with the highest levels being reached in June (180 spores/m³).

In total, 460 quarters of filters were cultivated in order to identify the different types of fungi present in the atmosphere, and so there were 1289 colonies counted. There is also an annual variation in the number of fungal and bacterial colonies developed in the culture media with two peak levels, one in March and another in December (Figs. 3 and 4). Overall, 75 taxa

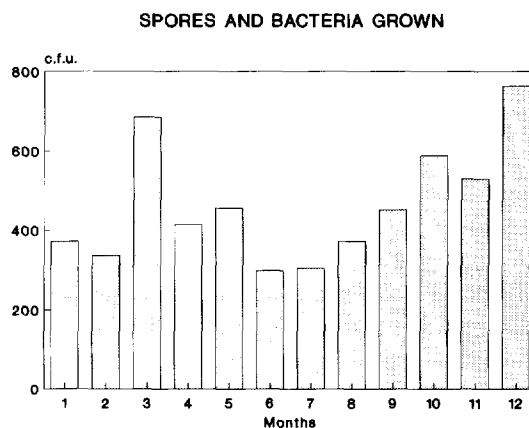


Fig. 2. Colonies grown monthly during 1992.

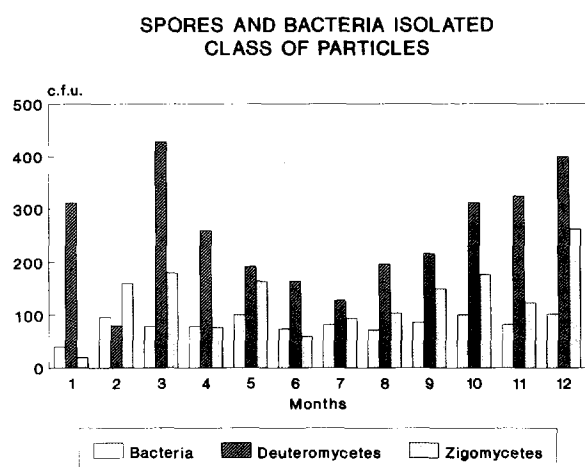


Fig. 3. Number of colonies of Bacteria, Deuteromycetes and Zigomycetes grown monthly in 1992.

corresponding to 34 genera were identified: 26 Deuteromycetes (54%), four Zigomycetes (28%) and four Bacteria (18%) (Table 1).

In Table 1, we list the genera with viable colonies that were identified in the different months in both culture media. Beside the generic name, there is a number in parenthesis corresponding to the number of species identified for each genus. The list of species of fungi is as follows: *Acremonium* sp., *A. murorum*, *A. strictum*; *Alternaria alternata*, *A. consortiale*, *A. tenuissima*; *Aspergillus candidus*, *A. flavus*, *A. fumigatus*, *A. nidulans*, *A. niger*, *A. repens*, *A. terreus*, *A. unguis*, *A. versicolor*; *Aureobasidium pullulans*; *Beauveria bassiana*; *Botrytis cinerea*; *Cloridium* sp.; *Chrysosporium pannorum*; *Cladosporium* sp., *C. cladosporioides*, *C. herbarum*, *C. macrocarpum*; *Curvularia lunata*; *Epicoccum* sp.; *Fusarium solani*; *Geotrichum candidum*; *Helminthosporium* sp., *H. solani*, *H. velutinum*; *Humicola brevis*, *H. fuscoatra*, *H. grisea*, *H. nigrescens*; *Leptosphaeria* sp.; *Monilia sitophila*; *Mycelia sterilia*; *Nigrospora* sp.; *Paecilomyces fulvus*, *P. varioti*; *Penicillium brevicompactum*, *P. camemberti*, *P. chrysogenum*, *P. diversum*, *P. expansum*, *P. frequentans*, *P. funiculosum*, *P. italicum*, *P. meleagrinum*, *P. notatum*, *P. purpureogenum*, *P. roseopurpureum*, *P. rubrum*, *P. thomii*, *P. vermiculatum*, *P. viridicatum*, *P. waksmanii*; *Phoma* sp.; *Stemphylium* sp.; *Trichoderma viride*; *Trichocladium* sp.; *Verticillium* sp., *V. albo-atrum*; *Absidia corymbifera*, *A. glauca*, *A. ramosa*, *A. spinosa*; *Mucor circillenioides*, *M. hiemalis*, *M. indicus*, *M. mucedo*, *M. racemosus*; *Rhizomucor pusillus*; *Rhizopus nigricans*.

The highest number of colonies corresponded to the genus *Mucor* with 25% of the colonies grown, followed by *Aspergillus* (23%), *Penicillium* (15%), *Absidia* (3%) and *Alternaria* (2%); the most abundant species were *Mucor racemosus*, *M. hiemalis*, *Aspergillus fumigatus*, *A. flavus*, *Penicillium frequentans* and *P. notatum*.

The most common Deuteromycetes belonged to genera *Alternaria*, *Aspergillus*, *Geotrichum*, *Helminthosporium*, *Penicillium* and *Phoma* (Fig. 5). Among Zigomycetes, *Absidia* and *Mucor* genera were the most frequently recorded (Fig. 6); among airborne bacteria the most common were *Actinomycetes*, *Bacillus*, *Pseudomonas* and *Xanthomonas* (Fig. 6).

In relation to the culture media used, in Sabouraud medium there is a better development of colonies of *Absidia*, *Actinomycetes*, *Bacillus*, *Xanthomonas*, *Penicillium* and *Phoma*; on the other hand, *Mucor*, *Pseudomonas*, *Alternaria*, *Aspergillus* and *Helminthosporium*, are best developed in Czapekdox medium. The genus *Geotrichum* was more abundant during the first semester in Sabouraud medium and during the second in Czapekdox medium (Figs. 5 and 6).

The results obtained by multiple regression analysis (Table 2) show that the concentration of spores is significantly correlated with minimum temperature and precipitation, so an increase in the value of these parameters is related to an increase in the spore concentration; the other meteorological parameters are not significant in estimating the fungal concentration. The wind direction is not correlated with the daily spore concentration (Table 3).

The number of colonies grown is significantly correlated with temperature (Table 4). Furthermore, there is a significantly larger number of colonies grown when the wind blows from the southwest (fourth quadrant) than when it blows from the southeast (third quadrant); similarly, the number of colonies grown when the wind blows from the northwest and southwest (first and fourth quadrants) is also large. The wind coming from the latter quadrant has more colonies growing in the culture medium (Table 5).

4. Discussion

The results of this work reflect the fungal spectrum of Palencia and show the dominant fungi and with a seasonal periodicity similar to that in other parts of the world (Hyde et al., 1956), characteristic of continental climates, in which the great decrease in winter temperature inhibits the enzymatic activity of many fungi (Aller et al., 1971; Ebner et al., 1989; Fernández González et al., 1993). There are low, more or less constant spore levels in the air during the first 4 months of the year; in June, a great increment is produced mostly by the genus forms *Cladosporium* and *Alternaria*, and then in August, a continuous decrease of spore concentrations begins until December when only a small proportion is left. Such annual variation is different in the Mediterranean coastal areas (Roses-Codinach et al., 1992) where the spore concentrations show little variation throughout the year.

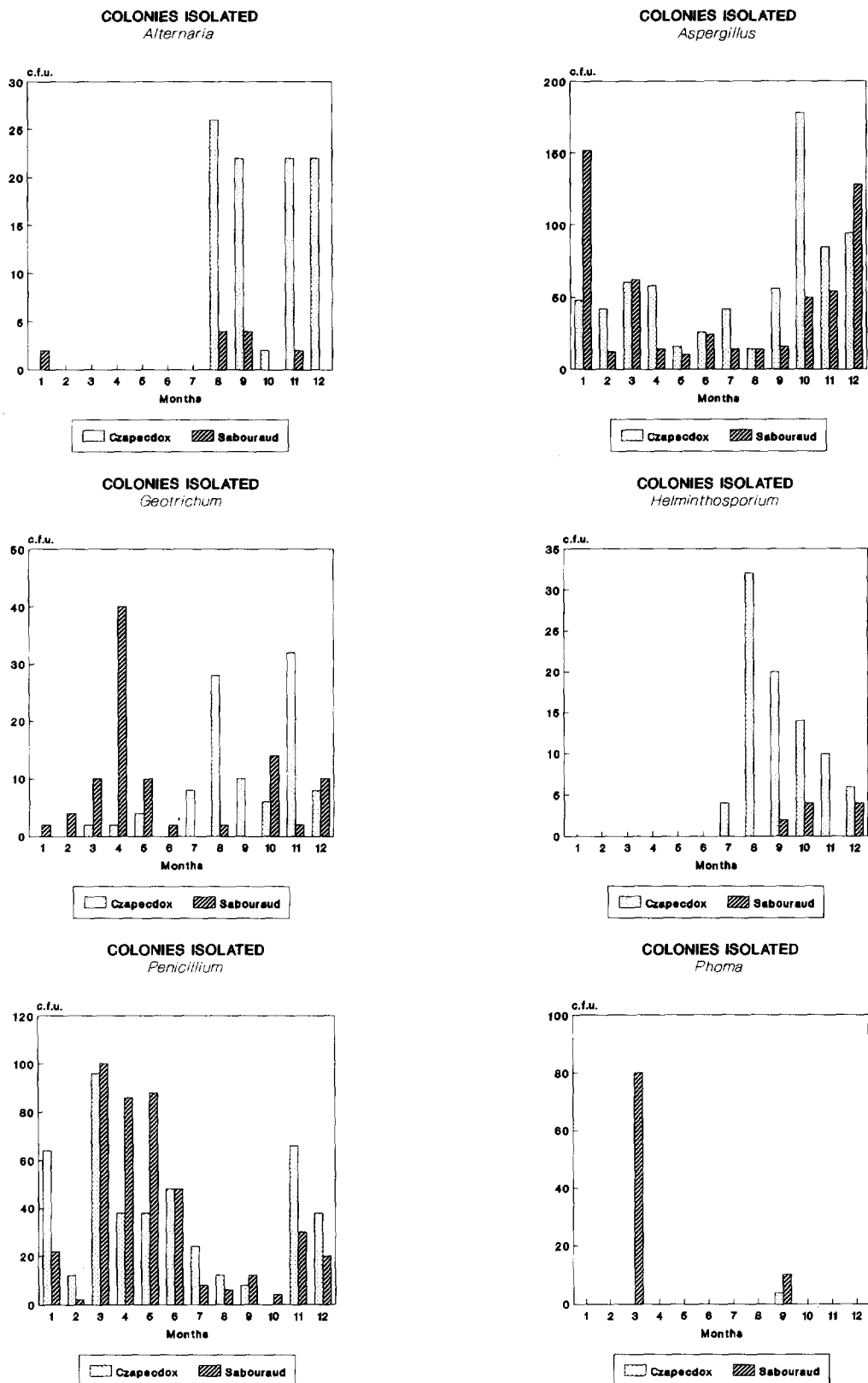


Fig. 4. Number of colonies isolated of the most abundant Deuteromycetes found in the atmosphere of Palencia in 1992.

Table 1
List of fungus and bacteria isolated during 1992

	Jan		Feb		Mar		Apr		May		Jun		Jul		Aug		Sep		Oct		Nov		Dec	
	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S
Deuteromycetes																								
<i>Acremonium</i> (3)																+	+				+			
<i>Alternaria</i> (3)		+														+	+	+	+	+		+	+	+
<i>Aspergillus</i> (9)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Aureobasidium</i> (1)												+	+	+	+	+	+						+	+
<i>Beauveria</i> (1)					+		+																	
<i>Botrytis</i> (1)														+	+									
<i>Cloridium</i> (1)																	+	+	+		+		+	+
<i>Chrysosporium</i> (1)																								
<i>Cladosporium</i> (4)				+			+	+	+				+									+	+	
<i>Curvularia</i> (2)			+								+					+		+						
<i>Epicoccum</i> (1)													+											
<i>Fusarium</i> (1)													+						+					
<i>Geotrichum</i> (1)		+		+	+	+	+	+	+	+		+	+		+	+	+		+	+	+	+	+	+
<i>Helminthosporium</i> (3)													+		+		+	+	+	+	+	+	+	+
<i>Humicola</i> (4)															+	+	+	+	+	+			+	
<i>Leptosphaeria</i> (1)																				+				
<i>Monilia</i> (1)															+									
<i>Mycelia</i> (1)	+	+		+	+	+		+		+	+	+	+			+		+						
<i>Nigrospora</i> (1)																+			+					
<i>Paecilomyces</i> (2)					+												+		+		+	+	+	+
<i>Penicillium</i> (17)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+
<i>Phoma</i> (1)						+											+	+						
<i>Stemphylium</i> (1)																								+
<i>Trichoderma</i> (1)													+	+										
<i>Trichocladium</i> (1)																		+	+	+				
<i>Verticillium</i> (2)	+						+		+	+	+					+			+					
Zigomycetes																								
<i>Absidia</i> (4)			+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
<i>Mucor</i> (5)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Rhizomucor</i> (1)																	+	+						
<i>Rhizopus</i> (1)			+																+		+			
Bacterias																								
<i>Bacillus cereus</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Pseudomonas</i>		+	+	+	+	+	+	+	+	+	+			+		+	+	+	+	+	+	+	+	+
<i>Xanthomonas</i>		+	+	+	+		+		+	+		+			+	+	+	+		+	+		+	+
<i>Actinomycetes</i>			+	+	+	+		+	+	+	+	+	+	+		+	+					+		+

No. of species identified in parentheses; +, present; C, Czapeckox medium; S, Sabouraud medium.

The statistical results, with a positive correlation between the spore concentration and minimum temperature, agree with expected fungal behaviour (Gregory, 1973; Trujillo et al., 1990), because most of the fungi found in the air are thermotolerant, that is, they have a growing range between 12°C and 55°C, although some of them, such as some *Cladosporium* and *Fusarium* species, belong to the psychrophilic group (i.e. they can develop in low temperatures, sometimes below 0°C, with an upper limit higher than 20°C). The same can be said in relation to rainfall, whose increase implies a great increase in relative moisture, over 70%, a value considered as the lower limit for fungal development. As far as the number of colonies developed in both culture media are concerned, we have observed that in general, they do not show a clear difference.

Some genus forms like *Alternaria* and *Helminthosporium* grow almost exclusively during the second half of the year, mainly because of environmental factors (Honda, 1969, Tanaka and Akai, 1963). Both taxa belong to the same family and their concentration in the air is higher between July and October because they are mainly cereal saprophytes and some *Solanaceae* saprophytes and parasites, but they appear in the culture media from August to December, probably as a consequence of the lower spore quantities of other fungi groups in competition with them.

Aspergillus, *Penicillium* and *Mucor* have been the most abundant in both culture media as they correspond to widely distributed fungi and are important as industrial or research organisms (Burge, 1986). The *Aspergillus* seasonal pattern confirms its characteristic winter be-

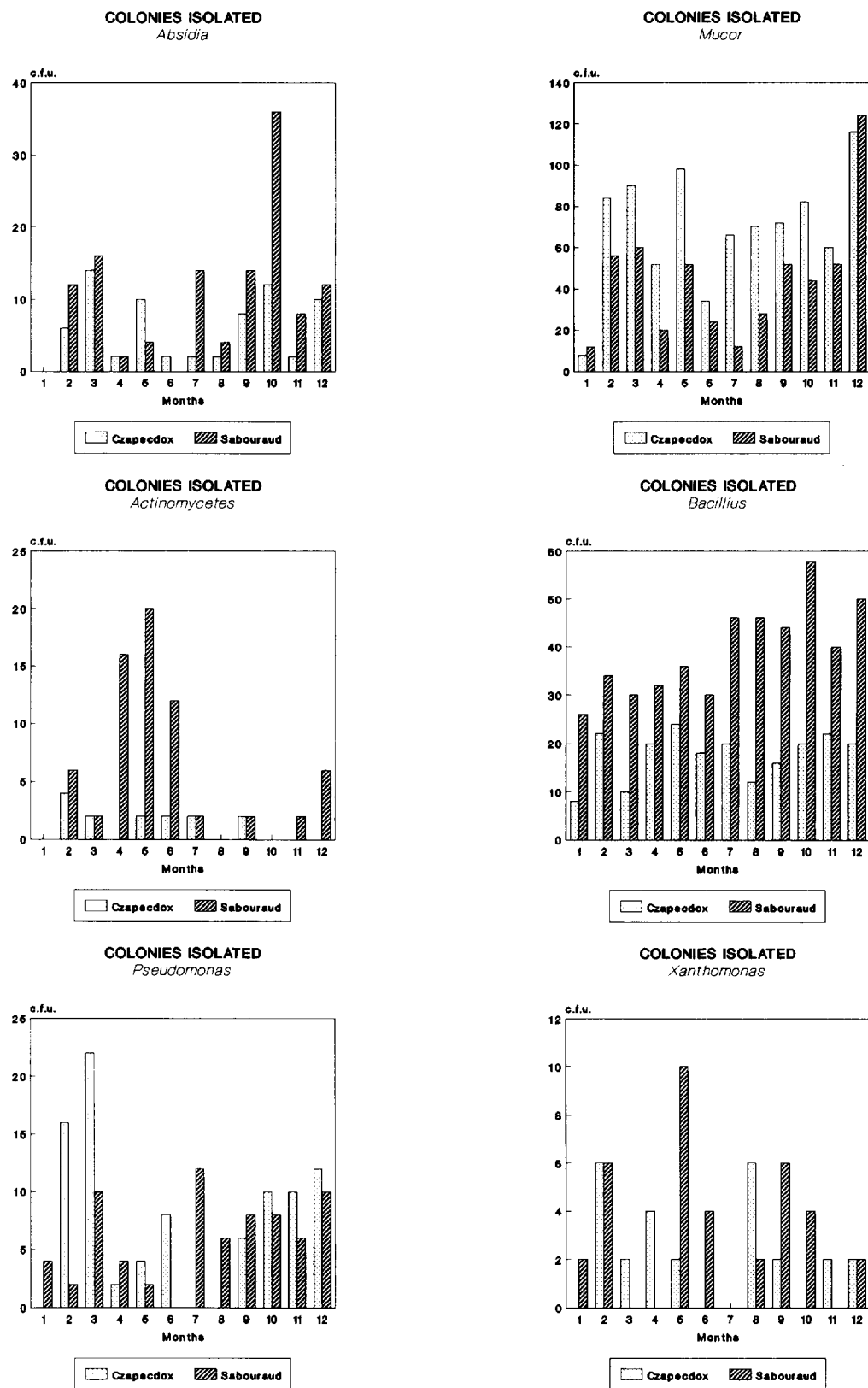


Fig. 5. Number of colonies isolated of the most representative Zigomycetes, Actinomycetes and Bacteria found in the atmosphere of Palencia.

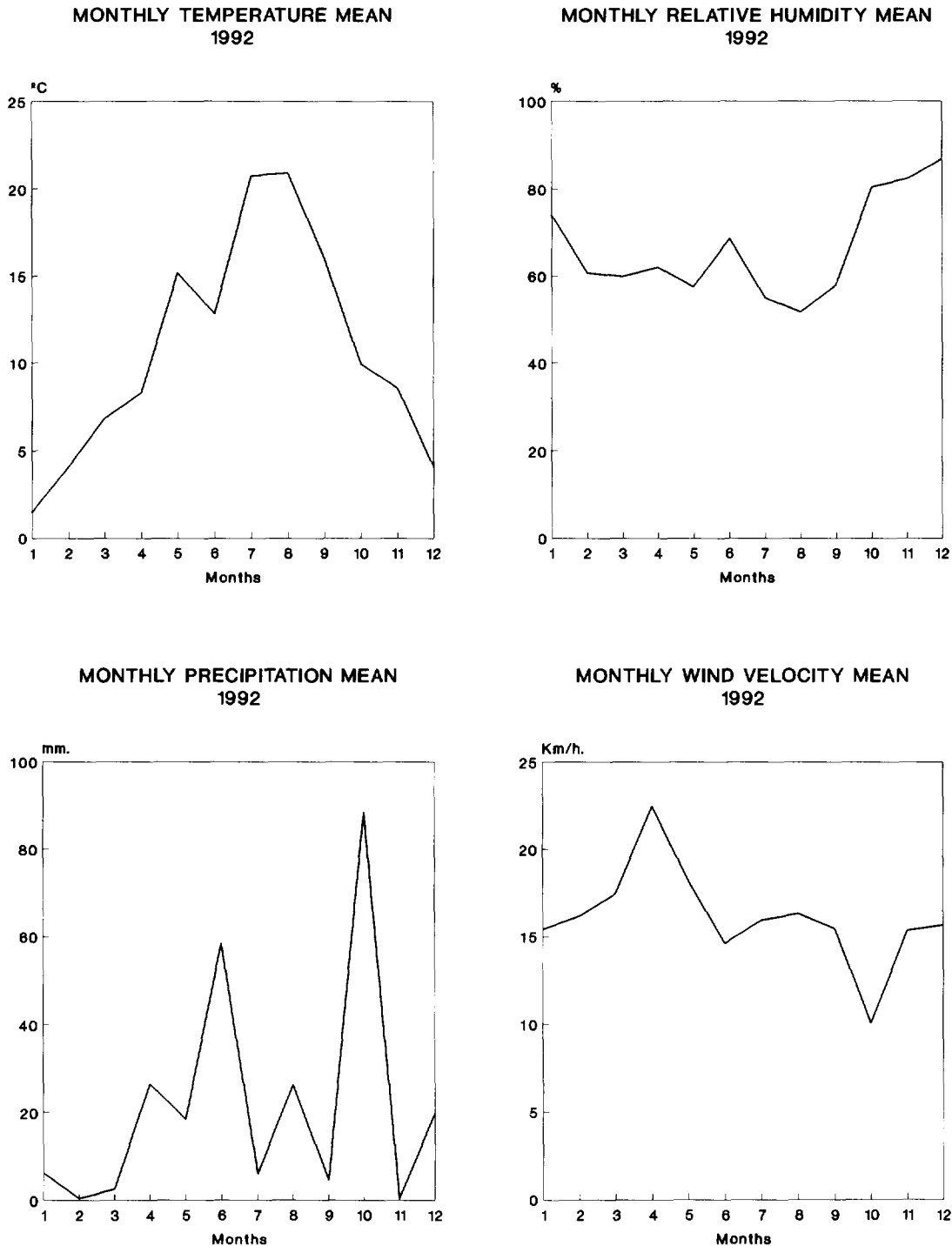


Fig. 6. Meteorological data for Palencia, Spain, during 1992.

haviour (Al-Doory et al., 1980; Rubulis, 1984). On the other hand, the *Penicillium* colonies were more abundant during the first half of the year, for groups there is scarce competition with other fungi. In any case, the maximum C.F.U. values were observed in which there is no clear explanation, unless, as in other groups there is scarce competition with other fungi. In any case, the maximum

C.F.U. values were observed in December with 764, followed by March with 686 and October with 588.

Relating to the culture media used, we have observed that, in both culture media, the Deuteromycetes have dominated over the other fungi groups, followed by Zygomycetes and Bacteria. Infante et al. (1992), also noted that Deuteromycetes are the most abundant fungi

Table 2

Variance in multiple regression analysis of meteorological factors in daily spore concentrations

R^2	0.163
n	230
T -values	
Maximum temp.	1.165
Minimum temp.	3.281*
Relative humidity	1.223
Precipitation	2.863*
Wind velocity	−0.608

*Significant for $\alpha = 0.05$.

Table 3

One factor variance analysis (one way) of spore concentrations and colonies grown versus wind direction

	Spores concentration	Colonies grown
F -value	0.579	3.286*
n	230	2787

*Significant for $\alpha = 0.05$.

when an agar medium is used with malt extract, but in our case the presence of Ascomycetes was zero. Some Ascomycetes require the presence of plant tissue for their development. Antibiotic activity in some media and the fast growth of other taxa prevent their occurrence.

The culture media used, also cause specificity in the development of some fungi. This has already been reported by other authors for artificial culture media (Subiza Martín and Jerez Luna, 1983; Horner et al., 1992). The genus *Cladosporium* has scarcely grown in the culture media used but has a higher score among the spores recognized with optical microscopy on the uncultured filter, reaching up to 43%.

Table 4

Correlation analysis of meteorological factors versus colonies grown

Humidity	Precipitation	Temperature	Wind velocity
−0.0169	−0.0175	−0.0905*	−0.0260

*Significant for $\alpha = 0.05$.

Table 5

Multiple range test analysis (Bonferroni and Duncan), colonies grown versus wind direction

Means		Bonferroni's test					Duncan's test				
		1	2	3	4	5	1	2	3	4	5
4.3558	Gr.1									*	
4.1343	Gr.2										
3.5231	Gr.3										
5.6543	Gr.4			*					*		
5.0000	Gr.5										

*Significant for $\alpha = 0.05$.

Taking into account all we have noted until now, we can deduce that the preponderance of some fungi taxa over others is different in the direct analysis of their spores compared to concentrations in the culture media, knowing that the results obtained in pure cultures do not agree with the fungi behaviour in nature. As was observed by Savino and Caretta (1992), analysis by optical microscopy gives more information on the spore concentration in the air and on the seasonal behaviour of the groups causing allergic problems.

The wide distribution of *Bacillus* in the soil and its resistant spores may be responsible for their abundance in the atmosphere of Palencia, and that agrees with the data found in Kuwait by Marafie and Ashkanani (1990) and in China by Yonqyi et al. (1993). *Pseudomonas* and *Xanthomonas* bacteria are abundant on plant leaves and in the soil, but they have been collected in less quantity in this study. So, geographical and environmental features may cause the prevailing atmospheric bacterial pattern that differs from place to place.

We can conclude: (1) The highest spore concentrations were registered in June, mainly by the genus *Cladosporium*. (2) Minimum temperature and precipitation are the meteorological factors that have the most influence on spore concentrations. (3) The culture media Czapeckdox and Saboureaud produce a selection of the development of the different spore types. (4) The highest number of viable colonies has corresponded to the genera *Mucor*, *Aspergillus*, and *Penicillium*.

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