

Mold allergy in the Mediterranean island of Crete, Greece: A 10-year volumetric, aerobiological study with dermal sensitization correlations

Michael I. Gonianakis, M.D., Ph.D.,* Ioannis K. Neonakis, M.D.,* Ioannis M. Gonianakis,* Maria A. Baritaki, M.D.,* Demosthenis Bouros, M.D., Ph.D.,# George Potamias, Ph.D.§ and Kalliopi S. Kontou-Fili, M.D., Ph.D.¶ (Greece)

ABSTRACT

Mold spores are universal outdoor and indoor components and generally are recognized as possible sources of respiratory allergies. A 10-year aerobiological study (1994–2003) was conducted in the city of Heraklion located at the center of the Mediterranean island of Crete, Greece. Eighteen mold species exhibiting a normal annual seasonal pattern have been identified and recorded. The most abundant mold species include (a) Cladosporium, (b) Alternaria, (c) miscellaneous ascospores (d) Leptosphaeria, and (e) basidiomycete Coprinus. In parallel, 571 atopic individuals were tested by skin-prick tests (SPTs). Among these 571 patients 42.5% showed dermal positivity to mold allergens. Most positive SPTs were those of (a) Alternaria, (b) Cladosporium, (c) Fusarium, (d) Aspergillus, and (e) Mucor. No linear relationship was noted between SPT frequencies and percentages of mold species. All of these aerobiological and sensitization data constitute a firm basis for further medical and biological research and application.

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Mold spores present a ubiquitous global distribution and consist an inevitable allergen source. The allergens yielded from the mold spores, as well as intact spores are dispersed in the air of outdoor and indoor environments. When genetically predisposed atopic individuals inhale these components, a significant percentage of them are sensitized to the corresponding allergens. Consequently, some atopic individuals will exhibit allergic manifestations from the upper and lower respiratory tract.^{1–3} The allergenicity of mold-dispersing units has been well established for only a small number of molds,^{4–7} whereas the ability of the vast majority of mold species to provoke allergic sensitization to atopic individuals still has not been well elucidated.^{8,9} To improve the diagnosis and treatment of allergic patients, aerobiological and clinical data should be combined.^{10,11}

To identify, quantify, and determine the seasonal pattern of allergenic mold spores in the city of Heraklion, a 10-year aerobiological study (1994–2003) was conducted. Heraklion is the major city located in the center of the island of Crete (Greece). This study represents the first long-term aerobiological investigation in southern Greece.

In parallel, the frequencies of positive skin-prick tests (SPT) in a population of 571 allergic individuals were assessed with a battery containing 10 mold extracts most often used by practicing allergists in the area. A correlation of these frequencies with air sampling data was attempted. Positive skin reactivity would be considered as an index of allergen exposure and sensitization.

MATERIALS AND METHODS

Location

The island of Crete is geographically located in the middle of the Mediterranean Sea. The city of Heraklion lies 30 m above sea level. The area is characterized by a mild temperate climate: mild winters and cool summers. The mean monthly temperatures generally do not fall below 10°C during winter and do not rise above 27°C in summer. Total monthly hours of sunshine are >350 hours in the summer and usually are

*The Heraklion Aerobiology Group, Heraklion, Greece, #Department of Pneumology, University of Alexandroupolis Medical School, Alexandroupolis, Greece, §Institute of Computer Science, Foundation for Research and Technology–Hellas, (FORTH), Heraklion, Crete, Greece, and ¶Aerobiology Laboratory, Department of Allergology–Clinical Immunology, Laikon, General Hospital of Athens, Athens, Greece
Address correspondence and reprint requests to Michael I. Gonianakis, M.D., Ph.D., 66 Thenon str, Heraklion, z.c. 71305, Crete, Greece
E-mail address: gonianos@forthnet.her.gr
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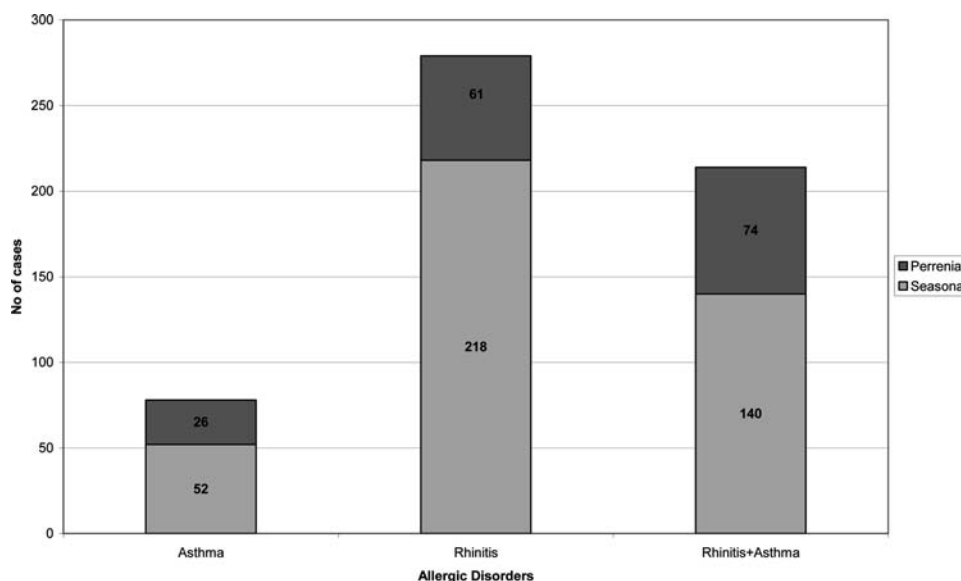


Figure 1. Distribution of allergic disorders in 571 selected patients.

not <105 hours in winter. Generally, the total monthly rainfall exceeds 27 mm in the spring, 40 mm in the fall, and 76 mm during winter; there is virtually no rain in June and July. The mean humidity is usually 63%.

Air Sampling

Allergenic airborne particles were sampled continuously since 1994, using a motorized Burkard 7-day spore trap. The Burkard trap was positioned on the roof of an 18-m-tall building, in the middle of the city, away from parks or fields. Airborne particles were sampled, mounted on slides, and recognized as previously described.¹² All identified particles were counted and the mean value of three transverses was recorded. The mean daily mold counts per cubic meter of air were calculated according to the formula previously proposed.¹¹ All identifiable mold spores, which could give a constant seasonal pattern, were analyzed. The number of various mold spores counted over this 10-year period has been calculated in absolute numbers and as a percentage of the total mold spore load. Ten-day moving averages for each year and collectively for all 10 years also have been drawn. Similarly, the minimal, the maximal daily concentration, and also the mean annual counts per cubic meter of each genus of mold per year have been determined.

Patient Group and SPTs

A total of 571 atopic outpatients of the Allergologic Clinic of the Asclepeion Hospital in Heraklion were examined. All patients were residents of Heraklion and the surrounding areas. The age range of mold-positive subjects was 6–65 years; 295 were male patients and 276 female patients. Complete convincing data regarding the atopic status of each individual were recorded

by reviewing personal and family histories. Distribution of the allergic disorders of these 571 patients is shown in Fig. 1. A complete physical examination was performed also. Patients were subjected to SPT with commercial extracts (Stallegens, Fresnes Cedex, France), including 50 common aeroallergens (tree, grass and weed pollens, mold spores, house-dust, mites, and epithelia) and positive (histamine) and negative (diluent) controls. All SPT results were read and recorded at 15 minutes. Positive controls were considered as the reactions that gave a wheal of >3 mm in diameter ($\pm$ erythema), with an absolutely negative diluent control. Mold spore extracts were those routinely used by the allergists and other physicians in our country over the study period, *viz.*, those of *Alternaria*, *Aspergillus*, *Botrytis*, *Cladosporium*, *Dreschlera/Helminthosporium*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus*, and *Stemphyllium* (Fig. 2).

RESULTS

Eighteen mold species exhibiting a normal yearly seasonal pattern have been identified and recorded (Figs. 3 and 4). They belonged to the groups Deuteromycetes (or Fungi imperfecti), Ascomycetes, and Basidiomycetes. The relevant aerobiological data are presented in Tables 1, 2, and 3, respectively. Because of their small size, members of the group Zygomycetes were only sporadically identified and could not give a graphical seasonal pattern.

In our area, the Deuteromycetes *Cladosporium* was by far the most numerous spore identified, followed in declining order by *Alternaria*, miscellaneous ascospores, *Leptosphaeria*, and the basidiomycete *Coprinus*. The contribution of all Deuteromycetes in total air mold flora was 60.24% (Table 1). The main spore load of

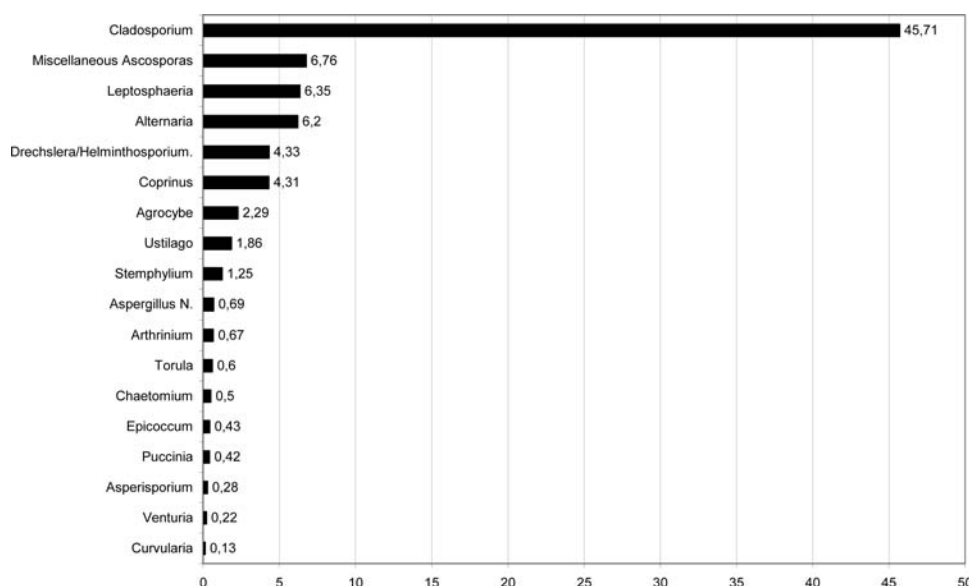


Figure 2. Mold spore species relevant frequencies (%) over a period of 10 years (1994–2003).

Cladosporium was observed during the summers. Its recorded counts never exceeded the level of 680 conidia/m³, except in 15 single days throughout the 10 years. *Alternaria* constitutes the second most frequently trapped spore type in this group. It has never exceeded the level of 90 conidia/m³ in absolute daily values, except in 17 single days. Main spore load of both *Cladosporium* and *Alternaria* was noticed during the summer and fall. With the exception of *Drechslera/Helminthosporium* that occasionally exceeded the level of 30 conidia/m³, all the other Deuteromycetes genera exhibited values lower than 8 conidia/m³ (Fig. 3).

Deuteromycetes were followed by Ascomycetes and Basidiomycetes in second and third rank, respectively. Cumulative results for the counted Ascomycetes are shown in Table 2. The mean daily concentration of all identified ascospores collectively was 26.61/m³ for the entire period, corresponding to 13.83% of the total fungal load. The predominant ascospores were those of *Leptosphaeria*, *Chaetomium*, and *Venturia*. Other genera encountered sporadically were *Pleospora*, *Pyrenophora*, *Chaetosphaerella*, *Ascobolus*, *Leptosphaerulina*, *Massaria*, *Sporormiella*, *Xylaria*, *Endophragmiella*. These genera, along with other ascospores that could not be identified, were grouped together under the general term “miscellaneous ascospores.” Schematic representation of the data from all 10 years showed two periods of ascospore activity: (a) midspring through summer, characterized by relatively “high” ascospore counts (15–33 spores/m³), and (b) the rest of the year, exhibiting lower activity (8–10 spores/m³).

Leptosphaeria spp. spores are found all year long with narrow fluctuations from 7–17 spores/m³ and peak values up to 50 spores/m³ in June. *Chaetomium* spp. and *Venturia* spp. spores were detected in very low

concentrations year round. Basidiomycetes with an all year round presence are *Agrocybe*, *Coprinus*, *Puccinia*, and *Ustilago*, while *Ganoderma*, *Boletus*, *Pleurotus*, and *Tilletia* were observed sporadically. Cumulative results of the first four genera are shown in the Table 3. *Coprinus* and *Agrocybe* showed a perennial distribution with very small fluctuations (4–10 spores/m³) and a small increase around December and January (up to 16 spores/m³). *Puccinia* and *Ustilago* also exhibited a perennial distribution, but the main mold spore loads, of low absolute values, are found during spring, summer, and fall. Even during its intense sporulation period, *Ustilago* did not exceed 10 spores/m³, and of *Puccinia* counts fluctuated ~2 spores/m³. The relevant frequencies of other spore species with no documented allergenic relevance were Myxomycetes, 14.69%; Peronospora, 2.19%; and Diplococcium, 0.12%.

In reference to the annual mold catch it should be noted that it did not show significant differences from year to year. Overall, the atmospheric spore concentrations are higher during the warmer months—April to October—with maximal load in June, July, and August (Figs. 3 and 4).

SPT

Among the 571 atopic patients tested by SPT, 243 gave a positive dermal reaction to at least one mold extract. The percentage distribution of positive SPT frequencies is shown in Fig. 5. The most commonly observed sensitizing allergens were in declining order: *Alternaria*, *Cladosporium*, *Fusarium*, *Aspergillus*, and *Mucor*. Among the 243 atopic individuals, 10 were exclusively sensitized to only one mold (4, *Alternaria*; 3, *Cladosporium*; 1, *Drechslera/Helminthosporium*; 1, *Penicil-*

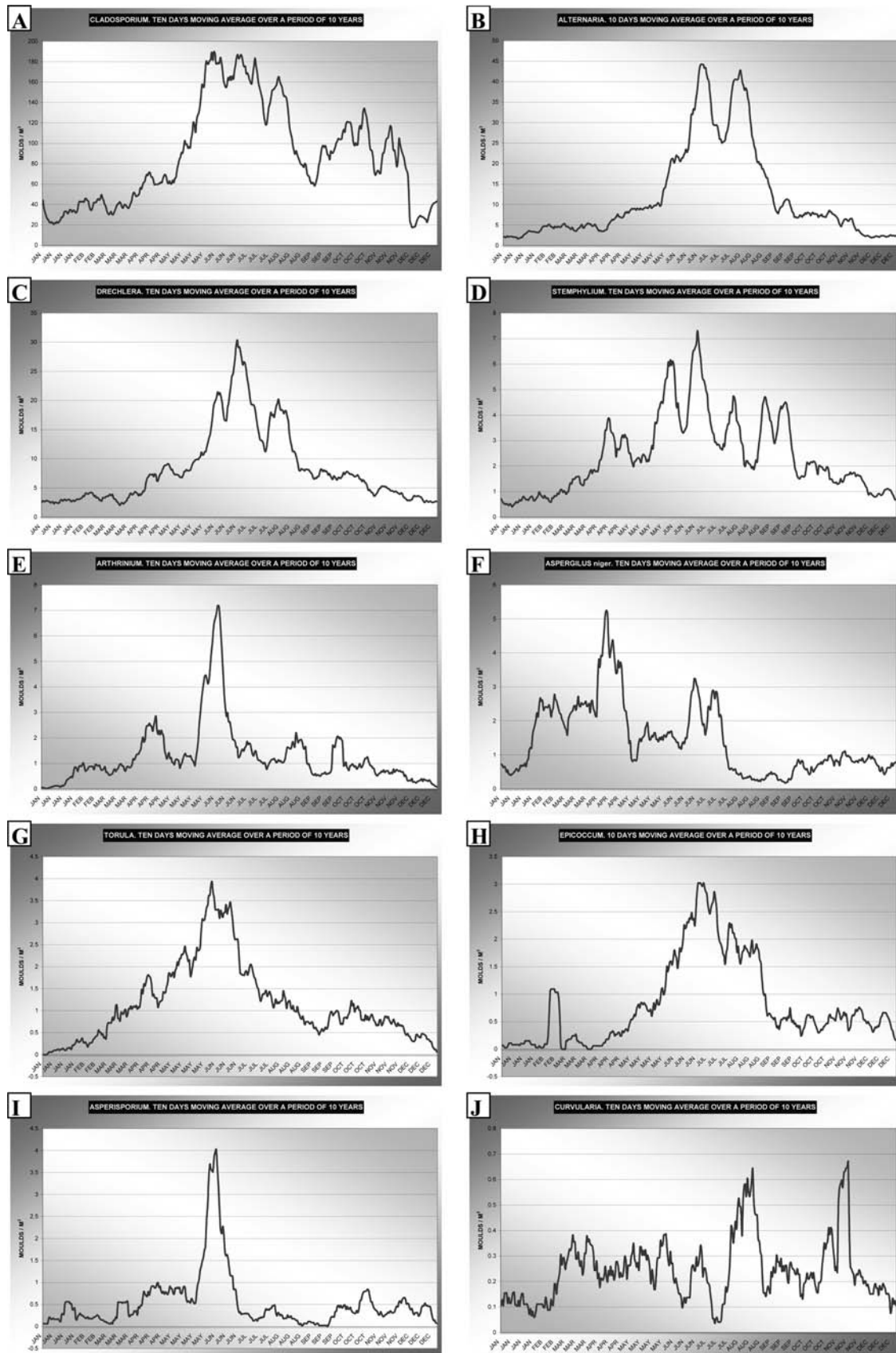


Figure 3. Deuteromycetes: 10-day moving averages over a period of 10 years (1994–2003). (A) *Cladosporium*, (B) *Alternaria*, (C) *Drechslera*; (D) *Stemphylium*; (E) *Arthrrium*; (F) *Aspergillus niger*; (G) *Torula*; (H) *Epicoccum*; (I) *Asperisporium*; (J) *Curvularia*.

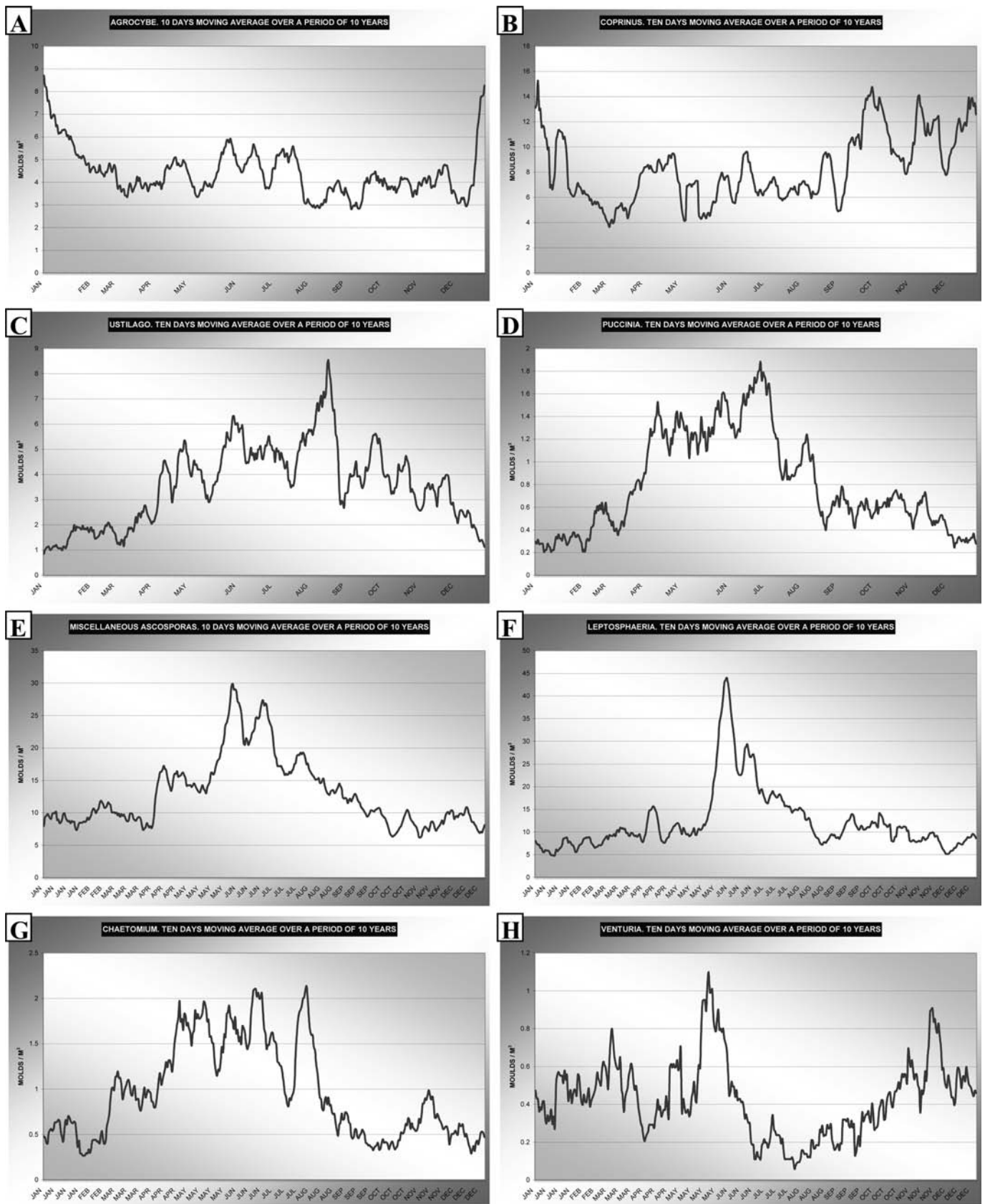


Figure 4. Basidiomycetes and Ascomycetes: 10-day moving averages over a period of 10 years (1994–2003). (A) *Agrocybe*; (B) *Coprinus*; (C) *Ustilago*; (D) *Puccinia*; (E) *Miscellaneous Ascosporas*; (F) *Leptosphaeria*; (G) *Chaetomium*; (H) *Venturia*.

Table 1 Deuteromycetes or fungi imperfecti: A 10-yr aerobiological study in Herakleion, Crete (1994–2003)

Deuteromycetes	Mean Daily	Mean Annual	Maximum Daily	Maximum Annual	Minimum Annual	Total 10-yr Load	%
<i>Cladosporium</i>	87.99	32134.1	2595	49479	16025	32,1341	45.71
Date			Dec. 4, 2000	2000	2003		
<i>Alternaria</i>	11.94	4358.6	193	5439	1881	43,586	6.20
Date			Jul. 6, 2001	1994	2003		
<i>Drechslera/</i> <i>Helminthosporium</i>	8.33	3043.5	191	5299	501	30,435	4.33
Date			Jun. 30, 2001	1996	2003		
<i>Stemphylium</i>	2.41	880.8	59	1524	157	8808	1.25
Date			May 30, 1996	1994	2003		
<i>Arthrinium</i>	1.29	472.1	95	697	144	4721	0.67
Date			Oct. 4, 2000	1996	2003		
<i>Aspergillus</i> N.	0.33	485.1	108	686	162	4851	0.69
Date			Apr. 9, 1994	1994	2003		
<i>Torula</i>	1.16	423.4	25	638	245	4234	0.60
Date			Mar. 10, 1997	1996	2003		
<i>Epicoccum</i>	0.83	301.4	30	522	162	3014	0.43
Date			Feb. 23, 1998	2001	2003		
<i>Asperisporium</i>	0.54	199.9	36	281	13	1999	0.28
Date			Jun. 17, 1994	1998	2003		
<i>Curvularia</i>	0.24	89.3	21	164	32	893	0.13
Date			Nov. 17, 1994	1994	2003		
Spore concentrations/m ³ .							

Table 2 Ascomycetes: A 10-yr aerobiological study in Herakleion, Crete (1994–2003)

Ascomycetes	Mean Daily	Mean Annual	Maximum Daily	Maximum Annual	Minimum Annual	Total 10-yr Load	%
Miscellaneous <i>Ascosporas</i>	13	4750.7	108	6798	1241	47,507	6.76
Date			Jun. 8, 1996	1994	2003		
<i>Leptosphaeria</i>	12.22	4464.9	363	9747	1020	44,649	6.35
Date			Jun. 15, 1994	1994	2003		
<i>Chaetomium</i>	0.96	350.8	21	569	50	3508	0.50
Date			Jun. 10, 1995	1996	2003		
<i>Venturia</i>	0.43	156.7	25	234	91	1567	0.22
Date			Apr. 30, 1996	2002	1994		
Spore concentrations/m ³ .							

lium; and 1, *Rhizopus*). The remaining 233 atopic individuals were polysensitized to various mold species and other airborne allergens (Table 4).

DISCUSSION

The association of mold concentration in the air of a given environment with allergic manifestation of the respiratory tract is not well established, even though several studies suggest a causative role of atmospheric molds to exacerbations of respiratory allergy.^{6,7,13–16}

The potential association of respiratory allergy with the mold spore load of an area underlines the importance of aerobiological data in a given site. The island of Crete, known for its temperate climate, is located in the Mediterranean basin with almost equal distances from three continental mainlands. The city of Heraklion was chosen as the site of the first aerobiological studies in Crete for two reasons: (a) because of its central location in the island, Heraklion is considered to be representative of the wider area of Crete; and (b) being a uni-

Table 3 Basidiomycetes: A 10-yr aerobiological study in Herakleion, Crete (1994–2003)

Basidiomycetes	Mean Daily	Mean Annual	Maximum Daily	Maximum Annual	Minimum Annual	Total 10-yr Load	%
<i>Coprinus</i>	8.29	3028.3	295	5405	1591	30,283	4.37
Date			May 15, 2001	2002	2003		
<i>Agrocybe</i>	4.40	1607.9	31	2294	263	16,079	2.29
Date			Jul. 17, 2000	2000	2003		
<i>Ustilago</i>	3.58	1308.8	126	1848	578	13,088	1.86
Date			Aug. 31, 2000	2001	2003		
<i>Puccinia</i>	0.8	293.3	14	443	110	2933	0.42
Date			Apr. 20, 2000	2000	2003		

Spore concentrations/m³.

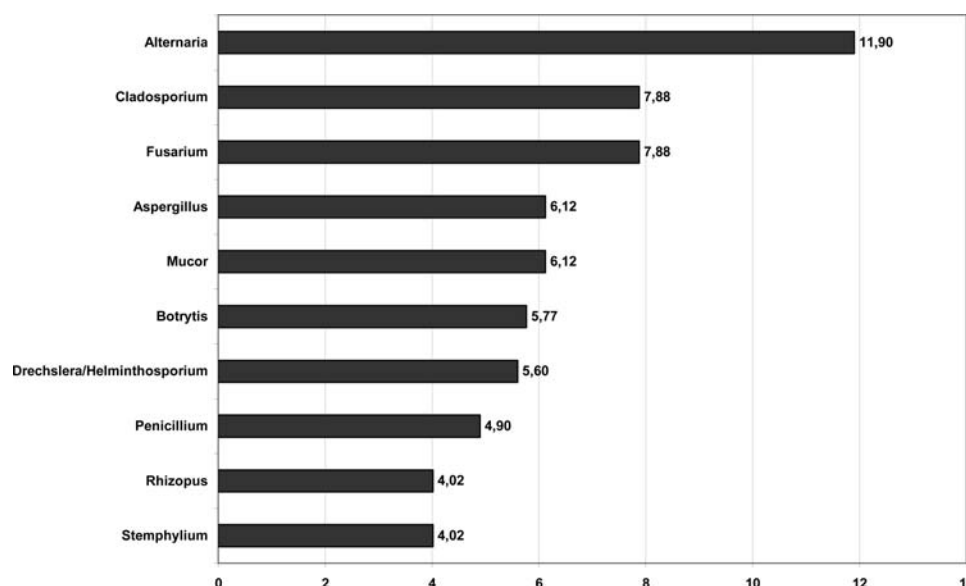


Figure 5. Percentage distribution of positive SPT frequencies on a mold allergic population of 243 individuals, subjected to SPT with the routinely used mold allergens.

versity town, as well as the largest commercial and cultural center of Crete, it attracts millions of visitors each year. Consequently, aerobiological data from this particular site are of interest for a larger number of allergic individuals, permanent or transient residents, and visitors.

In this study, all detected genera of Deuteromycetes, Ascomycetes, and Basidiomycetes showed lower values when compared with those of other aerobiological studies. D'Amato *et al.* reported from Naples, Italy, that the concentration of *Cladosporium* conidia exceeded 1000/m³ in April and reached 10,000/m³ between August and October.¹⁷ Likewise, Colakoglou from Istanbul, Turkey, reported that *Cladosporium* conidia concentrations reached a weekly average of 1500 conidia/m³.¹⁸ On the contrary, in our area, the concentration of *Cladosporium* conidia was only 70/m³ in April and reached 140/m³ from August to October. As for *Alter-*

naria conidia, D'Amato reported that they reached values of 100/m³ at the end of June and exceeded 500/m³ between August and September.¹⁷ In our area, maximal values were at the level of 45/m³ in June and August (Fig. 3) and declined to 7/m³ by the end of September. For the rest of the Deuteromycetes the same researcher reported that their levels fluctuate from 10 to 50 conidia/m³.¹⁷ In our area, with the exception of *Drechslera/Helminthosporium* that occasionally exceeded 30 conidia/m³, all the other Deuteromycetes genera exhibited values lower than 8 conidia/m³. Several studies^{5,19–21} have suggested the clinical relevance of air-transported basidiospores; it is therefore pertinent to determine the ratio of such spores in aerobiological studies. In our area this ratio was confined to 8.94%, which was low, compared with other locations. Davies *et al.* reported that basidiospores comprise 54 and 25% of the total spore counts in Liverpool and

Table 4 Distribution in categories of allergic sensitization in 243 out of 571 tested patients, who showed at least one positive response to mold extracts. Ten out of the sixteen patients sensitized only to molds were monosensitized.

Allergens	No of cases	Percentage (%)
Pollens + Molds + Mites + Epithelia	136	23.81
Pollens + Molds	69	12.08
Molds + Mites + Epithelia	22	3.85
Molds	16	2.80
Total	243	42.54

London, respectively,²² whereas in Kansas, this ratio constituted 24.3% of total fungal catch.²³ *Chaetomium* is an ascomycete rarely reported in aerobiological studies; it is a thermophilus mold with allergenic potency.²⁴ In our area this mold was detected year-round, even though the recorded counts seem to be very low (Fig. 5). The only previous report about the presence of *Chaetomium* in the Mediterranean basin is that of Minero *et al.* from Cadiz, Spain.²⁵

The load of atmospheric mold spores depends on the complex interplay of a series of meteorological parameters (temperature wind speed, relative humidity, rainfall, wind speed, and snowfall) and other factors (human activity, use of land, and others).^{26–32} Climatic and other conditions existing on the island of Crete, characterized by mild and temperate climate with low to modest rainfall and very dry summers, along with limited vegetation and almost nonexistent human outdoor activities particularly relating to cereal harvesting and no land disturbance, might explain the relatively low mold concentrations observed. Moreover, Crete is an island located far away from the European, the Asian, and the African mainland; thus, it is not seriously affected by continental mold spores, which could be transferred by the winds.

The SPT results suggest a polysensitization pattern, with no linear correlation to the observed mold counts for those types of mold spores that were adequately tested. However, it should be underlined that dermal sensitization to Basidiomycetes has not been properly evaluated, because the relevant extracts were not commercially available.

In conclusion, this 10-year aerobiological investigation represents the first long-term study in our area. Eighteen mold species exhibiting a seasonal pattern throughout the years have been identified and recorded. The most abundant of mold species were (a) *Cladosporium*, (b) *Alternaria*, (c) miscellaneous ascomycetes, (d) *Leptosphaeria*, and (e) the basidiomycete *Cop-*

rinus. In parallel, skin sensitivity to 10 mold species was evaluated with the corresponding extracts. Positive SPTs most frequently detected were those of (a) *Alternaria*, (b) *Cladosporium*, (c) *Fusarium*, (d) *Aspergillus*, and (e) *Mucor*. These aerobiological and sensitization data suggest the need for additional medical and biological research and application.

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