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Alternaria and *Cladosporium* Calendar of Western Thrace: Relationship With Allergic Rhinitis Symptoms

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Objectives/Hypothesis: *Alternaria* and *Cladosporium* are the most important outdoor moulds. The aim of this study was to present fungal spore monitoring data, investigate the relationship of fungal counts with climate conditions, and to explore the clinical significance of *Alternaria* and *Cladosporium* species monitoring in allergic rhinitis (AR).

Study Design: Analytic observational study.

Methods: A 7-day volumetric trap was used to collect circulating *Alternaria* and *Cladosporium* fungi. Sixty-nine AR patients were studied and recorded their symptoms by Total 5 Symptoms Score (T5SS). Sensitization prevalence to fungi species was detected by skin prick tests. Monitoring data were correlated to climate conditions and patients symptoms score.

Results: *Alternaria* and *Cladosporium* were detected throughout the calendar year in ranges of 0 to 217 spores/m^3 and 6.5 to 1,600 spores/m^3 per day, respectively. Highest daily concentrations of both fungi were detected during the summer (73.9 $\pm$ 34.4 spores/m^3 for *Alternaria* and 595.8 $\pm$ 288.0 spores/m^3 for *Cladosporium*) and lowest during the winter (2.4 $\pm$ 3.0 spores/m^3 for *Alternaria* and 24.3 $\pm$ 15.7 spores/m^3 for *Cladosporium*). Both were positively correlated to mean daily temperature and negatively to relative humidity (all $P < 0.001$). Clinically, a strong significant correlation between T5SS and airborne fungi levels, both for *Alternaria* ($r = 0.822$, $P = 0.001$) and *Cladosporium* ($r = 0.787$, $P = 0.002$) species was observed.

Conclusions: We found *Cladosporium* to be the most frequently detected airborne mould, whereas *Alternaria* was the most prevalent with regard to sensitization rate. Patients' symptoms score was significantly correlated to spore concentrations. Both fungi were clearly affected by climate factors, such as temperature and relative humidity. These findings are important in AR management.

Key Words: Allergic rhinitis, airborne mould, *Alternaria*, *Cladosporium*, skin prick tests, symptoms score.

Level of Evidence: N/A.

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INTRODUCTION

Allergic rhinitis (AR) represents a prevalent, however underappreciated, inflammatory disorder of the nasal mucosa[1] in response to environmental allergens, such as trees, grasses, weed pollens, and moulds.[2] Symptoms intensity depends on aeroallergen circulation density and on exposure duration.[2,3] Studies have shown significant changes in production, dispersion, and allergen content of pollens and fungal spores over the last decades, which may be region- and/or species-specific.[3,4] This may be attributed to climate changes that may impact the physiology and distribution of living organisms, such as plants and fungi.[3–6]

Among aeroallergens, fungal spores constitute a significant fraction of airborne bioparticles, being several times more numerous than other airborne particles, such as pollen grains.[7] For this reason, the World Health Organization has noted exposure to airborne fungi as an important public health risk.[8] Recently, the development of aerobiology networks has made a major contribution toward a more thorough monitoring of atmospheric levels of fungal spores in both inland[9,10] and coastal areas[11] of many countries. Although there is a wide variety of airborne fungal species,[12] only a few have been studied in detail. This might be partly attributed to the difficulty of detecting airborne spores when they are few in number.[12] Previous studies have shown that *Alternaria* and *Cladosporium* are the most prevalent outdoor moulds, whereas *Aspergillus* is a typical indoor mould.[12,13] Moreover, among fungi, *Alternaria* and *Cladosporium* species bear the highest clinical importance due to their high allergenicity.[14,15] This is indicated by the fact that globally an estimated 20% to 30% of sufferers from AR and/or asthma are experiencing symptoms after fungal spore exposure.[16] *Alternaria* in particular is considered as a risk factor for asthma triggering in both children and adults.[17] Given the large number of allergenic fungi and

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TABLE I.
Climatic Conditions in Western Thrace (northeastern Greece) (mean ± SD).

Month	Temperature (°C)	Rainfall (mm)	Relative Humidity (%)	Wind Speed (km/h)	Wind Direction
January	7.7 ± 3.5	3.8 ± 8.3	80.8 ± 7.8	5.9 ± 5.6	NE
February	7.5 ± 3.0	3.7 ± 5.9	79.3 ± 6.8	8.6 ± 6.0	NE
March	10.1 ± 3.3	2.5 ± 6.8	75.9 ± 7.6	7.4 ± 4.0	ENE
April	13.9 ± 3.1	0.8 ± 2.3	68.7 ± 10.4	7.0 ± 5.4	NE
May	20.0 ± 2.0	0.1 ± 0.5	64.6 ± 8.2	7.8 ± 5.4	SW
June	22.1 ± 3.1	2.5 ± 7.0	62.6 ± 11.4	6.1 ± 3.0	NE
July	25.3 ± 1.7	0.8 ± 0.5	49.3 ± 4.9	8.4 ± 6.0	NE
August	26.6 ± 1.3	0	47.7 ± 6.3	9.6 ± 5.6	NE
September	20.6 ± 1.7	1.2 ± 3.6	61.9 ± 11.4	4.0 ± 1.6	NNW
October	13.7 ± 2.9	2.2 ± 6.7	71.5 ± 11.7	4.7 ± 3.6	NE
November	12.2 ± 3.5	4.4 ± 9.3	79.7 ± 9.0	5.9 ± 5.6	NE
December	4.9 ± 2.8	0.2 ± 0.4	71.5 ± 13.3	7.1 ± 6.5	NE

SD = standard deviation.

differences in variation among countries, few studies have attempted to determine the clinical relevance of exposure to fungal allergens,[18] correlating symptom scores in AR patients and atmospheric fungal counts. Moreover, no recent data from Greece[19–21] exist and, continual fungal monitoring data have never been presented for the unique climate conditions of the large region of Western Thrace (northeastern [NE] Greece).

Accordingly, the aims of this study were: 1) to present annual fungal monitoring data for the Mediterranean climate conditions of Western Thrace, 2) to investigate the relationship of fungal counts with climate conditions, and 3) to explore the clinical significance of *Alternaria* and *Cladosporium*—the most frequently implicated in AR outdoor fungal species—using symptoms records in sensitized AR patients from the same region.

MATERIALS AND METHODS

Alternaria *and* Cladosporium *Monitoring Data of Western Thrace (NE Greece) Region*

This study was carried out in the department of otorhinolaryngology of the tertiary academic hospital of Alexandroupolis. We present records of samples acquired between January 1 and December 31, 2013. Fungal spores were collected using a volumetric spore sampler (Burkard Scientific Ltd., Uxbridge, UK)),[22] a standard device for aerobiologic sampling.[22–24] The trap was placed on the roof of the University Hospital of Alexandroupolis at a height of 20 meters above ground, according to the instructions provided by the manufacturer.[22] The unit sampled airborne particles continuously collecting 10 liters of air per minute. A strip of silicone-coated Melinex tape (DuPont Teijin Films Luxembourg SA, Luxembourg City, Luxembourg) was exposed to air for trapping air particles and was changed once a week. The exposed tape was cut into 48-mm segments, each representing a 24-hour period. These segments were mounted on microscopic slides using Gelvatol (Sigma-Aldrich, St. Louis, MO, USA) mixed with stain (acid fuchsin) to enable visualization under a high-resolution light microscope (Olympus BX40, Tokyo, Japan) at 400× magnification. Fungal spores' counts were expressed as fungi per cubic meter of air, according to the guidelines of the British Aerobiology Federation.[24]

Western Thrace region represents a geographic area located between Nestos and Evros Rivers in the northeastern part of Greece. Its geographic location is 40°51′N and 25°52′E, situated 40 km from the border with Turkey, 301 km from Thessaloniki, and 750 km from Athens. Alexandroupolis is the largest city of the region. The climate is typical Mediterranean, characterized by hot and dry summers, whereas winters exhibit moderate to low temperatures. Rainfall occurs mainly during winter months. Meteorological data were obtained from the regional station of the Hellenic National Meteorological Service, situated close to the sampling site (Table I).

Study Population

Four hundred and seventy-two AR patients were examined in the rhinology clinic of Alexandroupolis University Hospital. Among them, 69 patients of both sexes who were sensitized exclusively to *Alternaria* and *Cladosporium* fungi and lived permanently in the study area for at least 5 years were included in the study group and their symptom scores were analyzed. For all patients, a detailed medical history was obtained, and rigid nasal endoscopy, sinus computed tomography scanning, skin prick tests (SPTs) with a battery of common aeroallergens, and spirometry were carried out. Only subjects who fulfilled AR criteria according to Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines[25] and were sensitized exclusively to *Alternaria* and *Cladosporium* moulds were included. Patients sensitized to house dust mites, pollens, or animal epithelia were excluded from this study. Sensitizations other than fungi were examined by SPT's results and history and those patients were excluded in order to avoid any bias in symptoms results and be sure that symptoms were caused exclusively by moulds and only. Other exclusion criteria were: history of chronic rhinosinusitis with or without nasal polyps and malignancy, previous nasal operation, history of chronic urticaria/angioedema and/or symptomatic dermographism, immunotherapy treatment during the last 5 years, recent use of oral or nasal corticosteroids 4 weeks prior to inclusion, and oral H1 antihistamines 1 week prior to inclusion. Asthma diagnosis was based on Global Initiative for Asthma (GINA) guidelines.[26] Symptoms evaluation was made by the Total 5 Symptoms Score (T5SS), which includes the symptoms of nasal discharge (rhinorrhea), nasal congestion, itchy nose, sneezing, and itchy eyes.

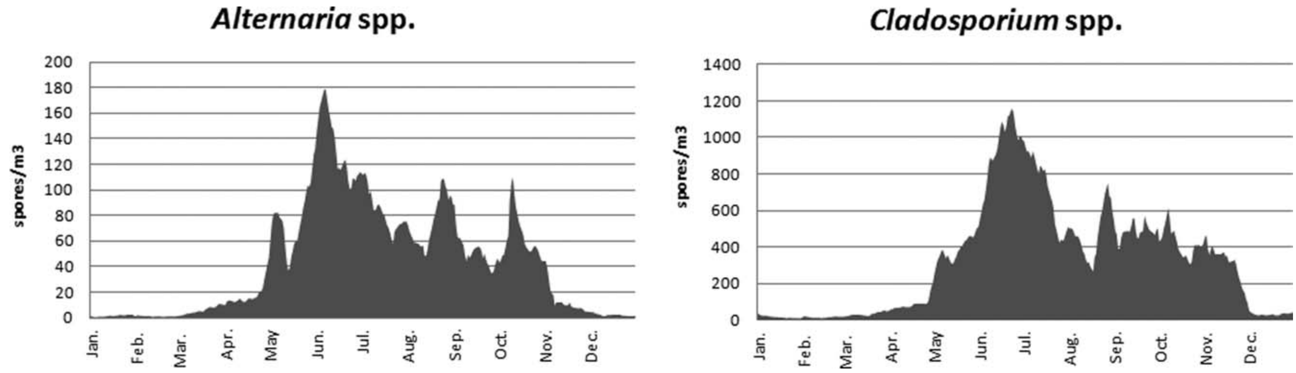

Fig. 1. Annual atmospheric distribution of *Alternaria* and *Cladosporium* (10-day averages).

All symptoms were graded from 0 (absent) to 3 (very troublesome), with total scores ranging from 0–15.[25] SPT's were performed and evaluated as described by the European Academy of Allergy and Clinical Immunology.[27] The allergen panel (HAL Allergy, Leyden, The Netherlands) consisted of the Pan European Global Allergy and Asthma European Network (GA2LEN) panel,[28] which contains common grass, tree and weed pollens, indoor and outdoor molds, house dust mites, and animal epithelia aeroallergens. The study protocol was approved by the local institutional review board. All subjects signed an informed consent form. The study was performed in accordance with the Declaration of Helsinki/Hong Kong.

Statistical Analysis

The 5% to 95% fungi count method was used to describe the main spore season.[29] In brief, the start of the season was defined as the date when 5% of the seasonal cumulative fungal count was reached, and the end of the season as the date when 95% of the seasonal cumulative fungal count was reached. This period includes the beginning, peak, and end of clinical symptoms in AR patients. Fungi spore concentrations are presented as moving average values per 10 days (running means) in accordance to the British Aerobiological Federation guidelines.[24] Running means or moving average values for a specific period of time is a statistic technique used for the presentation of values with great variation in order to smooth their variance. In this way, a general trend is observed that is generally more significant than daily variations. The correlation between meteorological parameters and fungal spore counts was assessed by regression analysis, using SPSS 15.0 (Chicago, IL) software package. Spearman rank correlation was carried out to assess the relationship between T5SS scores and fungal spore counts. A value of $P < 0.05$ was typically considered to be statistically significant.

RESULTS

Fungi Monitoring Data

Alternaria and *Cladosporium* outdoor moulds were monitored throughout the calendar year (January–December 2013) with their numbers ranging from 0 to 217 spores/m^3 and 6.5 to 1600 spores/m^3 per day, respectively. Atmospheric levels of both fungi were higher between July and September (peak: 73.9 ± 34.4 spores/m^3 for *Alternaria* and 595.8±288.0 spores/m^3 for *Cladosporium*) and lower between January and March (peak: 2.4 ± 3.0 spores/m^3 for *Alternaria* and 24.3 ± 15.7 spores/m^3 for *Cladosporium*). Graphical presentation of the concentrations of *Alternaria* and *Cladosporium* moulds are depicted in Figure 1.

With regard to the correlation of fungal spore counts and meteorological parameters, regression analysis revealed a statistically significant positive correlation ($P < 0.001$) of *Alternaria* with mean daily temperature (B coefficient = 4.486, 95% confidence interval [CI] = 3.953, 5.020). On the contrary, the correlation of *Alternaria* with relative humidity was negative ($P < 0.001$), with a B coefficient equal to −1.602 (95% CI = −1.950, −1.255). Similar results were obtained for *Cladosporium*; a positive correlation ($P < 0.001$) was observed with mean daily temperature (B coefficient = 32.049; 95% CI = 28.467, 35.631) and a negative one ($P < 0.001$) with relative humidity (B coefficient = −11.572; 95% CI = −13.947, −9.196).

From a clinical standpoint, according to the 5% to 95% prediction system[29] (Table II), the Main Spore Season (MSS) for fungal circulation in the atmosphere of Western Thrace was from May to November. The peak date for *Alternaria* was June 7, with 178 fungal spores/m^3; and for *Cladosporium* it was June 23, with 1159 fungi spores/m^3. The highest total amount of fungal spores for the whole year corresponded to *Cladosporium*, with 120.464 spores/m^3 (Table II). June was the month with the highest total concentrations of airborne fungi, with 4.019 fungal spores grains/m^3 of air for *Alternaria* and 28.239 fungal spores/m^3 for *Cladosporium* (Table III). This was followed by July (2.577 spores/m^3 for *Alternaria* and 22.111 spores/m^3 for *Cladosporium*). Characteristics of *Alternaria* and *Cladosporium* airborne distribution are presented in detail in Table III. Spearman's rho correlation analysis revealed a significant positive correlation between the levels of both fungi in the atmosphere (r = 0.885, $P < 0.001$).

Fungal Sensitization and Allergic Rhinitis in Selected Patients

Sixty-nine AR patients (mean age: 21.2 ± 16.4 years, range: 4–66; median: 14 years)—28 (40.6%) males of mean age 20.8 ± 16.7 years and 41 (59.4%) females of mean age 21.6 ± 16.4 years—were studied. They all suffered from perennial AR, whereas 18 (26.0%) had asthma (uncontrolled in 3 patients, partially controlled in 2 patients and controlled in 13). According to SPT results, 52 patients were monosensitized (N = 39 to *Alternaria*

TABLE II.
Characteristics of *Alternaria* and *Cladosporium* Airborne Distribution.

Fungi	Start Date (5%)	End Date (95%)	Peak Date	Peak Concentration*	Total Spores*
Alternaria	1 May	24 October	7 June	178	16040
Cladosporium	4 May	12 November	23 June	1159	120464

*Spores/m^3.

TABLE III.
Total and Average Fungi Counts Per Month: Fungal Spores/m^3 air.

	January	February	March	April	May	June	July	August	September	October	November	December
Alternaria												
Total	42.16	30.20	179	535.93	2396.48	4019.44	2577.32	2337.89	1541.99	1935.61	380.19	64.35
Average	1.31	1.12	4.57	13.55	38.92	137.62	86.15	72.79	61.46	55.64	19.31	2.53
Cladosporium												
Total	571.66	485.33	1090.57	2689.67	12037.46	28239.05	22110.78	14904.41	14634.02	13161.51	9481.88	1057.22
Average	23.59	17.86	30.72	74.31	339.74	856.63	788.97	472.14	518.96	384.68	376.15	37.38

and N = 13 to *Cladosporium*), whereas 17 patients were sensitized both to *Alternaria* and *Cladosporium* fungi. In total, sensitization to *Alternaria* was detected in 56 patients (20 males, 36 females). Accordingly, 30 patients were sensitized to *Cladosporium* (15 males, 15 females).

Mean values of T5SS for all patients based on their monthly sensitivities are presented in Table IV. Concerning seasonal distribution, higher T5SS were observed during summer and pre-autumn period (July to September) for both fungi.

Correlations Between Symptoms and Fungal Counts

Spearman's r correlation analysis revealed a strong significant correlation between symptoms score and fungi atmosphere levels for both *Alternaria* ($r = 0.822$, $P = 0.001$) and *Cladosporium* ($r = 0.787$, $P = 0.002$) species.

DISCUSSION

Here we constructed for the first time in Greece a fungal spore calendar for the Mediterranean climate conditions of the wider region of Western Thrace. Moreover, to our knowledge, this is the first time that documented airborne *Alternaria* and *Cladosporium* concentrations are correlated to symptoms score in AR patients who live in the same area for a long period, exploring the clinical significance of fungal spore monitoring and its usefulness in everyday clinical practice. Selection of these two spores is based on the fact that they are the most abundant outdoor fungal genera, with great clinical interest due to their high allergenicity.[14,15]

Fungal surveillance data for Western Thrace revealed levels of *Alternaria* between 0 and 217 spores/m^3 per day and levels of *Cladosporium* between 6.5 and 1600 spores/m^3 per day, depending on the season. We observed that *Cladosporium* was the taxon with the highest number of spores (120.464 fungal spores/m^3) compared to *Alternaria* (16.040 fungal spores/m^3). This can be attributed to the fact that it can release billions of spores per day.[15] The dominance of *Cladosporium* has also been observed in many countries, including Australia, Portugal, Spain, Turkey, and the United States,[30,31] as well as Greece.[20] However, there are differences in total counts.[7] In some European countries, *Cladosporium* may reach values of 700,000 spores/m^3 per year, whereas *Alternaria* varies between 20,000 and 30,000 spores/m^3 per year.[32] However, in the Iberian Peninsula the annual presence of both types varies from close to 70,000 spores/m^3 to more than 200,000 spores/m^3 per year,[33,34] with *Cladosporium* exceeding in some areas the level of 300,000 spores/m^3 per year.[34] Higher spore concentrations for *Cladosporium* can be attributed to region-specific climate conditions and ecology. It is also important to mention that in our study both fungi spores were present throughout the year, with

TABLE IV.
Total Five Symptoms Score in Total Study Group and in Patients Sensitized to the Most Significant Fungi Per Calendar Month (mean ± SD)

	January	February	March	April	May	June	July	August	September	October	November	December
Alternaria	5.0	5.0	7.0	6.8 ± 0.5	8.6 ± 2.1	9.2 ± 1.3	10.6 ± 0.8	12.0	11.5 ± 1.7	7.0 ± 0.8	10.0	5.0 ± 1.0
Cladosporium	5.0	6.0	7.0	8.0	9.0	8.5 ± 0.7	11.3 ± 0.6	11.8 ± 0.4	10.7 ± 2.5	7.0	7.0	6.0

SD = standard deviation.

Cladosporium being the dominant type. According to literature in all previous studies, no fungal spore free season was detected.[7]

With regard to clinical patterns, there is lack of information on clinical thresholds (i.e., the level of specific fungal spores' concentration able to induce symptoms in a sensitized subject), with different studies indicating that thresholds can be quite variable.[3] Previous studies mentioned *Alternaria* "symptoms triggering levels" between 50 and 100 spores/m3 and for *Cladosporium* up to 3,000 spores/m^3 of air.[35] In our study, concentrations exceeding 50 spores/m^3 were detected from May to November for *Alternaria*, whereas at the same period *Cladosporium* concentrations were more than 400 spores/m^3. This indicates that symptoms in sensitized individuals are expected to be elevated mostly during these periods. Concerning seasonal distribution, the highest daily concentrations of both fungi were detected during summer and the lowest during winter. This finding is in agreement with previous studies from areas with similar Mediterranean climate conditions such as Portugal[7] and Spain[15]; the highest airborne spore concentrations were found between late spring/early summer and early autumn (July to September), whereas the lowest concentrations were registered during winter. The MSS was observed between May and November for both fungi. This is in agreement with previous studies from cities with similar Mediterranean climatic conditions.[9,13,15] All these findings support the theory that fungal spores are prevalent in dry and humid places and therefore develop only under certain temperature and relative humidity thresholds. The importance of temperature and humidity in fungal spore discharge and dispersion has previously been reported[7,8,13,15,30]; correlation results of Mediterranean climate conditions to fungal levels are in agreement with our findings. Additionally, we observed that both spore types were correlated to each other. This possibly means that detection of one spore type might act as a bioindicator of the presence of the other type. Such bioindicators are normally of great practical use. For example, they could help establish a model for estimation of the concentration of one spore type based on the concentration of the other, obviating the need for additional microscopic examination. In case of *Cladosporium*, this would be especially interesting because of its small size and the frequently high atmospheric concentrations that it reaches (the most abundant spore type in most places studied). In a recent work, Recio et al.[13] presented a similar pattern.

The next important step in our study was to detect the clinical relevance of fungal surveillance plan provided herein. Therefore, a clinically important question was how all fungal concentrations are correlated to clinical patterns of AR sufferers. In order to answer this question, we first recorded fungal sensitization prevalence in a significant number of patients suffering from AR who lived in the study area. Despite the fact that *Cladosporium* was the highest spore counted in the atmosphere, we observed that *Alternaria* was the one mostly implicated in SPT's sensitivities. Among monosensitized patients, 75% were found sensitized to *Alternaria*. Similar findings were reported in a previous study by Gioulekas et al.,[20] who found skin sensitivities to be more frequent for *Alternaria*, although the highest spore counts were once again *Cladosporium*. This may be attributed either to higher *Alternaria* allergenicity or to *Alternaria* concentrations in the atmosphere that exceed threshold levels. In the same study among fungi, sensitization prevalence's to *Alternaria* species was observed in 177 out of 421 patients (42.0%), to *Cladosporium* in 98 patients (23.2%), to *Aspergillus* in 65 patients (15.4%), and 81 patients (19.2%) were sensitized to other species. In another study by M.I. Gonianakis et al.,[11] from Heraklion/Greece (1994–2003), the most frequent fungi sensitivities were found accordingly to *Alternaria*, *Cladosporium* and *Aspergillus* species. As far as patients' symptoms are concerned, we observed that high T5SSs were documented not only during MSS (June–September) but also outside this period. Moreover although *Alternaria* is less abundant than *Cladosporium* in the air samples, it is associated with more severe allergic respiratory symptoms. This comes to prove the higher *Cladosporium* threshold levels and the higher *Alternaria* allergenicity.[3] Brito et al.[18] showed that patients allergic to *Alternaria* had a more persistent and more severe clinical profile than those sensitized to other aeroallergens. Additionally, an important overall finding that emphasizes the clinical significance of the fungal calendar was the strong significant correlation between symptoms score and atmosphere fungi levels, both for *Alternaria* ($r = 0.822$, $P = 0.001$) and *Cladosporium* ($r = 0.787$, $P = 0.002$). This finding comes in accordance with a previous work of Brito et al.,[18] who found a significant correlation between fungal count and symptoms for *Alternaria*. All aforementioned findings emphasize the clinical significance of fungi continuous monitoring in daily clinical practice for physicians treating AR, asthma, and related symptoms.

CONCLUSION

In the present study, for the first time to our knowledge, outdoor fungal spores responsible for respiratory allergies were monitored in the Western Thrace region, an area with Mediterranean climate conditions. The most prevalent mould in atmospheric concentrations is *Cladosporium*, whereas *Alternaria* is most frequently detected in terms of SPT sensitivity. Spore concentrations are significantly correlated to symptoms in AR patients, finding that supports the clinical significance of fungi monitoring in everyday clinical practice. Moreover both fungi are clearly affected by climatic factors, such as temperature and relative humidity. In the future, national aerobiological networks with adequate numbers of monitoring stations are needed in order to acquire long datasets and provide information on trends and possible changes in fungal counts. This will be important for implementing models that are able to project future scenarios. Furthermore, additional studies are needed in order to further associate the onset, peak, and end of allergic symptoms with the relevant periods of fungal presence in the atmosphere.

BIBLIOGRAPHY

1. Greiner AN, Hellings PW, Rotiroti G, Scadding GK. Allergic rhinitis. *Lancet* 2011;378:2112–2122.

2. Raulf M, Buters J, Chapman M, et al. Monitoring of occupational and environmental aeroallergens-EAACI Position Paper. Concerted action of the EAACI IG occupational allergy and aerobiology & air pollution. *Allergy* 2014;69:1280–1299.
3. Cecchi L, D'Amato G, Ayres JG, et al. Projections of the effects of climate change on allergic asthma: the contribution of aerobiology. *Allergy* 2010; 65:1073–1081.
4. Reid CE, Gamble JL. Aeroallergens, allergic disease, and climate change: impacts and adaptation. *Ecohealth* 2009;6:458–470.
5. Damialis A, Gioulekas D, Lazopoulou C, Balafoutis C, Vokou D. Transport of airborne pollen into the city of Thessaloniki: the effects of wind direction, speed and persistence. *Int J Biometeorol* 2005;49:139–145.
6. Smith M, Jager S, Berger U, et al. Geographic and temporal variations in pollen exposure across Europe. *Allergy* 2014;69:913–923.
7. Oliveira M, Ribeiro H, Abreu I. Annual variation of fungal spores in atmosphere of Porto: 2003. *Ann Agric Environ Med* 2005;12:309–315.
8. Aira MJ, Rodríguez-Rajo FJ, Fernandez-Gonzalez M, et al. Spatial and temporal distribution of Alternaria spores in the Iberian Peninsula atmosphere, and meteorological relationships: 1993–2009. *Int J Biometeorol* 2013;57:265–274.
9. Aira MJ, Rodriguez-Rajo F, Jato V. 47 annual records of allergenic fungi spore: predictive models from the NW Iberian Peninsula. *Ann Agric Environ Med* 2008;15:91–98.
10. Gomez de Ana S, Torres-Rodriguez JM, Alvarez E, Mojal S, Bemonte J. Seasonal distribution of Alternaria, Aspergillus, Cladosporium and Penicillium species isolated in homes of fungal allergic patientes. *J Investig Alllergol Clin Immunol* 2006;16:357–363.
11. Gonianakis MI, Neonakis IK, Gonianakis IM, et al. Mold allergy in the Mediterranean study of Crete, Greece: a 10 year volumetric, aerobiological study with dermal sensitization correlations. *Allergy Asthma Proc* 2006;27:354–362.
12. WE Horner, A Helbling, JE Salvaggio, SB Lehrer. Fungal allergens. *Clin Microbiol Rev* 1995;8:161–179.
13. Recio M, Trigo Mdel M, Docampo S, et al. Analysis of the predicting variables for daily and weekly fluctuations of two airborne fungal spores: Alternaria and Cladosporium. *Int J Biometeorol* 2012;56:983–991.
14. Resano A, Sanz ML, Oehling A. Sensitization to Alternaria and Cladosporium in asthmatic patients and it's in vitro diagnostic confirmation. *J Invest Allerg Clin* 1998;8:353–358.
15. Reyes ES, de la Cruz DR, Merino ME, Sanchez JS. Meteorological and agricultural effects on airborne Alternaria and Cladosporium spores and clinical aspects in Valladolid (Spain). *Ann Agric Environ Med* 2009;16: 53–61.
16. RK Bush, JM Portnoy. The role and abatement of fungal allergens in allergic diseases. *J Allergy Clin Immunol* 2001;107:430–440.
17. Katotomichelakis M, Anastassakis K, Gouveris H, et al. Clinical significance of Alternaria alternata sensitization in patients with allergic rhinitis. *Am J Otolaryngol* 2012;33:232–238.
18. Feo Brito F, Alonso AM, Carnes J, et al. Correlation between Alt a 1 levels and clinical symptoms in Alternaria alternata-monosensitized patients. *J Investig Allergol Clin Immunol* 2012;22:154–159.
19. Gonianakis MI, Neonakis IK, Gonianakis IM, et al. Mold allergy in the Mediterranean Island of Crete, Greece: a 10-year volumetric, aerobiological study with dermal sensitization correlations. *Allergy Asthma Proc* 2006;27:354–362.
20. Gioulekas D, Damialis A, Papakosta D, Spieksma F, Giouleka P, Patakas D. Allergenic fungi spore records (15 years) and sensitization in patients with respiratory allergy in Thessaloniki-Greece. *J Investig Allergol Clin Immunol* 2004;14:225–231.
21. Gioulekas D, Damialis A, Papakosta D, et al. 15-year aeroallergen records. Their usefulness in Athens Olympics. *Allergy* 2004;58:933–938.
22. Burkard Scientific Ltd., Uxbridge, UK. Available at: http://www.burkardscientific.co.uk. Accessed August 24, 2015.
23. Bush R. Aerobiology of pollen and fungal allergens. *J Allergy Clin Immunol* 1989;64:1120–1124.
24. British Aerobiological Federation. Airborne Pollens and Spores: A Guide to Trapping and Counting. Rotherham, UK: British Aerobiology Federation; 1995.
25. Bousquet J, Anto JM, Demoly P, et al. Severe chronic allergic (and related) diseases: a uniform approach—a MeDALL—GA(2)LEN—ARIA position paper. *Int Arch Allergy Immunol* 2012;158:216–231.
26. Bateman ED, Hurd SS, Barnes PJ, et al. Global strategy for asthma management and prevention: GINA executive summary. *Eur Respir J* 2008; 31:143–178.
27. Dreborg S. EAACI Subcommittee on skin tests. Skin tests used in type I allergy testing. Position paper. *Allergy* 1989;44:S22–S31.
28. Burbach GJ, Heinzerling LM, Edenharter G, et al. GA2LEN skin test study II: clinical relevance of inhalant allergen sensitizations in Europe. *Allergy* 2009;64:1507–1515.
29. Driessen M, Van Herpen R, Moelands R, Spieksma F. Prediction of the start of the pollen season for the western part of the Netherlands. *Grana* 1989;28:37–44.
30. Erkara IP, Asan A, Yilmaz V, Pehlivan S, Okten SS. Airborne Alternaria and Cladosporium species and relationship with meteorological conditions in Eskisehir City, Turkey. *Environ Monit Assess* 2008;144:31–41.
31. Katial R, Zhang Y, Jones R, Dyer P. Atmospheric mold spore counts in relation to meteorological parameters. *Int J Biometeorol* 1997;41:17–21.
32. Nikkels AH, Terstegge P, Spieksma FTM. Ten types of microscopically identifiable airborne fungal spores at Leiden, The Netherlands. *Aerobiologia* 1996;12:107–112.
33. Oliveira M, Abreu I, Ribeiro H, Delgado L. Fungal spores in the atmosphere in the city of Oporto and its allergological implications. *Rev Port Imunoalergol* 2007;15:61–85.
34. Sabariego S, Diaz de la Guardia C, Alba F. [Aerobiological study of Alternaria and Cladosporium conidia in the atmosphere of Almeria (SE Spain)]. [Article in Spanish]. *Rev Iberoam Micol* 2004;21:121–127.
35. Caretta G. Epidemiology of allergy disease: the fungi. *Aerobiologia* 1992;8: 439–445.