

# Seasonal variations of allergenic pollen in a Mediterranean region – Alexandroupolis, north-east Greece

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Nikolaidis C, Katotomichelakis M, Nena E, Makris M, Tsakas M, Michopoulos I, Constantinidis TC, Danielides V. Seasonal variations of allergenic pollen in a Mediterranean region (Alexandroupolis, north-east Greece). *Ann Agric Environ Med*. 2015; 22(4): 685–689. doi: 10.5604/12321966.1185776

## Abstract

**Objectives.** The aim of this study was to describe the aerobiological characteristics of NE Greece, identify the most prevalent taxa, define the duration of the main pollen season and correlate allergen records with meteorological parameters.

**Materials and method.** A 7-day volumetric trap, running continuously throughout the year, was used to collect circulating pollen. Pollen taxa were characterized by standard protocols and counted as grains/m<sup>3</sup>. The main pollen season was deduced from these data and running means (10-day averages) were plotted against time. Correlations with climatic factors (temperature, rain, humidity, wind velocity) were assessed by single linear regression analysis.

**Results.** In total, 11 pollen families were identified, including 6 arboreal and 5 non-arboreal taxa. The 5 most prevalent taxa were Oleaceae, Fagaceae, Poaceae, Cupressaceae and Pinaceae. Peak pollen concentrations were detected in April and May, with daily averages exceeding 410 grains/m<sup>3</sup>. Compositae had the longest pollen season of 135 days and Oleaceae the shortest, extending to only 27 days. Correlations with meteorological parameters showed variable associations among different taxa, with mean temperature ( $p < 0.001$ ), relative humidity ( $p = 0.015$ ), and wind speed ( $p = 0.042$ ) emerging as the most significant determinants as regards total pollen counts.

**Conclusions.** Describing the aerobiological characteristics of NE Greece enabled the identification of allergenic risks that are specific for this region. Records generated in this study can be used to alert sensitized individuals of prevailing seasonal patterns, in order to take necessary precautions against imminent exposures. The monitoring system established here can serve as a reference guide for future epidemiological research focusing on allergic asthma and rhinitis.

## Key words

Allergenic pollen, seasonal trends, Mediterranean, NE Greece

## INTRODUCTION

The monitoring of aerobiological parameters is of paramount importance for the characterization of allergenic risks. Pollen allergy (hay fever/pollinosis) is caused by a hypersensitivity reaction of the respiratory tract and eye conjunctivae to pollen grains. It includes allergic rhinitis, allergic conjunctivitis, allergic bronchial asthma, and less frequently, urticaria [1]. Seasonal allergies are often characterized by the recurrence of symptoms which show periods of improvement and relapse [2]. The allergic manifestations are more prevalent during the plant pollination season; hence, accurate descriptions of its start and end periods are important for both patients and clinicians alike [3, 4, 5].

Particularities at the epidemiological level are directly linked to environmental factors which, in turn, are determined by the geographic zone and local vegetation

patterns. The phenological stage of plant species and climatic conditions are crucial for elucidating seasonal patterns and assigning patient records with respect to these variables. Information of this type can be used to establish early warning systems and alert public health authorities, advance our medical understanding of disease manifestations in a region, and promote population-based screening options that can ultimately lead to better treatment regimes [6].

Airborne particles can be dispersed in the atmosphere and travel great distances, although local sources are typically more potent in inducing allergenic reactions. Climatic factors, such as air temperature, humidity, rainfall and wind speed/direction are key modifiers of pollen dissemination, as well as of respiratory tract sensitization. They may cause rapture of pollen grains, releasing thousands of allergenic particles into the air and elicit acute reactions in sensitized individuals [7, 8].

The Mediterranean environment in particular is dominated by aeroallergens that correspond to a plethora of flora, including both arboreal and herbaceous taxa that flourish in the temperate climate throughout the calendar year [9]. This poses a challenge for individuals suffering from asthma

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Received: 09 October 2014; accepted: 19 January 2015



and rhinitis, as well as for medical institutions dealing with these types of morbidities.

## OBJECTIVES

The aim of this study was to analyze pollen dynamics in NE Greece, describe their seasonal trends and correlate them with meteorological parameters. These preliminary data can help establish a pollen monitoring system that alerts susceptible individuals, and can provide critical evidence for future epidemiological studies focusing on allergic respiratory diseases in the region.

## MATERIALS AND METHOD

The city of Alexandroupolis is situated in North-Eastern (NE) Greece, at 40.51' N 25.52' E. It has a typical Mediterranean climate, with an average daily temperature of 14.9°C, relative humidity of 67.4% and 549.3 mm total precipitation per year. It is situated near the delta of the Evros river, 40 km from the border with Turkey, 302 km West of Istanbul and 750 km North-East of Athens (Fig. 1). Local vegetation includes evergreen, deciduous and fruit trees (e.g. pines, cypresses, oaks and olives), as well as a variety of other shrubs, grasses and herbs (e.g. mugwort, laurel, mint, thyme) that create a diverse polinological environment.



Figure 1. Map of study region

For pollen capturing, a 7-day recording volumetric spore sampler (Burkard Scientific, Ltd.) was placed 21 m above the ground, on the roof of the University Hospital of Evros, which is 6 km West of the city of Alexandroupolis. The unit was operating continuously for one year (January-December 2013), collecting 10 liters of air per minute. A strip of silicone-coated Melinex tape was exposed to the air for trapping the spores, and was changed once a week. The exposed tape was cut into 48 mm segments representing 24 h periods. These segments were mounted on microscopic slides using Gelvatol mixed with a stain (acid fuchsin) to enable visualization

under a high resolution light microscope (Olympus BX40) at 400× magnification.

Pollens were identified following standard manuals [10] and counted according to the guidelines of the British Aerobiology Federation [11]. The spore counts were converted into the number of spores per cubic meter of air. Seasonal distribution of pollen counts was presented as 10-day-running average. The main pollen season (MPS) was characterized as 90% of the seasonal pollen count, starting on the day when 5% of the total pollen had been recorded, and ending on the day 95% of the total pollen had been counted.

Meteorological data were obtained from the regional station of the Hellenic National Meteorological Service situated close to the sampling site (<http://www.hnms.gr>). Daily mean temperature (°C), relative humidity (%), rainfall (mm), wind speed (km/h) and wind direction were recorded. The correlation between individual meteorological parameters and pollen counts was assessed by regression analysis, using SPSS 15.0® software package (<http://www.spss.com>). A value of  $p < 0.05$  was considered to be statistically significant.

## RESULTS

Airborne pollen belonging to 11 families were identified in the study, including 6 arboreal (Cupressaceae, Pinaceae, Fagaceae, Platanaceae, Betulaceae and Oleaceae), and 5 non-arboreal taxa (Poaceae, Plantaginaceae, Compositae, Chenopodiaceae and Urticaceae). Running means (or moving averages) for 10 days were used for smoothing the values, in order to make the trend of seasonal variation more easily observed (Fig. 2). Pollen taxa were widely distributed throughout the calendar year, and peaked during the spring and summer months (March-September).

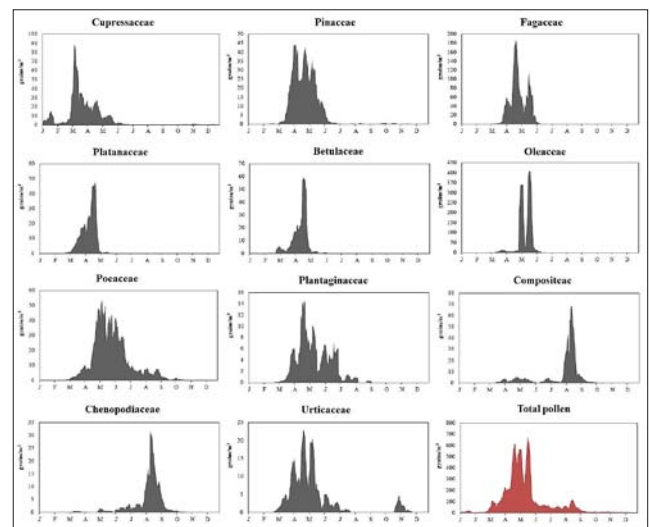
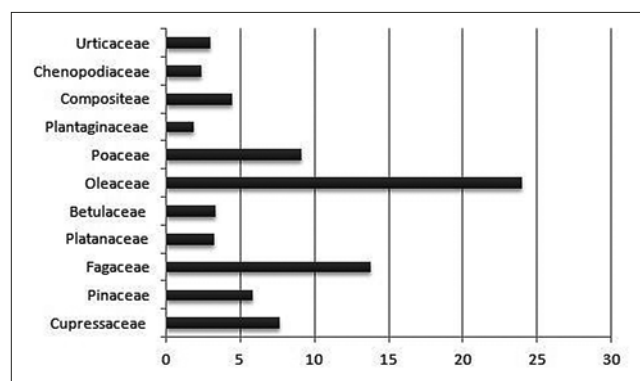


Figure 2. Pollen concentrations (grains/m<sup>3</sup>) as 10-day running averages

The major pollen producers were arboreal plants, namely Oleaceae (24.1%), Fagaceae (13.7%), Cupressaceae (7.6%) and Pinaceae (5.9%), which accounted for 51.3% of the total pollen grains (Fig. 3). The family Oleaceae was dominated by *Olea europaea* (olives), followed by *Fraxinus* and *Ligustrum* species. Fagaceae were principally represented by *Quercus*



**Figure 3.** Annual contribution (%) of individual pollen on total atmospheric load

spp. (oaks), Cupressaceae were associated with *Cupressus* or *Juniperus* spp. and Pinaceae included a mixture of *Pinus*, *Abies* or *Cedrus* taxa.

Among non-arboreal plants, pollen grains belonging to Poaceae (9.1%), Compositae (4.4%) and Urticaceae (3%) dominated the atmosphere during the study period. Poaceae generated a diverse sample, including both domesticated and wild flowering (monocotyledonous) plants. Compositae (also known as Asteraceae) included a large variety of taxa with a characteristic morphology (e.g. *Ambrosia* or *Artemisia* spp.). Finally, Urticaceae (*Parietaria* spp.) were the smallest pollen grain recorded, measuring only 12–15  $\mu\text{m}$  in diameter.

The main pollen season (MPS) was described as the time during which most pollen (90%) was recorded, starting when the sum of daily means concentrations reached 5% of the total sum, up to the point when the sum reached 95% [12]. The pollination of all arboreal species initiated and peaked during springtime, with the exception of Cupressaceae that started their circulation in January (Tab. 1). The pollen season of Pinaceae, Platanaceae, Betulaceae and Urticaceae started in March, of Fagaceae, Poaceae, Plantaginaceae and Compositae in April, whereas Oleaceae and Chenopodiaceae seasons initiated in May and June, respectively.

Termination of the pollen season for all arboreal taxa was observed in spring (or early summer in the case of Pinaceae). On the other hand, herbaceous taxa pollination extended to late summer (Poaceae) and early autumn (Compositae, Chenopodiaceae). Peak dates for all pollen types occurred in spring (April-May), with the exception of Compositae and Chenopodiaceae, which peaked in August. Moreover,

Compositae had the longest pollen season (135 days), followed by Urticaceae and Poaceae (134 days). Among arboreal taxa, Cupressaceae had the longest pollen season of 122 calendar days.

April and May were the months when the highest concentrations of airborne pollen (8 taxa) was observed. During these months, an average of 410 pollen grains per  $\text{m}^3$  circulated in the atmosphere. The highest concentrations of pollen counts (over 1,000 grains per  $\text{m}^3$ ) were recorded in May and the lowest (<1 grain per  $\text{m}^3$ ) in December. Notably, Oleaceae had the shortest pollen season of just 27 days, although they presented the maximum of pollen load in the atmosphere (7,742 grain per  $\text{m}^3$ ) during their seasonal circle.

In the present study, 4 climatic factors, namely average daily temperature ( $^{\circ}\text{C}$ ), relative humidity (%), rainfall (mm) and wind speed (km/h), were taken into consideration (Tab. 2). Based on these records, August was the warmest ( $26.6 \pm 1.3^{\circ}\text{C}$ ) and driest month of the year (0 mm rain,  $47.7 \pm 6.3\%$  humidity). January, on the other hand, was the wettest month ( $3.8 \pm 8.3$  mm rain,  $80.8 \pm 7.8\%$  humidity) and December the coldest ( $4.9 \pm 2.8^{\circ}\text{C}$ ). August was also the windiest month ( $9.6 \pm 5.6$  km/h) and September the calmest ( $4.0 \pm 1.6$  km/h). North-easterly winds dominated throughout the year which tended to disperse airborne pollen in south-western directions, away from the mainland and over the Aegean sea.

**Table 2.** Climatic conditions (mean $\pm$ SD)

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

**Table 1.** Characteristics of the pollen season

Pollen type	Start date (5%)	End date (95%)	Season duration (days)	Peak date	Peak concentration <sup>1</sup>	Total pollen <sup>1</sup>
Cupressaceae	23 January	24 May	122	15 March	310.5	2468.7
Pinaceae	29 March	5 June	79	11 April	133.9	1983.4
Fagaceae	5 April	27 May	53	27 May	432.0	4499.3
Platanaceae	19 March	30 April	43	26 April	119.3	1049.9
Betulaceae	9 March	4 May	57	26 April	204.1	1072.2
Oleaceae	1 May	27 May	27	8 May	1209.6	7741.9
Poaceae	8 April	19 August	134	15 May	184.1	2923.9
Plantaginaceae	3 April	6 July	95	25 April	61.0	608.12
Compositae	25 April	6 September	135	24 August	108.5	1433.2
Chenopodiaceae	13 June	9 September	89	22 August	52.4	752.2
Urticaceae	20 March	31 July	134	15 May	76.1	953.6

<sup>1</sup> grains/ $\text{m}^3$



**Table 3.** Impact of climatic factors on atmospheric concentration of pollen (results from single linear regression analysis; only significant associations are shown)

Pollen type	Climatic factor	B (95% CI)	p value
Cupressaceae	Relative Humidity	0.22 (0.06, 0.38)	0.009**
Oleaceae	Wind speed	3.55 (1.14, 5.95)	0.004**
Poaceae	Mean Temperature	0.77 (0.52, 1.01)	<0.001***
	Relative Humidity	-0.24 (-0.38, -0.11)	0.001**
Plantaginaceae	Mean Temperature	0.10 (0.03, 0.17)	0.006**
Compositae	Mean Temperature	0.71 (0.53, 0.89)	<0.001***
	Relative Humidity	-0.38 (-0.47, -0.28)	<0.001***
	Wind speed	0.42 (0.14, 0.69)	0.003**
Chenopodiaceae	Mean Temperature	0.39 (0.30, 0.47)	<0.001***
	Relative Humidity	-0.21 (-0.25, -0.16)	<0.001***
	Wind speed	0.17 (0.04, 0.30)	0.012*
Total pollen	Mean Temperature	5.14 (2.54, 7.75)	<0.001***
	Relative Humidity	-1.76 (-3.17, -0.35)	0.015*
	Wind speed	3.97 (0.15, 7.79)	0.042*

B: coefficient of linear regression, CI: confidence intervals, \*p<0.05, \*\*p<0.01, \*\*\*p<0.001

The association between individual meteorological parameters and airborne pollen concentration was assessed by single linear regression analysis. The results of significant associations ( $p<0.05$ ) are given in Table 3. Among arboreal taxa, Cupressaceae were associated with relative humidity ( $p=0.009$ ) and Oleaceae with wind speed ( $p=0.004$ ). Among non-arboreal taxa, Poaceae were associated with mean temperature ( $p<0.001$ ) and relative humidity ( $p=0.001$ ) and Plantaginaceae with mean temperature ( $p=0.006$ ). Compositae, on the other hand, were associated with mean temperature ( $p<0.001$ ), relative humidity ( $p<0.001$ ) and wind speed ( $p=0.003$ ). Equally, Chenopodiaceae correlated with mean temperature ( $p<0.001$ ), relative humidity ( $p<0.001$ ) and wind speed ( $p=0.012$ ). Finally, total pollen were associated with mean temperature ( $p<0.001$ ), relative humidity ( $p=0.015$ ) and wind speed ( $p=0.042$ ).

## DISCUSSION

Allergic respiratory diseases, such as asthma and rhinitis, affect up to 40% of the global population [13]. Atmospheric pollen is the single most important inducer of allergies in susceptible individuals; thus, tight monitoring of phenological parameters is necessary to predict public risks. Plant pollination occurs on a seasonal basis, according to ecological and climatic factors that are specific for a region [14, 15].

Local vegetation patterns and geographic variables regulate phenological characteristics and dictate pollen dynamics. Aerobiological databases are therefore particularly useful in characterizing the start, peak and end of pollen season, and in alerting patients and physicians to take necessary precautions (e.g. avoid exposures, prescribe medications, etc.).

As the presented study shows, Oleaceae was the dominant taxon reaching a peak of 1,210 grains/m<sup>3</sup> in the beginning of May. This is no surprise as the area surrounding the sampling station is planted with olive trees to a radius of at least 15 km. *Olea europaea* pollen is highly allergenic, albeit its pollination

period was rather short, averaging only 27 calendar days. Other arboreal taxa, including Fagaceae (mainly *Quercus* spp.), Cupressaceae and Pinaceae that are less allergenic, extended their pollination time for longer periods.

Fagaceae were present in the atmosphere for 53 days, and peaked at the end of May with an average of 432 grains/m<sup>3</sup>. Pinaceae circulated for 79 days, with a peak of 134 grains/m<sup>3</sup> recorded in mid-April, whereas Cupressaceae reached a maximum of 310 grains/m<sup>3</sup> in the middle of March, and were present in the atmosphere for a total of 122 days (January – May). Poaceae dominated the herbaceous taxa, reaching a maximum of 184 grains/m<sup>3</sup> in mid-May. Along with Compositae, that peaked at 109 grains/m<sup>3</sup> in the end of August, they had the longest pollen season among all other taxa recorded, extending to 134 and 135 days, respectively.

In total, pollen taxa were detected in the atmosphere of NE Greece for 229 days, which corresponds to approximately 63% of the calendar year. Pollen circulation is generally low in autumn and the beginning of winter. Hence, allergic reactions recorded in sensitized individuals during this period must be due to other factors, including fungi, dust and mites. On the contrary, April and May were the months that pose the highest risk for pollen-induced allergies, with averaging total values of over 400 grains/m<sup>3</sup> in the atmosphere.

Compared to previous studies conducted in Greece, a significantly higher number of pollen were detected in the presented study [16, 17, 18]. However, the previous studies were conducted at least 10 years ago, and recent evidence show a tendency for an increase in pollen counts across Europe [19, 20]. This increase may be due to changes in climatic factors or other anthropogenic forces, such as urban or industrial pollution. Pollen maturation, rapture grain and atmospheric dispersal are generally thought to be affected by these variables; however, this may not always be the case [21, 22].

In the present study, an association of pollen counts with meteorological parameters was attempted. With the exception of rainfall, all other climatic factors, namely average daily temperature, relative humidity and wind speed, were significantly associated with total pollen counts. Cupressaceae were associated with relative humidity and Oleaceae with wind speed. Poaceae and Compositae were associated with both average temperature and relative humidity, and the latter was also associated with wind speed. Interestingly, no association with climatic factors was found for Fagaceae and Pinaceae.

Several experiments have been conducted that used modeling approaches, with varying degrees of success, the discussion of which is beyond the scope of this study [23, 24]. Nonetheless, the information provided here can serve as a reference for both on-going and future research. The correlation of environmental and clinical data can generate epidemiological allergy records that are unique for this region. Evidence on seasonal aeroallergen levels can help generate an alarm system for sensitized individuals and clinicians. Protection from exposure and prophylactic medication can reduce referral to medical centres by as much as 50%, thus reducing the cost of treatment [25, 26].



## CONCLUSIONS

Respiratory allergies are undoubtedly a major health burden that affects the quality of life of millions of people worldwide. However, due to the geographic zone, climate and ecological heterogeneities, a universal chart cannot apply, and regional monitoring systems are the only common denominator. Initiatives like this actually pay-off at the social, medical and economic scale; hence, an input from scientific and academic institutions specializing in aerobiology and population health is extremely valuable.

The presented study described the major pollen types circulating in the atmosphere of NE Greece, an area characterized by typical Mediterranean climate. Seasonal trends have been monitored and correlated with prevalent meteorological parameters. Future epidemiological studies will establish connections between respiratory allergies and pollen counts, which will allow the elucidation of environmental and clinical patterns coinciding with morbidity. This overlap of information is mandatory in order to better prepare medical interventions and to improve health indicators in the community.

## Acknowledgments

This study was funded by the Ministry of Education of Greece (General Secretariat for Youth/No: 4636/18-12-2012), but they had no role in the study design, data collection and analysis, preparation of the manuscript or decision to publish.

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