The clinical significance of the pollen calendar of the Western Thrace/northeast Greece region in allergic rhinitis

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Background: There are major differences in the clinical pattern of allergic rhinitis (AR) patients among countries, reflecting local aerobiological conditions. We analyzed the correlation between airborne pollen concentrations of the Western Thrace/northeast (NE) Greece region with symptoms scores in AR patients. The above data is the first provided for the Mediterranean climate of Greece and reflects the clinical significance of pollen calendar in everyday clinical practice.

Methods: An annual pollen calendar of the most important outdoor aeroallergens (grasses, trees, and weeds) was developed, using a Burkard volumetric spore trap. A total of 168 AR patients were studied and asked to evaluate their symptoms and main seasonal appearance. Sensitization prevalence to pollen species was detected by skin prick tests. Symptoms were evaluated by Total 5 Symptoms Score (T5SS) and correlated to aerobiological data.

Results: As far as the pollen calendar is concerned, the highest total percentages of pollens were recorded for olive (24.02% of total), oak (13.74%), grasses (9.08%), and cypress (7.63%). Regarding patients' sensitivities, the most prevalent ones were to grasses (56.0%), olive (43.5%), wall pellitory (24.4%), and cypress (16.7%) antigens. A strong significant correlation between total pollen counts and patients' T5SS ($r = 0.874$, $p < 0.001$) was observed. Moreover, strong significant correlations between T5SS and pollen counts were also found for the most prevalent species, including grasses, olive, and *Parietaria* allergens ($r = 0.627$, $p = 0.029$; $r = 0.695$, $p = 0.012$; and $r = 0.656$, $p = 0.021$, respectively).

Conclusion: Patients' symptoms scores were found to be significantly correlated to pollen counts. Given data are important for the management of AR patients who live in similar Mediterranean climate conditions.

Key Words:
allergic rhinitis; aeroallergens; pollen calendar; grasses; trees; weeds; sensitivities; skin prick tests; symptoms score; Burkard spore trap

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Allergic rhinitis (AR) represents an increasing worldwide health problem with differences in clinical pattern among patients from different countries, and a considerable socioeconomic cost.[1–5] An allergic reaction occurs when sensitized individuals inhale airborne allergens, such as typically tree, grass, weed pollens, and some molds,[6] with the intensity of symptoms depending on the density of aeroallergen circulation and on the duration of exposure.[7,8] Accordingly, allergic exacerbations caused by pollen exposure increase the social and economic burden of allergic diseases.[9] Thus, pollen monitoring is very important for the management of respiratory allergy. Because the allergenic content of the atmosphere varies not only among countries

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but even in the same country, according to climate (temperature, winds, rains, and humidity), geography, vegetation, and cultural factors,[10–12] pollen calendars have been constructed in many countries all over the world with a variety in kind and concentration of airborne pollens.[10–28] However, there is no recent data for Greece,[21–24] and no pollen calendar has been presented for the large region of Western Thrace (northeast [NE] Greece). Additionally, the correlation between symptom scores in AR patients and atmospheric pollen counts has not been adequately addressed in the literature and needs to be explored further, in order to estimate the clinical significance of pollen monitoring in everyday clinical practice.

Accordingly, the aim of this study was to explore the clinical significance of pollen species detected in a well-conducted pollen calendar concerning the Mediterranean climate conditions of Western Thrace/NE Greece, using records of symptoms in AR sufferers who lived in the same area. All the above constitute new information provided, important for the practicing clinician for the management of AR patients.

Patients and methods

Study population

Sensitivities to the most common implicated pollens in AR and symptoms scores were analyzed in a group of 168 AR patients of both sexes, who were examined and diagnosed over the year 2013 in the University Department of Otorhinolaryngology of the tertiary academic hospital of Alexandroupolis. All patients had lived permanently in the study area for at least the last 5 years. Diagnosis was based on history, nasal endoscopy, sinus computed tomography scanning, skin prick test (SPT) with a battery of common aeroallergens, and pulmonary function tests by spirometry. All patients were asked to evaluate their symptoms and express the seasonal appearance. Only subjects who fulfilled the criteria of AR according to the Allergic Rhinitis and its Impact on Asthma Guidelines (ARIA) guidelines[29] and were sensitized exclusively to pollen species were included. Patients sensitized to house dust mites, molds, or animal danders were excluded from this study. Other exclusion criteria were history of chronic rhinosinusitis; nasal polyps or malignancy; previous nasal operation; history of chronic urticaria/angioedema and/or symptomatic dermographism; immunotherapy treatment during the last 5 years; and use of oral or nasal corticosteroids 4 weeks prior to inclusion or oral H1 antihistamines 1 week prior to inclusion. 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; this evaluation happened when the patients were referred at the hospital. All symptoms were graded from 0 (absent) to 3 (very troublesome), with total scores ranging from 0 to 15 30. SPTs were performed and evaluated as described by the European Academy of Allergy and Clinical Immunology.[30] The allergen panel (SUBLIVAC®; HAL Allergy BV, Leiden, The Netherlands) consisted of the Pan European GA2LEN panel,[31] which contains common grasses, trees, and weeds aeroallergens already registered in the calendar. We used the Pan European GA2LEN panel[31] because we did not have data on species endemic to the region that could cause allergenic symptoms as this is the first record of aerobiological measurements in this area. Besides, the Pan European panel contains a broad spectrum of airborne allergens that cover the vast majority of allergenic pollens. From the clinical point, in our outpatient clinic we have not noticed patients with seasonal symptoms that cannot be attributed to the pollen species included in Pan European Panel. Finally, the accumulation of data from different studies with the same single panel of allergens provides the opportunity for comparative evaluation of sensitization rate and differences among studied populations. The study protocol was approved by the local Institutional Review Board. All subjects signed an informed consent form. The study was performed in accordance to the Declaration of Helsinki/Hong Kong.

Pollen calendar of Western Thrace/NE Greece

Records of pollen grains were made using a Burkard 7-day recording volumetric trap (Burkard Scientific Ltd., Uxbridge, UK), a standard equipment for aerobiologic sampling worldwide.[32–35] We used preliminary 2013 calendar year records, starting from January 1, 2013, to December 31, 2013. The trap was placed on the roof of the University Hospital of Alexandroupolis at a height of 20 m above the ground, according to instructions provided by the manufacturer.[32] Alexandroupolis was chosen as the largest city of the Western Thrace/NE Greece region with a typical Mediterranean climate, characteristic of the studied area. It is characterized by hot/dry summers, whereas winters exhibit moderate to low temperatures. Rainfall occurs mainly during winter months. There is an average daily temperature of 14.9°C, a relative humidity of 67.4%, and 549.3 mm of total precipitation per year. Its geographic location is 40°51′N and 25°52′E, situated 40 km away from the border with Turkey, 346 km from Thessaloniki, and 750 km from Athens.

The Burkard spore trap sampled airborne particles continuously for periods of up to 7 days without attention, collecting 10 L of air per minute. A strip of silicone-coated Melinex tape (Burkard) was exposed to air for trapping the spores and was changed once a week. The exposed tape was cut into 48-mm segments representing 24-hour periods. These segments were mounted on microscopic slides using Gelvatol mixed with stain (acid fuchsin) to enable visualization under a high-resolution light microscope (Olympus BX40) at ×400 magnification. Pollen grain counts were expressed as pollen grains per cubic meter of air and the pollen calendar was created according to the guidelines of the British Aerobiology Federation.[35]

Aeroallergens selected are the most frequently implicated in AR, not only in the Mediterranean and European context,[10–25] but also worldwide.[26–28] They include grasses (family [f.] Poaceae/Gramineae), trees (f. Oleaceae/olive, f. Cupressaceae/cypress, f. Pinaceae/pine, f. Fagaceae/beech, oak, f. Platanaceae/plane), and weeds (f. Urticaceae/wall pellitory, *Parietaria* spp). The pollen graph was constructed using a specific scale, each level corresponding to a particular amount of pollen grains. The levels included a total sum of pollen grains per 10 days as follows: 1st level: 1 to 2; 2nd level: 3 to 5; 3rd level: 6 to 11; 4th level: 12 to 24; 5th level: 25 to 49; 6th level: 50 to 99; 7th level: 100 to 199; 8th level: 200 to 399; 9th level: 400 to 799; 10th level: 800 to 1599; and 11th level: a sum of more than 1600 pollen grains per 10 days.[16] This way, all interactions between external factors and pollen concentrations were significantly reduced, enabling the comparison of different concentrations of pollen species throughout the calendar year.[20]

Statistical analysis

The 5% to 95% pollen count method was used to describe the main pollen season.[36] In brief, the start of the season was defined as the date when 5% of the seasonal cumulative pollen count was reached, and the end of the season as the date when 95% of the seasonal cumulative pollen count was counted. This period should include the beginning, peak, and end of clinical symptoms in allergic patients. Correlations between T5SS scores and pollen grain levels were examined using the Spearman rank correlation. Correlation coefficients and p values were obtained and considered to be statistically significant at the $p < 0.05$ level. SPSS (v 17.0; SPSS Inc., Chicago, IL) statistical package was used for all statistical analyses.

Results

The study group included 168 AR patients (mean ± standard deviation) (age: 29.6 ± 15.8 years; range, 5 to 66 years; median, 25 years); 81 (48.2%) males of age 27.0 ± 16.2 years, and 87 (51.2%) females of age 32.1 ± 15.1 years. They all suffered from seasonal AR, whereas 15 (8.9%) had asthma (uncontrolled in 2 patients, partially controlled in 3 patients, and controlled in 10). According to the SPTs, 81 patients (48.2%) were monosensitized and 87 patients (51.8%) were polysensitized to pollens. Overall, the most prevalent sensitizations were found to grasses (56.0%), olive (43.5%), and wall pellitory (24.4%) pollens. Other less common sensitivities were detected to cypress (16.7%), pine (11.3%), oak/beech (11.3%), and plane (7.7%). All patients' sensitivities are presented in Table 1.

Based on symptom scores and seasonality as estimated and described by patients, mean values of T5SS for the total study group and for the patients with the most important pollen sensitivities, per calendar month are presented in Table 2. Higher T5SSs were detected during periods from March to May, either in total or in patients sensitized to grasses, olive, and *Parietaria* antigens (Table 2). There was a higher overall score for nasal congestion, rhinorrhea, and sneezing symptoms in the total study group (Table 2).

TABLE 1. Patients' sensitizations to pollens presented as percentages of patients examined and gender distribution

		Gender distribution	
Pollen	Sensitization, n (%)	Men, n (%)	Women, n (%)
Grasses (Poaceae)	94 (56.0)	40 (42.6)	54 (47.4)
Olive (Oleaceae)	73 (43.5)	33 (45.2)	40 (54.8)
Wall pellitory (Urticaceae)	41 (24.4)	21 (51.2)	20 (48.8)
Cypress (Cupressaceae)	28 (16.7)	11 (39.3)	17 (60.7)
Pinus (Pinaceae)	19 (11.3)	6 (31.6)	13 (68.4)
Oak, Beech (Fagaceae)	19 (11.3)	5 (26.3)	14 (63.7)
Plane (Platanaceae)	13 (7.7)	2 (14.3)	12 (85.7)
Others[a]	18 (10.7)	7 (39.9)	11 (61.1)

[a] Ragweed, mugwort (Compositeae), and goosefoot (Chenopodiaceae).

Pollen calendar

In total, 5 arboreal taxa (f. Oleaceae/olive, f. Pinaceae/pine, f. Platanaceae/plane, f. Fagaceae/oak, f. Cupressaceae/cypress) and 4 non-arboreal (f. Poaceae/grasses, f. Urticaceae/wall pellitory, *Parietaria* spp, f. Compositeae/ragweed, mugwort, f. Chenopodiaceae/goosefoot), the most common implicated in respiratory allergy symptoms worldwide, were counted and presented in this study. The graphical presentation of the concentrations of the 9 most important families of trees, grasses, and weeds in Western Thrace/NE Greece are given in the pollen calendar (Fig. 1).

During the year 2013, the principal aeroallergen pollination period ran from March to August with values representing 94.81% of the total pollen content in the atmosphere (Table 3). The highest concentrations of pollens were found during April (28.37% of total pollens) and May (41.68%) (Table 3). For the seasonal distributions, 3 main pollination periods can be distinguished: winter/pre-spring (January to March) for the Cupressaceae taxon; spring/pre-summer (April to June) for the Poaceae, Urticaceae, Oleaceae, Fagaceae, Pinaceae, and Platanaceae taxa, and summer/pre-autumn (July to September) for the Compositeae and Chenopodiaceae taxa. Monthly distribution and total percentage of selected taxa, as well as their allergenic potential according to Grant-Smith[37] are presented in detail in Table 3.

From a clinical standpoint, according to the 5–95% prediction system,[36] results are presented in details in Table 4. As we can see, the highest levels of pollen grains in the atmosphere of Western Thrace/NE Greece occurred for all 7 taxa from March to May, whereas for the remaining

TABLE 2. T5SS in total study group and in patients sensitized to the most significant pollens per calendar month*

	January	February	March	April	May	June	July	August	September	October	November	December
Grasses	7.0 ± 1.0	6.5 ± 0.7	10.0 ± 2.6	11.6 ± 1.3	12.1 ± 2.2	9.4 ± 1.0	7.5 ± 0.6	8.0	9.6 ± 1.3	7.0 ± 1.2	8.0	5.7 ± 0.5
Olive	6.0	6.8 ± 0.5	11.6 ± 1.0	10.8 ± 1.2	11.6 ± 1.9	8.2 ± 1.1	8.0	9.0	9.4 ± 1.4	8.0	8.0	5.5 ± 0.6
Parietaria	10.0	7.0	10.7 ± 1.4	12.2 ± 1.2	10.0	8.6 ± 0.5	7.0	8.0	8.5 ± 0.6	6.0	6.0	6.0
Total	6.7 ± 1.8	6.5 ± 0.5	10.3 ± 2.0	11.2 ± 1.8	12.1 ± 2.0	8.6 ± 1.3	7.5 ± 0.6	8.6 ± 0.5	9.3 ± 1.4	6.8 ± 0.9	8.0 ± 2.0	5.8 ± 0.5
Rhinorrhea	2.0 ± 0.5	2.7 ± 0.5	2.8 ± 0.4	2.9 ± 0.3	2.9 ± 0.4	2.0 ± 0.7	2.0 ± 0.0	1.8 ± 0.5	2.6 ± 0.5	2.5 ± 0.5	2.3 ± 0.4	2.0 ± 0.0
Sneezing	2.0 ± 0.9	2.5 ± 2.7	2.9 ± 0.5	2.6 ± 0.7	3.0 ± 0.0	2.4 ± 0.5	2.0 ± 0.7	2.0 ± 1.8	2.6 ± 0.5	2.5 ± 0.7	1.9 ± 0.8	1.0 ± 0.0
Itchy nose	0.4 ± 0.5	0.2 ± 0.4	1.1 ± 0.7	1.6 ± 0.8	1.8 ± 1.0	0.9 ± 0.4	0.6 ± 0.5	0.75 ± 0.5	0.7 ± 0.7	0.4 ± 0.5	0.3 ± 0.4	0
Nasal congestion	2.4 ± 0.5	2.7 ± 0.0	2.9 ± 0.3	3.0 ± 0.2	3.0 ± 0.2	2.8 ± 0.9	2.8 ± 0.4	2.8 ± 0.5	3.0 ± 0.0	2.6 ± 0.5	2.7 ± 0.5	2.8 ± 0.5
Itchy eyes	0.4 ± 0.5	0.2 ± 0.4	0.9 ± 0.9	1.1 ± 0.7	1.6 ± 1.0	0.7 ± 0.6	0.4 ± 0.8	1.0 ± 0.8	0.4 ± 0.5	0.1 ± 0.3	0.3 ± 0.4	0

*Values are mean ± SD.
SD = standard deviation; T5SS = Total Five Symptoms Score.

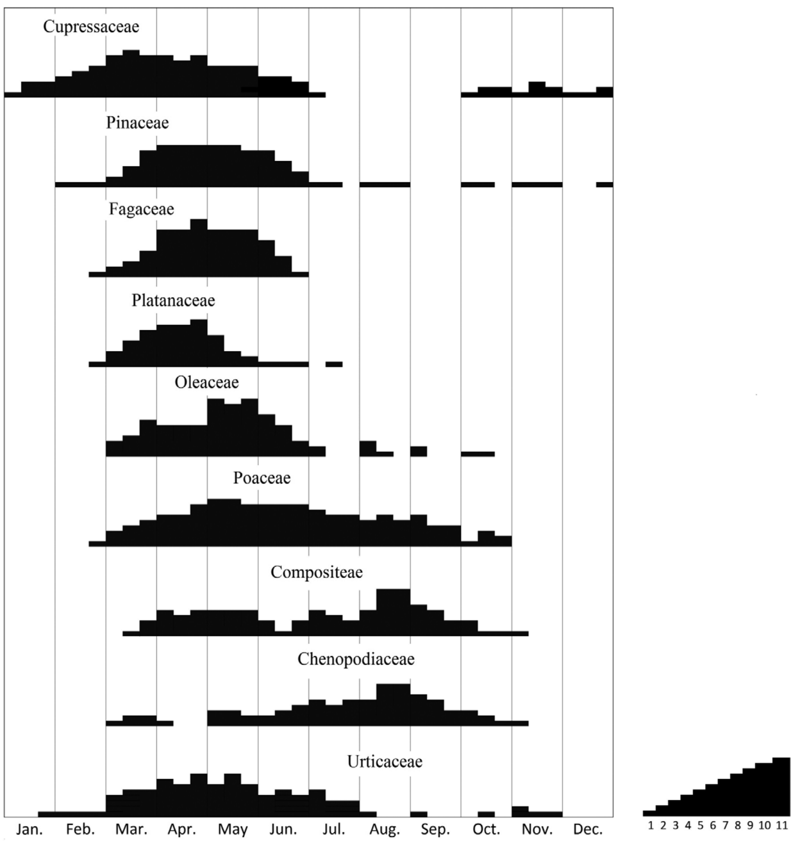

FIGURE 1. Pollen calendar of Western Thrace/northeast Greece in 10-day exponential scale.

2 taxa (ragweed/mugwort and goosefoot) the highest levels occurred in August. May was the month with the highest total concentrations of airborne pollen (4 taxa) with 1902 pollen grains/m^3 of air (Table 4). This was followed by March (311 pollen grains/m^3 of air) and then April (253 pollen grains/m^3 of air) (Table 4). The highest total amount of pollen grains documented for the whole year was 8153 grains for olive and the lowest was 808 grains for goosefoot. As far as separate taxa concentrations are concerned, Oleaceae (olive) was the taxon with the highest pollen count (8153 pollen grains/m^3), followed by Fagaceae (oak/beech) and Poaceae (grasses) (4666 and 3083 pollen grains/m^3, respectively). Cupressaceae was the taxon with the longest pollen period, with a total of 2590 pollen grains/m^3. Annual values for all other taxa were significantly lower (Table 4).

TABLE 3. Monthly distribution and total percentage of selected taxa and their allergenic potential

Pollen type	January	February	March	April	May	June	July	August	September	October	November	December	Total (%)	Peak concentration (pollen grains/m^3)	Allergenic potential[a]
f. Oleaceae (olive)	–	–	0.38	0.82	22.26	0.56	–	–	–	–	–	–	24.02	8 May	+++, ++
f. Fagaceae (oak)	–	–	0.19	7.49	5.58	0.48	–	–	–	–	–	–	13.74	27 May	++
f. Poaceae (grasses)	0.06	–	0.21	1.13	3.72	2.42	0.80	0.42	0.28	0.05	–	–	9.08	15 May	+++, ++
f. Cupressaceae (cypress)	0.58	0.2	3.9	1.95	0.78	0.12	–	–	–	0.02	0.04	0.02	7.63	15 March	++
f. Pinaceae (pine)	0.04	0.01	0.41	2.74	2.26	0.35	–	0.02	–	0.02	0.01	–	5.86	11 April	+
f. Compositeae (mug-wort/ragweed)	0.02	–	0.03	0.23	0.32	0.05	0.20	3.23	0.33	0.03	–	–	4.43	24 August	+
f. Platanaceae (Plane)	–	–	0.49	2.56	0.18	–	–	–	–	–	–	–	3.23	26 April	++
f. Urticaceae(wall pellitory)	–	–	0.26	1.21	0.99	0.24	0.11	–	–	–	0.15	–	2.96	15 May	++, +
f. Chenopodiaceae (goosefoot)	–	–	0.03	–	0.06	0.08	0.23	1.49	0.44	0.03	–	–	2.38	22 August	++, +
Others[b]	0.42	0.12	0.96	10.38	5.56	2.17	3.24	1.81	0.89	0.48	0.39	0.25	26.67		
Total	1.41	0.33	6.74	28.37	41.68	6.42	4.61	6.99	1.96	0.63	0.60	0.27	100		

[a]According to Grant-Smith[37] (1990): +++ = high; ++ = medium; and + = low.
[b]f. Betulaceae, f. Salicaceae, f. Aceraceae, f. Ulmaceae, f. Plantaginaceae, etc.

TABLE 4. Dates of onset, peak, and end of aeroallergen pollination season*

Pollen type	Onset date (5%)	Peak date	End date (95%)
Cypress (Cupressaceae)	23 January (136)	15 March (311)	24 May (2590)
Pine (Pinaceae)	29 March (105)	11 April (134)	5 June (1990)
Oak, beech (Fagaceae)	5 April (246)	27 May (432)	27 May (4666)
Plane (Platanaceae)	19 March (58)	26 April (119)	30 April (1097)
Olive, ash (Oleaceae)	1 May (429)	8 May (1210)	27 May (8153)
Grasses (Poaceae)	8 April (162)	15 May (184)	19 August (3083)
Ragweed, mugwort (Compositeae)	25 April (79)	24 August (109)	6 September (1505)
Goosefoot (Chenopodiaceae)	13 June (43)	22 August (52)	9 September (808)
Wall pellitory (Urticaceae)	20 March (53)	15 May (76)	31 July (1006)

*The actual values (pollen grains/m^3) for 5%, peak, and 95% of total counts are given in brackets.

Correlations between symptoms and pollen counts

A strong significant correlation between total pollen counts in the atmosphere and T5SS was found (Spearman's rank correlation, $r = 0.874$, $p < 0.001$) (Fig. 2). Moreover, a strong positive correlation was also observed between symptoms in AR patients being sensitized to grasses, olive, and Parietaria antigens and the respective pollen counts ($r = 0.627$, $p = 0.029$ for grasses; $r = 0.695$, $p = 0.012$ for olive; and $r = 0.656$, $p = 0.021$ for *Parietaria* spp).

Discussion

It is widely accepted that differences in climate may act alone or in concert with other parameters in different aeroallergenic patterns among regions, which in turn impacts the prevalence or severity of allergic illness via sensitization and response pathways.[8–12] The unique aerobiological conditions of an area clearly affect the clinical manifestations of respiratory allergy and explain the great

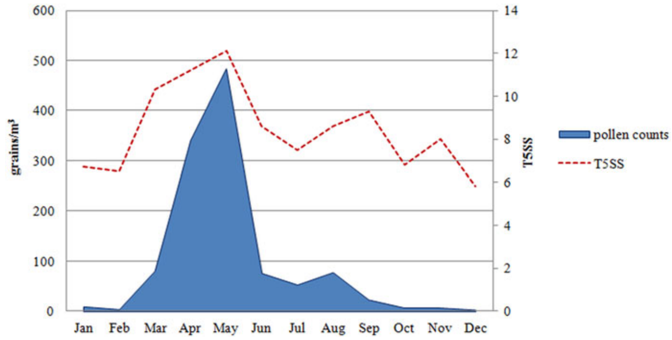

FIGURE 2. Average pollen counts (grains/m^3) and T5SS. T5SS = Total 5 Symptoms Score.

number of studies for pollen concentrations, even within the same country.[12–28] Therefore, the knowledge of the pollen calendar of a region is important both for the patients in terms of taking personal preventive measures, and for the clinicians in terms of diagnostic and therapeutic implications. However, the interpretation of pollen counting into clinically meaningful information for both patients and doctors needs to be further explored. This is the reason why registered airborne allergen concentrations of the most important pollen species[10–12,21–24] are correlated to symptoms in AR patients who live in the same area for a long time period, spanning more than 5 years. Moreover, this study represents the first conducted in Greece since 2004, and the only one that presents aerobiological data for the Western Thrace/NE Greece region, an area with Mediterranean climate conditions, which makes the results useful not only in Greece but also beyond its borders.

The first clinically important point to mention regarding pollen monitoring was that certain pollen species were detected throughout the year with values greater than 43 pollen grains/m^3. Previous studies have shown that 20 pollen grains/m^3 is usually sufficient for the onset of respiratory allergy symptoms in sensitized individuals.[38] This clinically means that aeroallergens are permanently found in the atmosphere, albeit in low concentrations, and they may still cause clinical disease in sensitized individuals. Generally, in our region 3 main pollination periods were distinguished: winter/pre-spring (January to March) for Cupressaceae taxon; spring/pre-summer (April to June) for Poaceae, Urticaceae, Oleaceae, Fagaceae, Pinaceae, and Platanaceae taxa, and summer/pre-autumn (July to September) for Compositeae and Chenopodiaceae taxa. The highest concentrations of pollens were found during April and May. We are the first to observe a longer duration of the pollination period (March to August) compared to previous studies in Greece (March to June).[23,24] There is also a shift of peak periods toward the late spring and early summer months (April, May, and June) with higher peak concentrations for most of the trees and grasses species. Similar findings regarding the main pollination period were observed in a study of Kizilpinar et al.[26] from the neighboring region of Turkey. However, there were differences in counting. All these differences among regions, although with similar climate conditions, over time may be partly attributed to climate changes, flowering seasons, vegetation, etc. Therefore, from a clinical standpoint, these findings are of great importance and add new knowledge to what is already known for pollen periods generally in Mediterranean areas and specifically in Greece. This elongation of the pollination period in Greece, since 2004, may be at least partly attributed to climate change and suggests that aerobiological conditions change with the potential for increased concentrations of allergenic pollen per season, and even wider distribution in the atmosphere. In more detail, we observed that Oleaceae (olive) was the taxon with the highest amount of pollen (8153 pollen grains/m^3), representing 24% of the total pollen concentration in the atmosphere. Oleaceae is the taxon with the highest allergenic potential as described by Grant-Smith[37] and was detected mainly in May, peaking during the first week of that month. Indeed, olive (*Olea europaea*) pollen is considered as 1 of the most important causes of AR in the Mediterranean region.[19,20] Previous studies from Spain,[16–18] Italy,[13,14] Greece,[21–24] and Turkey[26] confirmed olive pollen as the most important cause of pollinosis. Although there were small differences in the duration of olive pollination period among studies, we detected Oleaceae in significantly higher concentrations than previous studies, a finding that strengthens the allergenic potential of this taxon in the studied area. This was followed by 2 taxa, Fagaceae (oak/beech) (April to May) and Poaceae (grasses) (April to August), both with high allergenic potential[37] and both with values reaching their peak in May. Indeed, grass pollen has been found to be the major cause of pollinosis in many parts of the world and also in Europe.[20] Previous studies from Greece[21–24] have shown that the pollination period of herbaceous grasses lasted approximately 4 months (March to June). However, nowadays the pollination period for grasses is even longer (March to August) and their pollens are in higher total concentrations in the atmosphere. All other taxa (Cupressaceae, Platanaceae, Urticaceae, Chenopodiaceae, Pinaceae, and Compositeae), were detected in lower amounts. Furthermore, it is interesting to mention that Cupressaceae had the longest pollination period among all studied taxa (January to May), even longer compared to similar records from Italy or Spain.[13–17] Referring to Urticaceae, the pollination period in Greece seems different (March to July) compared to Italy (August to September).[13,14] Differences in the prevalence of aeroallergens may affect skin sensitivities, severity of symptoms, time of symptoms appearance, and eventually, for doctors, the time of starting treatment, the allergen used for sublingual immunotherapy, and the time for patients to take preventive measures. This is the reason why pollen maps are considered clinically useful tools, both for patients to take precautions and for doctors to adjust treatment.

The clinically important question that now should be answered is how all the above aerobiological data are correlated to clinical pattern of AR sufferers. In order to answer this question we detected sensitization prevalence to pollens, during the period of the pollen study, in a significant number of patients suffering from AR, who lived in the Western Thrace/NE Greece region for more than 5 years. The allergens selected were the most frequently implicated in AR and of species that were counted in the atmosphere. We clearly observed that the frequency of SPT sensitization did not necessarily present a positive relationship with pollen grain concentrations in the air. Therefore, although many AR patients presented a high SPT sensitivity to grasses (56.0%) and wall pellitory (24.4%), pollen concentrations were ranked lower in aeroallergen circulation in the studied area. On the contrary, SPT sensitivity to oak was only 11.3%, despite the fact that it is the second most frequently detected aeroallergen in the region. In a study by Gioulekas et al.[24] from the neighboring region of Thessaloniki, however, 10 years before, sensitizations to grasses (40.4%), olive (31.8%), and wall pellitory (15.3%) were mostly observed, as in our study group, but presented a significantly lower prevalence than in our region. Similarly, differences were observed in a study by Terzioglu et al.,[39] who reported a high sensitization to Parietaria pollen (52%) in the Aegean region of Turkey, which has a typical Mediterranean climate. In another study from Turkey,[40] *Olea europaea* was reported to be an important allergen. D'Amato and Lobefalo[41] show that skin sensitization to different pollens is changing from area to another area in Mediterranean region. The results suggested that sensitization to pollens in respiratory system allergies might be related to differences in the macroenvironmental (ie, climate) and microenvironmental (city or rural area) characteristics of an area.

As far as patients' symptoms are concerned we observed that the highest T5SSs were detected not only during the main pollination period (April and May), but also a month earlier, namely in March. This means that according to patients' evaluations, the symptoms period was longer than the main pollination period as indicated by the pollen calendar. The most troublesome symptoms were proven to be the nasal ones (nasal congestion, rhinorrhea, and sneezing, consecutively). Additionally, an important overall finding that emphasizes the clinical significance of the pollen calendar was the finding that total airborne pollen concentrations were strongly correlated to T5SS of AR patients ($r = 0.874$, $p < 0.001$). More specifically, concerning the most important pollens, a strong positive correlation between patients' symptoms and respective pollen counts in the atmosphere for grasses ($r = 0.627$, $p = 0.029$), olive ($r = 0.695$, $p = 0.012$), and Parietaria ($r = 0.656$, $p = 0.021$) species was observed. This finding is in accordance with previous studies of D'Amato et al.[38] and Brito et al.,[42] who found significant correlations between pollen counts and symptoms for *Parietaria* spp and grass pollen species, respectively. Our findings emphasize the clinical significance of pollen calendar in daily clinical practice as far as symptoms are concerned. However, patients' symptoms occur throughout the year, leading us to the hypothesis that pollen grains can rupture and release particles that enter the respiratory system, even during periods when pollen grains are absent. This comes in agreement with a recent work of Brito et al.,[42] and might also be explained by the minimal persistent inflammation concept, especially if the patient has not been treated properly during the high-pollen season.[43] Further long-term studies need to be conducted in order to determine the correlation of the onset, peak, and end of symptoms in each patient to specific allergens and pollen counts.

Conclusion

In the present study, for the first time the clinical significance of the pollen calendar of Western Thrace/NE Greece, an area with Mediterranean climate conditions, was explored. The most frequently detected pollen grains were olive, oak/beech, grasses, and cypress, whereas most of the pollens reached higher concentrations and had longer pollination periods compared to previous data from at least a decade ago. The clinical importance of pollen monitoring was proved by the finding that total pollen concentrations were significantly correlated to patients' symptoms. Moreover, significant correlations between olive, grass, and *Parietaria* pollens and symptoms scores in AR patients' was observed. Our clinical data allow us to support the clinical significance of pollen calendars in everyday clinical practice.

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