

# Allergenic fungi spore records (15 years) and sensitization in patients with respiratory allergy in Thessaloniki-Greece

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## Summary

**Background:** The prevalence of respiratory allergy to fungi spores (FS) is not precisely known but is estimated at 20-30% of atopic patients. There are no aerobiological records, necessary for respiratory allergy diagnosis and treatment, in Thessaloniki and generally in Greece.

**Aim:** Creation of a database on FS circulation and investigation of skin sensitivity (SS) of asthmatics by using skin prick tests (SPT).

**Material and methods:** Daily records and identification of 15 airborne FS species were conducted, using a Burkard trap during 1987-2001. SS to 5 most common FS extracts was investigated, by using SPT in a total of 1311 asthmatics with atopy, submitted to the Out-Patient Clinic of Asthma (Pulmonary Dept, Aristotle Univ, Thessaloniki) in 1990-2001.

**Results:** The FS recorded in the 15-year period were as follows: *Cladosporium spp.* (72.2%), *Alternaria spp.* (9.8%), *Ustilago spp.* (8.1%), *Ascospores* (2.7%), *Agrocybe spp.* (1.5%), *Helminthosporium spp.* (1.4%), *Leptosphaeria spp.* (1.2%), *Agrocybe spp.* (1.1%), whereas the species *Botrytis*, *Stemphylium*, *Pleospora*, *Nigrospora*, *Epicoccum*, *Fusarium*, *Torula* and *Phoma* presented concentrations <1%. The highest numbers of airborne FS were recorded during summer. Positive skin reaction to FS was observed in 421 (32%) patients of the 1311 asthmatics. Positive skin reaction to *Alternaria* species was observed in 177 patients (13.5%), in 98 (7.4%) to *Cladosporium*, 65 (5%) to *Aspergillus*, 45 (3.4%) to *Fusarium* and 36 (2.7%) to *Rhizopus*. FS sensitivity is much more frequent in younger men.

**Conclusions:** For the first time in Thessaloniki-Greece, 15 allergenic FS species circulation has been recorded for the last 15 years. SS was more frequently detected for the species of *Alternaria*, *Cladosporium* and *Aspergillus*.

## Keywords:

Fungi spores, asthma, respiratory allergy, skin prick tests, Greece, *Alternaria*, *Cladosporium*, *Aspergillus*, *Fusarium*, alveolitis, *Rhizopus*.

## Introduction

Epidemiological surveys support that asthma affects 5-30% of children and 2-30% of adults [1, 2]. This wide range of inter-survey fluctuation is mainly due to the different geographic distribution and to the use of different criteria among studies. Researchers characterize it as "a chronic allergic disease of airways" [3]. Rhinitis affects 40% of the population.

The term respiratory allergy refers to clinical manifestations of both upper and lower respiratory tract due to allergy and is extensively used by scientists worldwide.

It is well established that inhalation of fungi spores induces respiratory allergy symptoms in sensitized individuals; among all patients suffering from respiratory allergy symptoms, 20-30% of them are sensitized to fungi spores [4]. These symptoms are clinically manifested as

rhinitis, bronchial asthma [4, 5, 6-10] and alveolitis [6, 9]. Many authors correlate the onset of respiratory allergy symptoms with the presence of fungi spores in the atmosphere [5, 6, 11-13]. A lot of different species of fungi spores disperse in the atmosphere, which are characteristic of each region. Fungi spores involved in allergic diseases belong to 80 genera, the most significant of which belong primarily to *Ascomycetes* and *Deuteromycetes* and, secondly, to *Basidiospores* [4-6, 9]. They are either specialized single cells or, more often, composed of branching threads, termed hyphae; they are responsible for the reproduction and growth of fungi [5-7, 9]. Fungi spore antigenicity appears to significantly differ from that of mother cultures [4-6, 9]. The recording and forecasting of fungi spore dispersion in the atmosphere attract the interest of most scientists and specialists, since they form the basis of diagnosis and treatment of allergic diseases caused by fungi spores [6, 7, 9, 11, 14, 15].

## Aim of the study

Until today, no long-term records of allergenic fungi spore circulation in the atmosphere has been conducted in Greece, so as to draw useful conclusions for the protection of relevant allergic diseases. There is, however, a study of ours published in 1996 [16], referring to 4-year records in the area of Thessaloniki. We therefore decided to continue this research and create a database including long-term records of allergenic fungi spores performed on a daily basis in the city of Thessaloniki (Fungi Spore Calendar). It was considered necessary to simultaneously detect allergic patients sensitivity to the fungi spores most commonly involved in respiratory allergy symptoms in the region of Thessaloniki [5-8,10].

## Study area

Thessaloniki is an old city that celebrated its 2300-year anniversary of foundation in 1984. The city is built in the heart of Thermaikos Gulf in the Aegean Sea and its geographical location is 40° 37'N and 22° 57'E. Nowadays, Thessaloniki is an industrialized modern city of 1.1 million inhabitants; it is the second biggest city in Greece, the cultural and economic center of Macedonia and northern Greece and an important seaport.

In the north-east of Thessaloniki there is a large forest of pines, cypresses, cedars and oaks, called "Kedrinis Lofos". In the western side, where the industrial zone is well developed, the city is in contact with extensive fields of cultivated cereals. The eastern part of the city is close to grass areas and olive trees, neighbouring the peninsula of Chalkidiki. To the south the city borders the Mediterranean Sea.

## Material and methods

### *The study design included:*

- A. Daily records and identification of more than 40 fungi spore species belonging to different families. Selection of the 15 most significant for presentation, as far as their allergenicity is concerned.
- B. Creation and presentation of Fungi Spore Calendar for the 15 most allergenic fungi spore species in the area of Thessaloniki.
- C. Detection of skin sensitivity (allergy) to fungi spores in asthmatic population in our region, simultaneously with the records of fungi spores.

### **A. Records, identification and presentation of fungi spores in the air of Thessaloniki.**

This study began in September 1986, when the Burkard volumetric spore trap was installed in the center of Thessaloniki. Daily sampling of airborne fungi spores started on February 1st, 1987 and continues until today. In this study, 15-year records are presented, a period long enough for the creation of Fungi spore Calendars in an area [17].

The records of fungi spores were conducted using a 7-day recording Burkard volumetric spore trap, standard equipment for aerobiological sampling worldwide [5-7, 9, 17-18]. A Burkard volumetric trap was placed on the roof of a six-floor building in the center of Thessaloniki and at a height of 25 m above the ground, measuring concentrations of pollen grains in the ambient air throughout the study [7, 18]. Fungi spore counts are expressed as fungi spores per cubic meter of air [6-7, 18].

The aeroallergens selected for presentation comprise the most commonly implicated fungi spores to respiratory allergy symptoms both in European countries and worldwide [5-6, 9, 12, 19]. These are: *Agrocybe spp.*, *Alternaria spp.*, *Ascospores*, *Botrytis spp.*, *Cladosporium spp.*, *Drechslera/Helminthosporium spp.*, *Epicoccum spp.*, *Fusarium spp.*, *Leptosphaeria spp.*, *Nigrospora spp.*, *Phoma spp.*, *Pleospora spp.*, *Stemphylium spp.*, *Torula spp.* and *Ustilago spp.*

### **B. Creation and presentation of the Fungi Spore Calendar.**

Data of fungi spore records were inserted in an automated computer worksheet and processed with Microsoft Excel for Windows [17, 20]. The Fungi Spore Calendar was created according to worldwide-established methodology [17]. Fungi spores were expressed in daily annual values and then, in our case, as summed average values per 10 days for the 15-year period (1987-2001). They were presented on a particular scale, each level corresponding to a specific total amount of spores. In this way, all interactions between external factors and fungi spore concentrations were significantly decreased, therefore enabling us to compare different fungi spore species concentrations throughout the year.

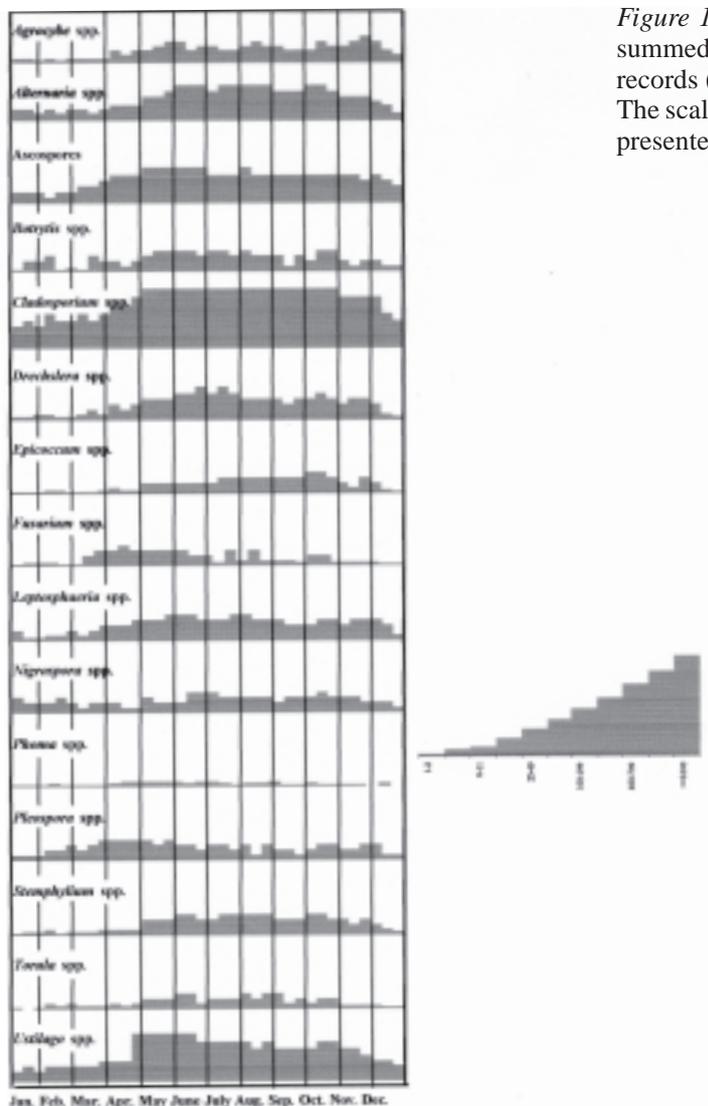


Figure 1. Fungi Spore Calendar of 15 fungi spore species in summed average values per 10 days for the 15-year period of records (1987-2001) in the area of Thessaloniki. The scale with the particular levels of total amount of spores is presented.

### C. Detection of skin allergic sensitization to fungi spore extracts in asthmatic patients.

Skin sensitivity to fungi spores was detected in a sample of 1311 asthmatics admitted to the OutPatients Clinic for Asthma of the Pulmonary Department of Aristotle University of Thessaloniki in the General Hospital "G. Papanikolaou" from 1/1/90 until the end of 2001. The patients included in the study had to present annual periodicity, seasonal occurrence of symptoms and/or worsening of respiratory allergy symptoms. The examination included:

- Filling of a questionnaire of 200 questions specific for asthma. The aim was to examine the relation between the skin allergic sensitization (skin prick tests) and the onset of clinical symptoms.
- Clinical examination.
- Spirometry and, in some cases, bronchial challenge test with metacholine or histamine.

- Chest x-rays film.
- Specific IgE (detected only in some cases).
- Skin tests. Skin tests were performed by a modified skin prick technique [21- 25]. The most common inhalant and food allergens were used, counting up to a total 55 extracts, among which 5 fungi spore extracts. The selection of fungi spore species was made according to expected sensitization, as documented by past studies made in our country, Europe and the U.S.A. [5-7, 9, 12, 19, 23, 26, 27]. The allergens were either isolated or in groups. All the allergens were produced by Allergofarma®, Germany. The fungi spore extracts used were: *Alternaria* spp., *Cladosporium* spp., *Aspergillus* spp., *Fusarium* spp. and *Rhizopus* spp. Skin positivity was recorded 15 minutes after the test and the occurring wheal and flare reaction was

impressed on a transparent cello tape. Positivity was expressed in wheal diameter > 3 mm and flair diameter > 10 mm compared to negative and positive controls [21- 25].

## Results

### A. Aerobiological records of fungi spores

(Fungi spore records are presented in Table 1)

More than 40 fungi spore species were recorded and identified during the 15-year period sampling, however, the 15 of them, which are the most common implicated in respiratory allergy symptoms worldwide, are presented in this study. The airborne fungi spore concentrations in the area of Thessaloniki during the 15-year period 1987-2001 are presented in Table 1.

### B. Fungi Spore Calendar

In Figure 1, the Fungi Spore Calendar including all recorded aeroallergens is displayed.

### C. Detection of skin allergic sensitization to fungi spores.

(Patient sensitization to fungi spores is presented in Table 2)

The sample of asthmatics examined for allergic sensitization to fungi spores consisted of 1744 patients suffering from respiratory allergy and displaying clinical

symptoms mostly during May-October. Their average age was about 35 years old (6-68 years old) and average duration of asthma was 23 months (3 months to 10 years). Skin positivity to one or more allergen extracts of the 55 tested occurred in 1311 (75.2%) patients, while 433 patients (24.8%) did not react to the skin tests at all. About 421 (32%) out of the 1311 patients reacted positively to at least one fungus spore extract, from whom 172 (40.9%) were women, whereas 249 (59.1%) were men. On the contrary, regarding the 433 patients having presented negativity to skin prick tests, 260 (60%) were women and 173 (40%) were men.

Among the above 1311 patients, 177 (13.5%) presented positive skin allergic reaction to *Alternaria* spp., 98 (7.4%) to *Cladosporium* spp., 65 (5%) to *Aspergillus* spp., 45 (3.4%) to *Fusarium* spp. and 36 allergic patients (2.7%) displayed skin sensitivity to *Rhizopus* spp.

In Table 2, age and sex distribution of sensitized allergic patients is also presented for all fungi spore extracts.

## Discussion

Not all components of the atmosphere present the same allergenic significance to humans. Pollen grains have been extensively studied as aeroallergens. On the contrary, allergy to airborne fungi spores has hardly been studied, as the cause of respiratory allergy symptoms. It must be mentioned that bronchial asthma incidents related to fungi spore allergy very often increase in summer. Moreover, sensitivity to some particular species, such as *Alternaria* spp., is one of the most severe

Table 1. Annual sum of fungi spore concentrations, their annual average values and their percentages (%) for each one of the 15 fungi spore species recorded in the area of Thessaloniki during 1987-2001

Fungi Species	Sum	Average	%
<i>Cladosporium</i> spp	1882560	125504	72,22%
<i>Alternaria</i> spp	255895	17060	9,82%
<i>Ustilago</i> spp	210970	14065	8,09%
<i>Ascospores</i>	70375	4692	2,70%
<i>Drechslera/Helminthosporium</i> spp	36190	2413	1,39%
<i>Leptosphaeria</i> spp	32175	2145	1,23%
<i>Agrocybe</i> spp	29925	1995	1,15%
<i>Botrytis</i> spp	16770	1118	0,64%
<i>Stemphylium</i> spp	16750	1117	0,64%
<i>Pleospora</i> spp	15235	1016	0,58%
<i>Nigrospora</i> spp	14705	980	0,56%
<i>Epicoccum</i> spp	9770	651	0,37%
<i>Fusarium</i> spp	7640	509	0,29%
<i>Torula</i> spp	6170	411	0,24%
<i>Phorma</i> spp	1630	109	0,06%
<b>Total</b>	<b>2606760</b>	<b>173784</b>	

Table 2. Patient sensitization to 5 fungi spores, presented as percentages of the total 1311 patients with respiratory allergy examined. Sensitization distribution between both sexes is also presented

Fungi Species	Number of patients	% patients	Men	%	Women	%
<i>Alternaria</i> spp.	177	13.5%	112	63.3%	65	36.7%
<i>Cladosporium</i> spp.	98	7.4%	54	55.1%	44	44.9%
<i>Aspergillus</i> spp.	65	5.0%	41	63.1%	24	36.9%
<i>Fusarium</i> spp.	45	3.4%	22	48.9%	23	51.1%
<i>Rhizopus</i> spp.	36	2.7%	20	55.6%	16	44.4%

forms of asthma, under conditions of high fungi spore concentrations in the atmosphere [11, 28, 29, 30, 31].

Allergy caused by fungi spores is necessarily relevant to regional vegetation and to the way and duration of exposure to each aeroallergen [5, 7, 8, 14, 20, 29]. Some fungi spore species may provoke respiratory allergy symptoms, as they enter the houses through air-conditioning equipment [19, 30, 32, 33].

For the first time in the area of Thessaloniki, and more generally in Greece, 15-year allergenic fungi spore records have been conducted. Allergenic fungi spores recorded in the area of Thessaloniki are usually present throughout the year (Figure 1). However, high concentrations of fungi spores appear in the atmosphere of the city at the beginning of March and spore circulation ends by the end of October (Figure 1). Similar results, concerning the very same fungi spore species, are also documented by more researchers in other areas, hence, with climatological conditions and prevailing meteorological factors very much like ours [14]. However, spore concentrations within the same species greatly differ, a fact due to the variety of the vegetation existing in each region [14, 35].

The exact numbers of fungi spores that are going to disperse in the air and the duration of their seasonal circulation in a specific region depend on the wind direction and speed, the presence of rain and its duration and relative humidity during each season [6, 9, 14, 19, 20].

As far as skin prick tests are concerned, they present a high diagnostic value, especially if combined with the patient's medical history [2, 8, 36]. Positivity in skin tests is not usually observed in individuals lacking clinical symptoms [2, 24, 37]. The method of modified skin prick test proves to be a reliable tool for detection of type I sensitivity of the human body worldwide. Also, the results can be stored in transparent cello tapes, which enables us to compare the results of the test at a given time with those at different periods or with other patients' tests, even from abroad [21, 22, 23, 37, 38]. The positivity of skin prick tests strongly correlates with bronchial hyper-responsiveness and other clinical aspects of bronchial asthma. Moreover, it is reported that skin prick tests are more reliable than detection of

total and special IgE tests with Phadiatop [37]. In a study of D'Amato et al [8], European citizens were examined (among whom Greek patients were also tested by our research team); the relationship between skin prick test sensitivity to fungi spores and specific IgE was particularly investigated. As documented, increase in skin prick test sensitivity to fungi spores is not necessarily followed by a relevant increase in IgE; hence, this study refers only to fungi *Alternaria* and *Cladosporium*. In everyday medicine and asthma diseases, the problem of respiratory allergy caused by fungi spore inhalation is often confronted. Not many references exist in Greek literature about fungi spore allergy [8]. In a study, 32% of patients were discovered to present at least one positive skin prick test to fungi spores, a fact revealing the necessity of detection of sensitization to fungi spores in patients suffering from respiratory allergy. Recent Greek references document increase in sensitization to airborne fungi spores of Greek citizens [39, 40]; children, particularly, are greatly sensitive (19.5%) to *Alternaria* spp. [41]. Our data of skin prick test positivity are considered quite useful, since they derive from a sample of 1744 patients with respiratory allergy. They correspond to individuals from all over the county of Thessaloniki and were obtained during the same period of fungi spore aerobiological records. The data concern sensitization to the fungi spores most commonly involved in respiratory allergy symptoms worldwide [6-8, 11, 14-15, 18, 32, 34, 36, 38].

Sensitization provoked by *Alternaria* spp. spores counts up to 13.5%. It is worth mentioning that younger individuals suffer mostly from allergic diseases due to fungi spores (Table 2). Furthermore, men are more usually offended by respiratory allergy than women (Table 2), especially in the case of *Alternaria* spp. [11, 15, 31, 38, 42]. The frequent occurrence of skin positivity to *Alternaria* extracts is observed in other countries as well. In the USA, the allergenic factor most commonly reported as involved in respiratory allergy symptoms is the spores of the fungus species *Alternaria*. [5, 10, 11, 15, 31, 36], while in California it is the third most significant factor of occurrence of clinical symptoms, just after the pollen grains of grass and

ragweed [43]. On the contrary, in the Netherlands, *Alternaria*'s role as an aeroallergen proves to be of minimal importance [19], resulting from the low airborne concentrations of fungus spores.

As far as *Cladosporium* spp. is concerned, it is also considered as a major allergenic factor responsible for causing of respiratory allergy symptoms in our area, with a percentage of skin positivity in asthmatics of about 7.4%. The spores of *Cladosporium* spp. are the most abundant in the air of all countries worldwide [5, 6, 7, 9, 14, 19, 20, 34]; however, their allergenicity appears to be rather low [7, 23, 34, 36]. Therefore, although in Thessaloniki 72.2% of the total amount of fungi spores circulating in the air belong to *Cladosporium* spp. (Table 1), there was not a corresponding hypersensitivity in the asthmatics examined (Table 2).

*Aspergillus* spp. extract was included in the study of patient sensitization because it comprises a common indoor fungus spore species (house dust, flowerpots etc), but also because it is often involved in various bronchopulmonary diseases (bronchopulmonary aspergillosis, aspergilloma, alveolitis, etc.) [4, 6, 9]. Skin prick test sensitivity to *Aspergillus* spp. reached up to 5% in asthmatic individuals (Table 2). This percentage might have been expected to be higher, inasmuch as *Aspergillus* spores are very common in domestic environment. Unfortunately, their very small size does not permit us to record their concentrations in the ambient air with the methodology used [5, 6]. Similarly to *Alternaria* spp., skin positivity was more often observed in men than in women (Table 2).

The fungus species of *Rhizopus* spp. belongs to *Zygomycetes* and comprises the most common indoor allergenic fungus spore family, along with *Mucor* species, altogether known as moulds [6, 7, 9]. *Rhizopus* spp. offends cultivations and stored agricultural products (e.g. potatoes, peanuts, cereals, peaches etc). Its frequent existence in domestic environment urged researchers to detect its sensitization in asthmatics [5, 6]. Likewise, *Fusarium* spp. is also growing and reproducing in agricultural products, such as potatoes, cotton, tomatoes, garbage, watermelons, bananas, etc. [5, 6, 12, 26]. Skin sensitization to the fungi spore extracts of *Rhizopus* spp. and *Fusarium* spp. were positive only for 3.4% and 2.7% of asthmatic patients respectively, percentages not very high compared to those reported in other countries worldwide [6, 34].

Finally, in a study of ours in 1994 [44], we investigated the relationship between skin prick test sensitivity to fungi spores, specific IgE positivity and positive medical history concerning respiratory allergy symptoms occurring during the main circulation season of fungi spores. No positive correlation was found between skin prick test sensitivity and specific IgE sensitivity. Particularly, *Alternaria* presented skin prick test positivity in 20 out of 21 patients, whereas specific IgE was found positive only in 15 patients. In contrast, *Aspergillus* presented skin prick test positivity in 8 out of 21 patients, whereas specific IgE was found positive in

17 patients. It is worth mentioning that specific IgE generally revealed a low level of reaction. It is therefore concluded that there is no certain relation between skin prick test sensitivity and specific IgE sensitivity, a fact possibly due to the lack of completely purified fungi materials used in both techniques.

## Conclusions

Fifteen allergenic fungi spore species were recorded in the area of Thessaloniki for the last 15 years (1987-2001) and are presented for the first time in a Fungi Spore Calendar, a fact very important for our city and country, both from the medical and biological viewpoint.

Sensitization (allergic reactions) to 5 fungi spore species was detected in patients suffering from respiratory allergy symptoms in the region of Thessaloniki. Clinically significant are mostly the fungi spores of *Alternaria* and *Cladosporium*, as evidenced both by great skin prick test positivity and by their abundant concentrations in the air of the city.

However, no safe conclusions were drawn concerning the relationship between skin prick test sensitivity and specific IgE sensitivity.

Finally, this kind of data is being presented for the first time in our area and could constitute the infrastructure of many medical, biological and other scientific field applications.

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