

Aeroallergens in West Crete, Greece: A five year (2010–2014) aerobiological study

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Abstract The objective of the analytic observational study was to present air-pollen counting program results for a 5-year period. Airborne pollens and fungi collection, from both urban and sub-urban areas, were obtained using a special Burkard pollen trap installed on the roof of Chania General Hospital. Aeroallergen concentration measurement was made in a standardized way with fixation of the material collected and then counting using an optical microscope. Annual and total circulating pollen and fungi counts for the study period are presented. In the year 2014, the highest total annual count was recorded, while 2013 was the year with the lowest one. Months with the highest average concentrations were June for the years 2010 and 2011 (1291 and 1114.6 grains/m³, respectively) and May for the consecutive 3 years 2012–2014 (1120, 890 and 1353.1 g/m³, respectively). Peak periods for circulating aeroallergens were April–June. Trees pollen accounted for the majority of circulating aeroallergens (615.9 and 677.1 g/m³ during peak periods in the years 2012 and 2014), while fungi accounted for the majority of circulating aeroallergens (818.5, 729.4, 890.7 spores/m³), during the peak periods in the years 2010, 2011 and 2013. Variability

in peak airborne allergen periods could be partly explained by the differences in climatic conditions during the study period.

Keywords Aeroallergens · Pollen count · Pollen forecast · Airborne moulds · Allergic rhinitis · Mediterranean

Introduction

Aeroallergens are light substances, pollens or spores, transported by air and can cause hypersensitive reactions such as allergic rhinitis and asthma [1]. Pollen and mold spores have sizes ranging from 2 to 60 µm and contain proteins (allergens) with molecular weight from 10,000 to 40,000 Da [2]. The airborne particles and mold spores are therefore closely related to hypersensitivity of the upper respiratory tract due to continuous exposure [3, 4]. Climate alterations lead to longer exposure to irritating agents with concurrent upper respiratory tract symptom exacerbations [5]. Variations in the amount of allergen air load should be taken under consideration when correlating allergens' atmospheric concentrations with symptom severity in patients with allergic rhinitis [6, 7]. Allergic rhinitis is the most common immune disorder in humans [7]. The most usual disease-related symptoms are sneezing, nasal obstruction and watery or mucous pre- or postnasal discharge mediated by an immunoglobulin E (IgE) inflammation of the nasal and paranasal sinus mucosa [8]. According to the unified airway theory (one airway, one disease), allergic rhinitis and asthma seem to be related [9]. Anatomically, the two airways have common respiratory epithelium and common disease susceptibility [10]. About 40 % of patients with allergic rhinitis have asthma, and

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50–80 % of patients with asthma report symptoms of allergic rhinitis [11–13]. Inflammation in one of the two anatomic parts affects both locations. The degree of inflammation in asthma is strongly dependent on the severity of inflammation in the nose, so a common strategy is needed to deal with upper and lower respiratory tract disease [14]. The development of allergic rhinitis and asthma requires coexistence of genetic predisposing factors and exposure to airborne allergens [11]. Allergic rhinitis and asthma affect sufferers' quality of life at both psychological status and level of daily activities, and can be ameliorated with appropriate medical treatment [15, 16]. There is therefore a clear need for aeroallergen monitoring of trees, weeds, grasses and fungi endemic in the certain area. Information concerning the concentration of allergens in the atmosphere can provide to sufferers from allergic rhinitis irritant avoidance and prompt initiation of appropriate medical treatment, leading to better disease management [16–19].

Accordingly the aims of the present study were to present the pollen and fungi calendar for the Mediterranean climate conditions of Western Crete, analyze aeroallergen dynamics and describe their seasonal trends for a 5-year period. These data can help establish a monitoring system that alerts susceptible individuals and provides critical evidence for future epidemiological studies regarding allergic respiratory diseases in the study area.

Materials and methods

Climate conditions in West Crete

Ground morphology and location of Crete in the center of the Mediterranean area have a direct impact on the Chania climate, characterized as mild temperate Mediterranean, particularly dry and warm, with sunshine covering 70 % of the days of the year. In more detail, in April, the weather is sweet and pleasant. In October, it rarely rains and the weather stays warm and mild, whereas May and September are usually sunny, but not too warm. Summer, however, is quite hot and dry. June, July and August are the hottest months of the year without rainfall [20, 21].

Main Chania Crete vegetation–flora features [data from Chania Mediterranean Agronomic Institute (MAICH)]

The extent of settlements represents around 5 %, while cultivated areas cover about 30 % of the county. Throughout the county, the main cultivated species are olive (*Olea europaea*), vines (*Vitis vinifera*), citrus (*Citrus* spp.) and avocado (*Persea americana*) in the northern

lowlands. Fewer areas are cultivated with horticultural species and other fruit trees. Chestnut (*Castanea sativa*) exists in a limited area, especially on acidic soils, with high rainfall in the N–NW side of the White Mountains often together with Oak species *Quercus* spp. Chania forests are mainly forests of cypress (*Cupressus sempervirens* var. *horizontalis*), located on the east side of White Mountains, often with maple (*Acer sempervirens*) or holm oaks (*Quercus coccifera*) and cedar (*Juniperus oxycedrus* sub sp. *oxycedrus*). Natural pine forests with pine (*Pinus brutia*) are mainly found in the southern part of White Mountains, while artificial pine forests are found in small areas in many villages. In favorable humidity conditions, species such as trees (*Platanus orientalis*), poplars (*Populus* spp.), willow (*Salix* spp., *Styrax officinalis*), the wickers (*Vitex agnus castus*), Oleander (*Nerium oleander*) and more rarely elms (*Ulmus minor*) are found.

In areas with intense human activities, nitrofila weed species such as nettles (*Urtica* sp.), *Parietaria* spp., *Amaranthus* sp., *Echallium elaterium*, *Marrubium vulgare*, as well as other weed species of the family of Graminae grow.

Geographic location of Chania, Crete, is 35° 30' 47'' N and 024° 01' 05'' E.

Study material

For pollen and fungi capturing, the study material consisted of a Burkard 7-day recording volumetric spore trap, Melinex Tape, double-sided adhesive tape, transparent cutting block, scissors, brush, forceps, slides, Gelvatol and light microscope with magnification of 400×, provided to the ENT Department of Chania General Hospital by the Region of Crete/Chania Prefecture. The Burkard Pollen Sampler was placed outdoor on the roof of the hospital at a height of about 20 m above the ground in an area particularly favorable for collecting information from both the urban center of Chania and the suburban hospital area, ensuring representative indications for the entire region.

The study was conducted for five consecutive years from January 2010 through December 2014 and the spore trap ran continuously during this period without any interruption. Special characteristics of the device are standard orifice 2 mm × 14 mm and drum speed 1 revolution in 7 days at 2 mm/h for a throughput of 10 l/min. Every week, a collecting drum was prepared with Melinex tape fixed to the device with double-sided adhesive tape in such way as to avoid leaving an empty gap to the drum, and then coated with a thin layer of silicone grease. Particles are impacted on that adhesive-coated transparent plastic tape supported on a clockwork-driven drum. The drum was removed every Tuesday at 8:00 AM (GMT + 2) and replaced immediately with the second prepared one.

Particular attention was given to tune the device clock so as not to interrupt rotation during the week. The Melinex tape from the removed drum was then cut into 48-mm-long segments corresponding to 24-h periods, placed on plain microscope slides, mounted and counted according to certain protocol. Pollen and mold spores were counted with 400× magnification and recorded as counts per cubic meter of air per hour [22, 23].

The particle allergens were collected and analyzed. They were divided into four main irritants groups: trees, weeds, grasses and fungi. The length of the pollen seasons was calculated by the 95 % method [24]. The pollen forecast used in our department is from the National Pollen Aerobiology Center, University of Worcester: low, less than 30 pollen grains per m³ of air; moderate, 30–49 pollen grains per m³ of air; high, 50–149 pollen grains per m³ of air; very high, 150 or more pollen grains per m³ of air. Most sufferers of hypersensitivity of the respiratory tract will start to experience symptoms when the count reaches the moderate category [25]. However, it should be noted that sometimes and for certain types of pollen grains, local or regional threshold levels able to trigger symptoms should be defined. Factors that affect pollen counts are abundance of the given species in a particular area, the presence of other species that might cause cross-reactions, presence of atmospheric irritants and special weather conditions. These are some important factors from the pool of factors involved in the appearance of allergic rhinitis symptoms in sensitized patients.

Statistical analysis

Monthly aeroallergen concentrations were taken into account, as well as absolute spore counts. Pollen seasons were calculated according to the 5–95 % count method [23]. According to this method, the pollen season starts on the date when 5 % of the average annual pollen count is reached and lasts until the atmosphere concentration reaches 95 %. Pollen and fungi concentrations throughout the studied period were presented as running means or moving (10 days) average values (British Aerobiological Federation guidelines) [26]. This is a statistical method of short-term forecasting, attributed to measures with random variation to produce trend patterns during a given time period.

Results

During the study period (2010–2014), 981.345 grains/m³ were recorded in total. The year with the lowest annual circulating aeroallergen counts was 2013 (150.778 grains/m³), whereas the highest total annual count was detected in 2014 (239.592 grains/m³). Daily mean counts (grains/m³),

both group specific and total, were used to calculate 10-day arithmetic means per year and for the whole study period, and the results were presented in the form of a histogram.

Total grain distributions through the years 2010–2014

In the year 2010, June was the month with the highest mean concentration of aeroallergens (1291 g/m³) with fungi showing the highest percentage (818.5 spores/m³), followed by trees (386.5 g/m³), weeds (57.2 g/m³) and grasses (28.8 g/m³). In 2011, maximum concentrations were reached in June (1114.6 g/m³) with fungi showing the highest percentage (729.4 spores/m³), followed by trees (278 g/m³), weeds (59.8 g/m³) and grasses (47.1 g/m³). In 2012, May was also the month with the highest circulating allergen concentration (1120 g/m³) with trees showing the highest percentage (615.9 g/m³), followed by fungi (391.7 spores/m³), weeds (61.4 g/m³) and grasses (50.9 g/m³). In 2013, May was the month presenting the highest aeroallergen concentrations (890.7 g/m³) with fungi showing the highest percentage (529.3 spores/m³), followed by trees (285.5 g/m³), weeds (39.5 g/m³) and grasses (36.4 g/m³). 2014 was the year with the highest reported aeroallergen concentration (1353.1 g/m³) in May, with 50 % (677.1 g/m³) attributed to tree allergens, followed by fungi (525.7 spores/m³), weeds (110 g/m³) and grasses (40.2 g/m³).

Group-specific grain distributions through the years 2010–2014 and the pollen seasons

Group-specific and total grain distributions through the years 2010–2014 are presented in Figs. 1, 2 and 3. In total, the months with the lowest aeroallergen concentration were January, February and March, whereas pollens and spores remained at concentrations >200 g/m³ from mid-March to mid-November, peaking in May–June, months with the greater reported concentrations. According to 10-day moving average graphs, weed pollens were present in concentrations >40 g/m³ from the beginning of May, peaked in mid-May and mid-June and stayed at concentrations >50 g/m³ up to the end of June. Tree pollens were present through the year, but increased to concentrations of >100 g/m³ in March, peaking in mid-May and remained at concentrations of ~200 g/m³ up to the end of July. Aeroallergens from fungi were present from about mid-March up to the end of April (200–300 spores/m³), when they started to increase, peaking in mid-June and staying in concentrations of ~400 spores/m³ up to November. Finally, grass pollens were present from April, peaking in May and June, with a relatively low concentration (20–30 g/m³) up to mid-August.

Pollen and fungi seasons for each grain for the years 2010–2014 are presented in detail in Tables 1, 2, 3 and 4.

Fig. 1 Total pollen 10-day average concentration graph (averaged for 2010–2014)

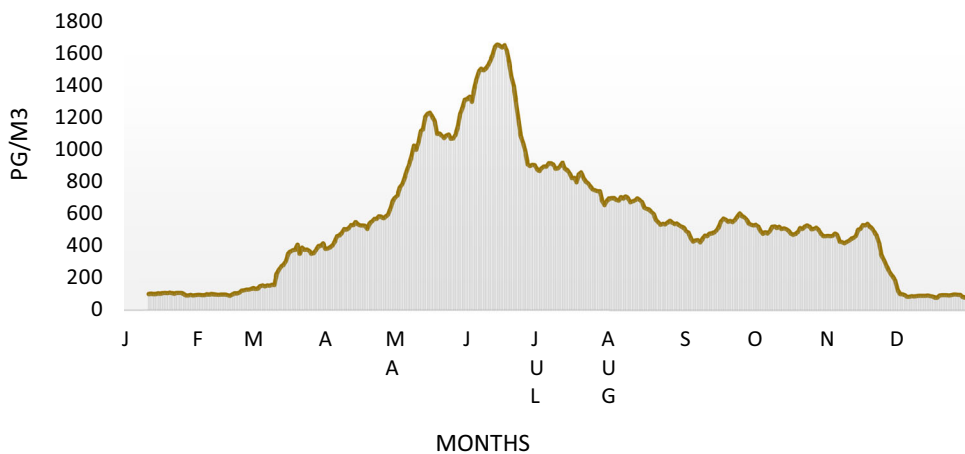


Fig. 2 Weeds', trees' and grasses' 10-day average pollen concentration graph (averaged for 2010–2014)

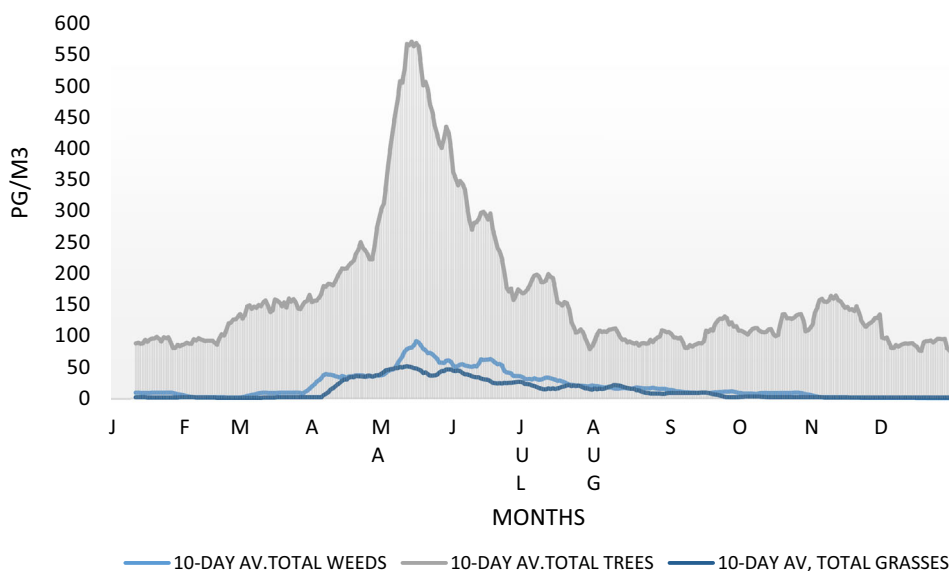


Fig. 3 Fungi's 10-day average mould spore concentration graph (averaged for 2010–2014)



Table 1 Weeds' pollen seasons

| Weeds | Start date | End date | Peak month | Peak mean concentration (SD) (pg/m ³) | Peak total spores/month |
|-------|------------|----------|------------|---|-------------------------|
| 2010 | 21/3 | 17/6 | 5 | 112.2 (52.3) | 956.6 |
| 2011 | 15/3 | 11/6 | 5 | 166.5 (24.2) | 1434.1 |
| 2012 | 18/3 | 25/6 | 5 | 232.7 (57.1) | 1153 |
| 2013 | 5/4 | 24/6 | 5 | 127.05 (74) | 743 |
| 2014 | 13/4 | 8/6 | 5 | 282.15 (44.1) | 2066 |

Table 2 Trees' pollen seasons

| Trees | Start date | End date | Peak month | Peak mean concentration (pg/m ³) | Peak total spores/month |
|-------|------------|----------|------------|--|-------------------------|
| 2010 | 3/2 | 5/6 | 5 | 541.2 (176.4) | 6038.5 |
| 2011 | 20/1 | 1/6 | 5 | 1095.6(267.2) | 8802.8 |
| 2012 | 23/1 | 1/5 | 5 | 1874.4 (175.2) | 11,492 |
| 2013 | 1/2 | 10/6 | 5 | 904.2 (166.4) | 5256 |
| 2014 | 21/1 | 15/5 | 4 | 1365.2 (398.8) | 8583.4 |

Table 3 Fungi's pollen seasons

| Fungi | Start date | End date | Peak month | Peak mean concentration (pg/m ³) | Peak total spores/month |
|-------|------------|----------|------------|--|-------------------------|
| 2010 | 6/4 | 2/11 | 6 | 1636.8 (531) | 17,777.2 |
| 2011 | 30/3 | 10/11 | 6 | 1689.6 (293.6) | 13,397.1 |
| 2012 | 13/4 | 11/11 | 6 | 1357.6 (207) | 8704 |
| 2013 | 10/4 | 21/11 | 6 | 1531.2 (215,2) | 8888 |
| 2014 | 20/3 | 14/11 | 6 | 1420.6 (413) | 14,091 |

Table 4 Grasses' pollen seasons

| Grasses | Start date | End date | Peak month | Peak mean concentration (pg/m ³) | Peak total spores/month |
|---------|------------|----------|------------|--|-------------------------|
| 2010 | 13/4 | 3/6 | 5 | 108.9 (8.6) | 785.4 |
| 2011 | 30/4 | 14/6 | 5 | 127.5 (32.3) | 1001.5 |
| 2012 | 15/4 | 17/6 | 5 | 101.6 (23) | 977 |
| 2013 | 13/4 | 3/7 | 5 | 70.95 (18.4) | 730 |
| 2014 | 12/4 | 20/6 | 4 | 77.6 (19.8) | 754 |

Discussion

This is the first study to provide airborne allergen concentration count for a 5-year period 2010–2014 for the Mediterranean climate conditions of the West Crete region. Data obtained could be used as a prognostic risk factor for allergic rhinitis symptoms exacerbation in sensitized patients. Symptom triggering and intensity in patients with allergic rhinitis are associated with negative impact on physical, social and mental well-being, that is to say exert a negative impact on the patient's quality of life [27]. Therefore, it seems of considerable value to know the period in which this phenomenon has high probability to occur.

At this point, it is useful to remind that according to what has been previously reported, cross-reactivity is observed between different genus, family or subfamily. Botany taxonomic ranks are as follows: kingdom, phylum, class, order, family, subfamily, tribe, genus and species [28]. The pattern of allergenic cross-reactivity is characterized by strong cross-reactions between the same tribe and genus due to closely related compositions and protein structure, moderate cross-reactions between the same family or subfamily due to similar compositions and finally from low or absence of cross-reactions between different subfamilies or families due to distinct compositions and structures with minimal similarities [29].

Accordingly, we present the concentrations of the four main airborne allergen families, specifically, weeds, trees, fungi and grasses, as recorded and measured by airborne allergen collection station installed on the roof of Chania General Hospital, specifically for the years 2010–2014. May and June were found to be the months with the higher allergen mean concentrations in all years. Moreover, in 2013, the lowest average concentrations were reported. The analysis of our results begins with the pollen season calculation, according to the 5–95 % method for the main families. Pollen season for weed pollens through the study period always started in the second half of March and finished between 8 and 25 of June, peaking always in May. Tree pollen season through the study period started always in the last 10 days of January and the first week of February, peaked at April in 2014 or May in 2010, 2011, 2012 and 2013, and ended within the first half of May. Grass pollen season through the study period always started in the second half of April, peaked at May except in 2014 when the peak month was April and ended in June, with the exception of the year 2013, when the end date was early July (03/07/2013). Finally, for mould spores, the spore season through the study period started between 20th of March (2014) and 13th of April (2012), peaked always at June and ended at maximum on 21 November in 2013. Variations in starting dates and peak total spores per month are attributed to climate conditions and alterations that induce changes in plant flowering periods, mostly by lengthening them. During pollen seasons, all sensitized patients have severe probability of allergic rhinitis symptoms and exacerbation and that fact makes information provided particularly useful for patients and physicians dealing with upper respiratory tract hypersensitivity. Our study data are mostly in accordance with recent pollen and fungi count studies in Greece [30, 31] and effort was made to collect data from other areas that have not been studied to have an overall estimation of Greece, contributing in this way to improvement in patients' quality of life.

According to the total 10-day average concentration, the months with higher aeroallergens count for the study period were April, May, June and mid-July. During April, May and June, airborne allergens from all families (weed, trees and grasses) were in highest concentrations compared to the annual variation, and undoubtedly in concentrations capable of triggering symptoms in patients with hypersensitivity of the upper respiratory tract. Fungi spores reached higher concentration ratios from mid-May to August and peaked in June with concentration that reached more than 1200 spores/m³ in total.

As a limitation of this study we should mention the temporary lack of records of symptoms in AR sufferers who lived in the same area, in order to explore the clinical

significance of the calendar, finding out local threshold levels that could create difficulties in AR patients.

As we already know, the probability of symptom exacerbation is a complex phenomenon that depends on the sensitivity of each patient to a particular allergen, the atmospheric concentration of allergens, their allergenicity and weather conditions that can facilitate or prevent their manifestation. Finally, airborne pollen and fungi allergenicity is always an unpredictable factor which prevents an accurate and factual prediction. In this study, we analyzed the concentrations of airborne allergens and, in a subsequent study that is already underway, we will try to highlight the clinical relevance of these concentrations to patient's symptom intensity and correlate them with weather conditions. In this way, we could in future have a potential prediction of the number and type of allergens circulating at a certain time period, under specific weather conditions.

Conclusions

It is well known that in patients with allergic rhinitis symptom induction depends on several factors: One of the most important is high airborne allergens concentration and in exactly these time periods patients require early and careful management and treatment. This study provides recent data for the Mediterranean climate conditions of the West Crete area and follows already published data from other areas of Greece, with only minor deviations related to different West Crete area conditions and could be a part of a wider study of the aerobiological map of Greece in relation to specific climatic conditions of each area. In addition, this study could contribute to determining local threshold levels for airborne aeroallergens. The study data could also be a useful guide for patients and physicians for early start of preventive measures and, when necessary, for early onset of appropriate medical treatment depending on the patient's symptom severity and duration.

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Compliance with ethical standards

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Conflict of interest None.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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