



Pollen season identification for three pollen taxa in Thessaloniki, Greece: a 30-year retrospective analysis

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Received: 5 September 2018 / Accepted: 26 August 2019
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Abstract To examine the applicability of the recently proposed criteria by the European Academy of Allergy and Clinical Immunology (EAACI) for pollen season (PS) definition, we applied them to a 30-year time series of daily concentrations of cypress (Cupressaceae), olive (Oleaceae), and grass (Poaceae) airborne pollen from a Mediterranean city, Thessaloniki, Greece. These criteria led to PS definition for every year in the case of grasses, but they did not result

to a defined pollen season in each year for 10% of the examined period in the case of cypress (Cupressaceae) and even for 60% in the case of olive (Oleaceae). Application of the EAACI thresholds to data series of Thessaloniki shows the need for an accurate analysis and revision of the symptom thresholds for PS definition for olive and cypress in the Mediterranean area. Given that the PS, as defined after the EAACI criteria, corresponds to only the part of the PS inducing allergy symptoms, a different term ‘allergy symptom-inducing pollen season’ is proposed so as to avoid confusion but also make clear that, depending on the purpose of the study, different criteria may be used for PS definition.

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Keywords Allergy symptom-inducing pollen
season · Allergic rhinitis · Cypress · EAACI criteria ·
Grasses · Mediterranean · Olive

1 Introduction

Knowledge of the start, duration, and end of the pollen seasons is of paramount importance for the management of allergic diseases, such as allergic rhinitis and asthma, and plays a key role in medical praxis like in the case of nasal allergen challenges (Augé et al. 2018) and in allergen immunotherapy (Pfaar et al. 2018). It is equally important for other purposes like for assessing the effect of climate change on plants.

Several approaches have been proposed for the definition of a taxon's pollen season start and end. Definitions differ according to their aim, for instance, to get an indicator of flowering phenophases, describing the time period when pollen is present in the air, or to define the period in which symptoms are expected to occur in sensitized persons. In the first case, the start and end are usually defined after the pollen season (PS) ends, since they are proposed to correspond to percentages of the annual sums of pollen concentration like 5% and 95% (Nilsson and Persson 1981) or 2.5% and 97.5% (Andersen 1991) or 1% and 99% (Galán et al. (1995), respectively, as is also the case for the definition of flowering times (Blionis et al. 2001; Petanidou et al. 1995). However, this percentage approach may not be useful when aiming to describe the period within which symptoms are manifested. Definition of a taxon's pollen season based on a concentration threshold that holds for a number of consecutive days has been applied for PS definition with symptoms in mind. For instance, the pollen season for Poaceae (Sánchez-Mesa et al. 2003; Giorato et al. 2000) is proposed to be the period within which the daily pollen concentration exceeds 30 pollen grains (pg) m⁻³, and similarly for Urticaceae, whereas for other families, the threshold is set to 20 pg m⁻³ (Giorato et al. 2000). Taking explicitly symptom manifestation into consideration, the European Academy of Allergy and Clinical Immunology (EAACI) developed a set of criteria for PS definition focusing to describe 'the time period in which placebo- and active-treated groups in allergen immunotherapy (AIT) studies should be compared' (Pfaar et al. 2018).

Although the pollen presence in the air is a measurable variable, pollen season is a devised one and depends on the purpose of a study. From a plant-ecology point of view or for statistically oriented reasons or for forecasting and other modeling purposes, a PS definition that is based on pollen presence is generally advantageous. On the other hand, if the allergic population is the focus, then it is preferable to define the pollen season in relation to allergic symptom manifestation. Recently, Bastl et al. (2018) concluded that both types of approach are legitimate, but stressed the need for accurately defining pollen seasons in either case depending on the goals of the study.

From the allergological point of view, the analysis of a 3-year grass pollen concentrations' dataset, coming from 40 pollen monitoring stations in Germany (Karatzas et al. 2018a), proved the robustness of the EAACI criteria for pollen season definition of this taxon (Pfaar et al. 2017). In addition, a thorough analysis of 3-year data from the Berlin-Brandenburg area in Germany (Karatzas et al. 2018b) showed that these criteria mirror symptom scores for grass and birch pollen-induced rhinitis. Moreover, a detailed study in three additional geographic areas in Europe (in Austria, Finland, and France) for the aforementioned three-year period, provides evidence that the EAACI criteria are effective and can be used in these four countries, while they are the best suggestion for European countries, in terms of AIT studies and management of Allergic Rhinitis (AR) symptoms (Pfaar et al. 2019). Nevertheless, the efficiency of these criteria in defining the pollen season still needs to be examined in an even wider range of geographic areas and climatic regimes as initially suggested by Pfaar et al. (2017), and also for pollen data corresponding to longer periods.

Climatic conditions of the Mediterranean region, being characterized by mild winters and hot and dry summers, are different from those in other parts of the European continent. A taxon's pollen phenological and quantitative features may differ from region to region as a result of differences in the flowering season and of the taxon's representation, distribution, and abundance. In fact, Smith et al. (2014) showed that pollen concentrations for Betulaceae, Oleaceae, Poaceae, and Asteraceae differ in different parts of Europe and so did Sikoparija et al. (2017) for *Ambrosia* pollen. Given that, we examined the applicability of the EAACI criteria for the definition of the pollen season to three of the five pollen taxa suggested by Pfaar et al. (2017). These are taxa with allergenic pollen (Gioulekas et al. 2004) at high concentrations in the air of Thessaloniki (Damialis et al. 2007; Charalampopoulos et al. 2018), with representatives typically found in Mediterranean areas. For this analysis, we employed a time series one order of magnitude longer (i.e. 30 years long) compared to the one used in a previous analysis for PS identification according to the EAACI criteria (Pfaar et al. 2017) in the temperate-climate Germany (Karatzas et al. 2018b).

Table 1 Definition of pollen seasons for cypress, olive and grasses according to the EAACI position paper (Pfaar et al. 2017)

Taxa	Pollen season
Cypress (Cupressaceae)	Start: 1st day of 5 days (out of 7 consecutive days), each of these 5 days with ≥ 20 pollen/m ³ and with a sum of these 5 days of ≥ 200 pollen/m ³
Olive (Oleaceae)	End: last day of series of 5 days (out of 7 consecutive days), with ≥ 20 pollen/m ³ and with a sum of these 5 days of ≥ 200 pollen/m ³
Grasses (Poaceae)	Start: 1st day of 5 days (out of 7 consecutive days), each of these 5 days with ≥ 3 pollen/m ³ and with a sum of these 5 days of ≥ 30 pollen/m ³ End: last day of series of 5 days (out of 7 consecutive days), with ≥ 3 pollen/m ³ and with a sum of these 5 days of ≥ 30 pollen/m ³

2 Materials and methods

2.1 Study area

Thessaloniki is the second largest city in Greece. Located in the Central Macedonia region, it is an urban agglomeration with more than 1,000,000 inhabitants. The city is bordered by the sea to the south and southwest (Thermaikos Gulf), the Chortiatis Mountain to the southeast, and the Seich-Sou forest to the northeast. It has a Mediterranean-type climate with hot, dry summers (yet with some thunderstorms) and mild, wet winters, with some morning frost events. The annual mean temperature is 15.6 °C while mean relative humidity ranges from 53.2% (July) to 78% (December), with an annual mean rate of 67.3% (HNMS 2018). Thessaloniki has been an urban center for 2500 years, but in the recent years, its sprawl has been accompanied by a decrease of green spaces. It is estimated that there are on average 2.6 m² of green-space per inhabitant (Vlachokostas et al. 2014).

2.2 Pollen data

Airborne pollen has been collected by use of a 7-day Hirst-type volumetric trap (Hirst 1952), placed on the roof of a building in the center of the city, during the period 1987–2016. Pollen monitoring and microscopic pollen identification were performed after the standard guidelines of the European Aerobiology Society (Galán et al. 2014). Results are expressed as mean daily pollen concentrations (number of pollen grains per m³ of air per day). Although the procedure is not automated, collected data should be considered quite homogeneous because it was elaborated and

implemented by few, highly experienced scientists for the whole 30-year period. The dataset is complete and error-free; the years 2011 and 2015 were excluded from the analysis, as there were large gaps because of machinery failure.

2.3 Pollen season identification criteria

The EAACI criteria (Pfaar et al. 2017) for pollen season (PS) definition were applied on the daily pollen data, for the whole period under investigation, for grasses (Poaceae), olive (*Olea* sp.), and cypress (*Cupressus* sp.) (Table 1). Cupressaceae pollen ranks first in the pollen spectrum of Thessaloniki among the woody taxa and overall, grasses rank second among the herbaceous taxa (after Urticaceae), whereas olive pollen ranks fifth among the woody taxa (Charalampopoulos et al. 2018; Damialis et al. 2007). We did not apply these criteria on the data for *Betula* and *Ambrosia*, which complete the set of taxa studied by Pfaar et al. (2017), because they have low and very low representation, respectively, in the vegetation of the study area and in its surroundings, and very low airborne pollen concentrations recorded throughout the study period. According to Pfaar et al. (2017), the definition for cypress pollen is only valid for Mediterranean areas, where *Cupressus* species are the dominant Cupressaceae representatives. The reason for this clarification is because it is not feasible to distinguish *Cupressus* pollen from that of the other Cupressaceae genera via optical microscopy. *Cupressus* is indeed the dominant representative of the family in Thessaloniki, but we must note that under the common name cypress, pollen from all Cupressaceae is considered in this paper.

Similarly, pollen from *Olea* dominates Oleaceae pollen in the study area. More precisely, Charalampopoulos et al. (2018) recorded at least ten times more pollen from *Olea* than from the other Oleaceae representatives in the air of Thessaloniki. Given this, we used an approach similar to the one for *Cupressus*/Cupressaceae: under the common name olive, pollen from all Oleaceae representatives is considered in this paper.

2.4 Data analysis

We applied the EAACI PS criteria (Table 1), calculated the PS start and end events for the three taxa, for every year of the study period, and identified the cases (taxon-year) for which no PS could be defined. We used regression analysis to examine whether there are trends of change for the PS features of these three pollen taxa and we used the PS data to make pollen calendar plots for all years of the study period.

We also examined how much different are the pollen season features of these three taxa when using the 30-year dataset and applying the EAACI criteria from those published earlier (Damialis et al. 2007; Gioulekas et al. 2004) based on shorter datasets (18 and 15 years, respectively) and on the percent-of-the-total-pollen approach for defining pollen seasons in the area of Thessaloniki.

3 Results

Descriptive statistics of the pollen concentrations for the three taxa under investigation are given in Table 2.

Table 2 Descriptive statistics of the three pollen taxa under investigation for the period 1987–2016 (no data available for the years 2011 and 2015); minimum values for all three taxa are equal to 0

Pollen taxon	Pollen grains m ⁻³ day ⁻¹	
	Average value $\pm$ SD	Maximum value
Cypress	11.80 $\pm$ 75	2627
Olive	2.00 $\pm$ 9.6	452
Grass	1.43 $\pm$ 4.3	76

Values per day refer to the entire year (365 days), not only to the pollen season of each taxon

Cypress has the highest average and maximum values of airborne pollen concentration.

The PS start and end dates and duration per pollen taxon and year are presented in Table 3. Cypress has the earliest PS starting date, in the second half of February, whereas olive has the latest, in mid-May; PS for grasses starts in the first half of May. Regarding PS duration, cypress and grasses are of the same length, as their seasons last around 40 days; olive has a much shorter PS duration, only 14 days, on average.

Regression analysis showed no significant trend of change for the start of the pollen season for any of the three pollen taxa (Fig. 1); regarding its duration, a significant trend for longer seasons was observed in the case of cypress ($p = 0.003$). Results regarding the pollen season characteristics are visualized in Fig. 2, which contains the pollen calendars of the three taxa for all years of study. Application of the EAACI criteria could not identify a pollen season for cypress, for three of the 28 years examined (1988, 1989, 1991), and for many more years (1987–1991, 1994–1995, 1998, 2001–2003, 2005, 2009–2010, 2013–2014, 2016), 17 in total, for olive. In contrast, a pollen season was defined for every year in the case of grasses.

4 Discussion

Application of the EAACI criteria for a 30-year period on data for grasses from Thessaloniki, Greece, could define the pollen season for all years of study, as it was also the case for grasses from Germany (Karatzas et al. 2018b). In contrast, it did not result to a defined pollen season in each year for 10% of the period under consideration in the case of cypress and for most of the period (60%) in the case of olive. The EAACI criteria that describe the pollen season as a function of threshold pollen values and number of consecutive days with concentrations above these thresholds do not seem adequately applicable on data for these two taxa from Thessaloniki. These criteria should be tested on data from other areas so as to examine if this result is a peculiarity of only the specific site or if it holds true in other locations as well.

Accurate pollen season definition with respect to symptom manifestation is important for clinical praxis and clinical trials. We do not have data on the manifestation of allergy symptoms in the area of

Table 3 Pollen season (PS) start and end dates and duration per year for the examined pollen taxa

Year	Cypress			Olive			Grasses		
	PS start (Date)	PS end	PS duration (days)	PS start (Date)	PS end	PS duration (days)	PS start (Date)	PS end	PS duration (days)
1987	11-Feb	25-Feb	14				17-May	15-Jun	29
1988							11-May	28-May	17
1989							13-Jun	27-Jun	14
1990	25-Feb	4-Mar	7				10-May	28-May	18
1991							22-May	29-Jun	38
1992	28-Feb	19-Mar	20	30-May	9-Jun	10	2-Jun	8-Jun	6
1993	23-Apr	30-Apr	7	28-May	5-Jun	8	21-May	1-Jun	11
1994	4-Mar	10-Mar	6				19-May	6-Jun	18
1995	16-Feb	22-Feb	6				27-May	3-Jun	7
1996	2-Mar	11-May	70	3-May	5-Jun	33	3-May	28-Jun	56
1997	10-Feb	11-May	90	23-May	4-Jun	12	1-May	4-Jul	64
1998	12-Feb	18-Feb	6				11-Apr	20-Jun	70
1999	1-Mar	17-Mar	16	15-May	6-Jun	22	11-Apr	3-Jul	83
2000	2-Mar	30-Apr	59	19-May	30-May	11	5-May	15-Jun	41
2001	7-Feb	7-Mar	28				10-May	4-Jun	25
2002	20-Feb	7-Mar	15				12-May	20-Jun	39
2003	30-Mar	5-Apr	6				7-May	29-Jun	53
2004	26-Feb	15-Apr	49	23-May	4-Jun	12	6-May	5-Jul	60
2005	22-Feb	1-Apr	38				30-Apr	4-Jul	65
2006	26-Feb	24-Apr	57	23-May	6-Jun	14	12-May	20-Sep	131
2007	8-Feb	6-May	87	27-May	5-Jun	9	30-Apr	7-May	7
2008	20-Feb	24-Apr	64	15-May	2-Jun	18	26-Apr	30-Jun	65
2009	24-Feb	15-May	80				9-May	16-Sep	130
2010	25-Feb	30-Mar	33				12-May	5-Jul	54
2011	Pollen data not available								
2012	19-Feb	4-May	75	13-May	23-May	10	12-May	26-May	14
2013	8-Feb	15-Mar	35				9-May	21-May	12
2014	1-Feb	3-Apr	61				8-May	28-May	20
2015	Pollen data not available								
2016	9-Feb	21-Apr	72				9-May	24-May	15
Mean			40.0 ± 5.9			14.5 ± 2.2			41.5 ± 6.4

It is indicated when data are not available (years 2011 and 2015). Missing values correspond to the absence of PS events according to the EAACI criteria for the PS definition. Mean values correspond to mean PS start and end dates and duration ($\pm$ s.e.) (corresponding to a non-leap year)

Thessaloniki over the study period, so we cannot check whether they would match those of the pollen season, as derived by use of the EAACI criteria. However, pollen concentration from both cypress and olive rank high throughout the 30-year period (on average more than 25% and 3% of the total annual concentrations, respectively), their airborne pollen are

important aeroallergens in the study area (Gioulekas et al. 2004; Rachovitsas et al. 2017) and there are verified cases of rhinitis induced by them like olive-related AR (Sichletidis et al. 2004; Rachovitsas et al. 2017). According to Gioulekas et al. (2004), sensitization to olive pollen is very high, 31.8% in a population of 1311 atopic patients from northern

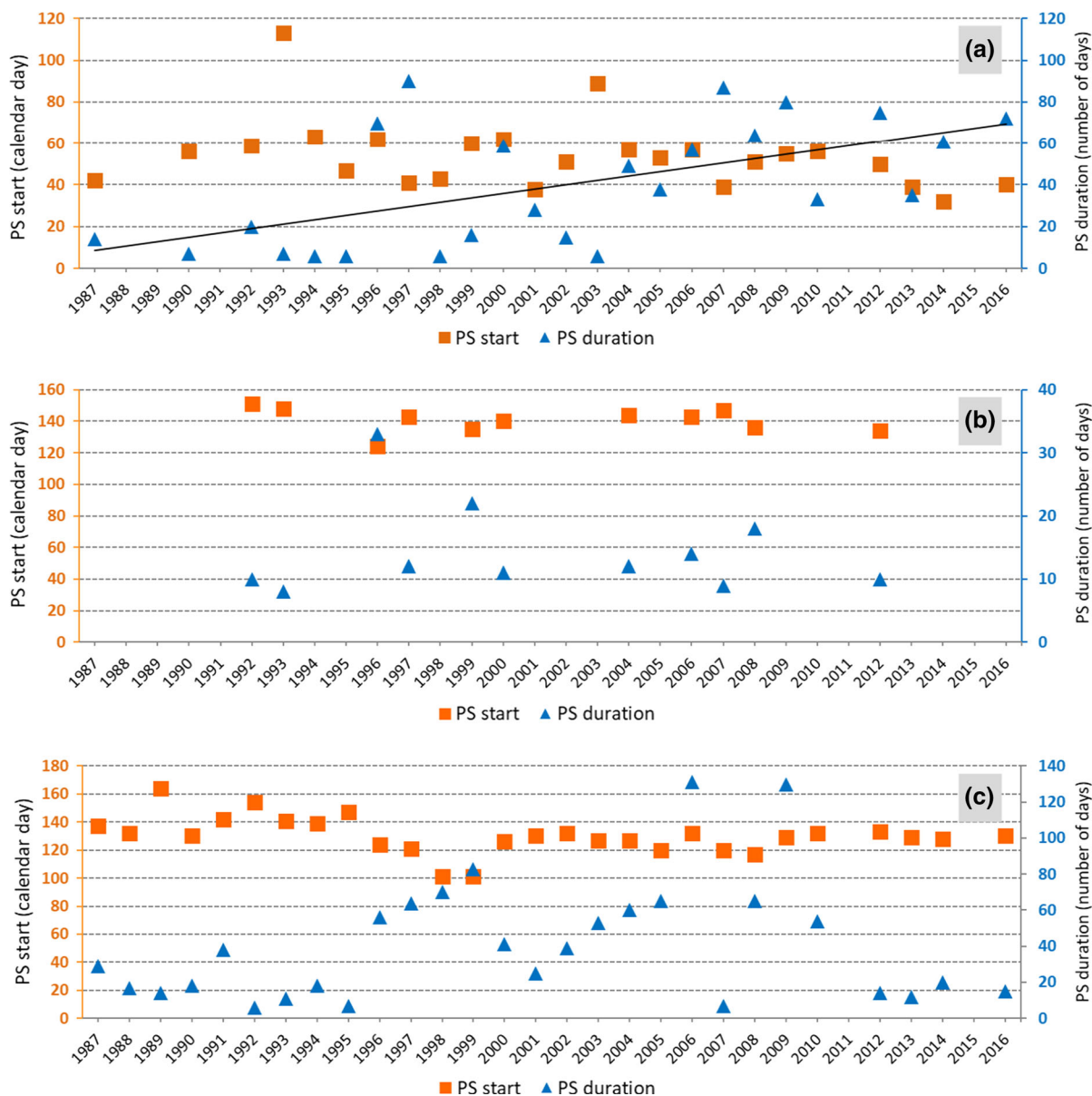


Fig. 1 Graphical representation of the pollen season (PS) start and of the PS duration for cypress (a), olive (b) and grasses (c) over all years of study. The x-axis denotes years, while the y-axis on the left denotes numbered calendar days (1 = January 1st) and on the right number of days. Regression analysis gave

significant results ($p = 0.003$) only in the case of PS duration for cypress; the linear regression line is added in the latter graph. Blank points correspond to missing values (no data for years 2011 and 2015 and no PS event in all other cases)

Greece, mainly from the region of Thessaloniki, second only to that from grasses (40.4%), while quite high is also sensitization to cypress pollen (12.7%). Given the above, it is unlikely that there is no AR symptom-inducing olive pollen season for 17 of the 30 years of study.

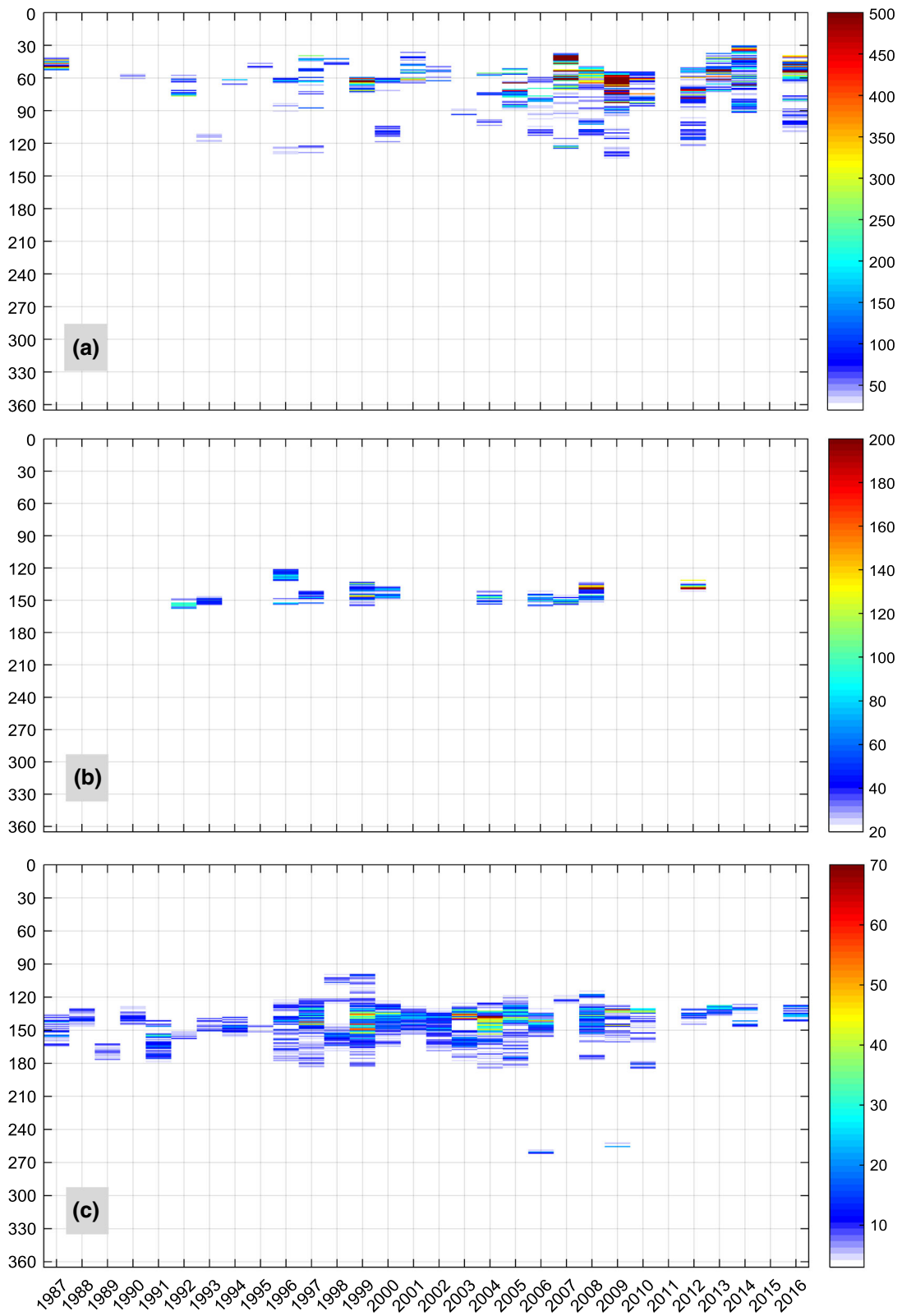
Trying to identify the reasons for the different results of the application of the EAACI criteria in describing the pollen seasons for the three taxa examined, we explored the thresholds published in the literature for symptom manifestation as compiled by de Weger et al. (2013). For Poaceae, thresholds range from > 1 (Thibaudon 2003) up to 52 pg m^{-3}

(Lipiec et al. 2005), as daily pollen concentrations, for low symptom intensity up to all patients experiencing symptoms, respectively, without a considerable difference of values between the north of Europe and the Mediterranean region. For Cupressaceae, the range is from 7 up to $> 141 \text{ pg m}^{-3}$ (Thibaudon 2003), with thresholds in the Mediterranean part of France being one order of magnitude lower than the ones in the north of the country. For olive, thresholds range from $> 4 \text{ pg m}^{-3}$ for initiation of symptoms in Israel (Waisel et al. 2004) up to $> 227 \text{ pg m}^{-3}$ in France (Thibaudon 2003) for high symptom intensity, or even $> 400 \text{ pg m}^{-3}$ for mild symptoms in Spain (Florido et al. 1999). From these threshold values, it appears that the range for Poaceae as compared to that of other taxa (de Weger et al. 2013) is short, definitely shorter than that for the other two pollen taxa, despite the fact that far more species are included in this pollen taxon than in the other two. For Cupressaceae, apart from the much wider range of thresholds, there seems to be a marked difference of sensitivity between the Mediterranean and northern populations of France toward pollen from their representatives. Remarkably, olive pollen is associated with the widest threshold range although it corresponds to only one Mediterranean species. Even if we exclude the highest and lowest values that were obtained with different methodologies (de Weger et al. 2013), the range still remains very large (from 6 up to $> 227 \text{ pg m}^{-3}$). This clearly suggests that even within the Mediterranean area, the different populations respond very differently to pollen and that other more specific environmental conditions including pollutants and other particles in the air may play an important role in pollen allergenicity. Given the above, it seems that the symptom thresholds, which are the basis for specifying the criteria for PS definition of olive and possibly for Cupressaceae, should be revisited.

Jato et al. (2006) suggested that decisions taken regarding pollen season definition should not be universal in nature, but vary as a function of both the pollen type under study and of the particular features of the study (sampling) area. In addition, meteorological factors like relative humidity may influence considerably symptom manifestation (Damialis et al. 2019), whereas the interaction of pollen grains with air pollutants or other airborne biological particles may modify pollen allergenicity (Gruijthuisen et al. 2006; Sedghy et al. 2018). The cumulative effect of pollen in

the manifestation of allergy symptoms should be embedded into the process of defining the pollen thresholds that delimit the pollen season. Nevertheless, as clarified by Bastl et al. (2018), pollen season definition could vary depending on the purpose of the study. Hence, symptom data should not be necessarily taken into consideration. For instance, if climate change and its effect on plants is the focus, symptom data are not useful in delimiting the pollen season. On the other hand, it is not possible to develop a pollen season definition for medical use solely on the basis of pollen concentrations without taking into account symptom data and the influence of medication taken by the subjects who report their symptoms. To avoid confusion, a different term should be better used when research focuses on the extent of exposure to airborne pollen and on the manifestation of symptoms, as is the case with the EAACI PS definition. For this case, we propose use of the term allergy symptom-inducing pollen season (ASIPS) rather than pollen season (PS) alone.

Comparing the data of this research with those that were derived earlier (Damialis et al. 2007; Gioulekas et al. 2004), we find that the rank of the three taxa with respect to airborne pollen abundance has remained the same in Thessaloniki over the years. Regarding the phenological features of the pollen season, there seems to be a number of differences in the start and end and, hence, the duration of the pollen season when applying the EAACI criteria as compared to those derived by use of the percent-of-the-total-pollen approach (Gioulekas et al. 2004); these seem to be pronounced in the case of olive (later start with EAACI criteria) and grasses (shorter duration as of later start and earlier end with EAACI criteria). However, these visually noted differences were not validated by statistical analysis. There was no significant trend for earlier (or later) PS start over this period (Fig. 1), in accordance to the results reported previously for the same area (Damialis et al. 2007). Regarding the PS duration, only for cypress there was an increasing trend over the last 30 years ($p = 0.003$). This was not observed previously (Damialis et al. 2007) when using a shorter time series and the 2.5–97.5% approach for PS definition. There are two possible explanations for this outcome operating independently or in combination with each other: Incorporation of recent data might have made detectable a change in the flowering phenology due to climate change and, hence, in the pollen season;



◀ **Fig. 2** Pollen calendar for cypress (a), olive (b) and grass (c) for the Thessaloniki area, Central Macedonia Region, Greece, and for the years 1987–2016. The EAACI pollen season criteria have been used per pollen taxon. The horizontal axis corresponds to the years of study and the vertical axis to calendar days (1 = January 1st). White color corresponds to no data (for years 2011 and 2015) or data below the pollen count threshold of the criteria per pollen taxon (i.e. 20, 20 and 3 pollen grains m^{-3} for cypress, olive, and grasses, respectively)

alternatively, this could be related to the different pollen season definition method applied. How sensitive are the different methods of PS definition in detecting PS-associated impacts of climate change is an issue to be explored.

The PS definition criteria by EAACI for the taxa addressed in this study (and similarly for other taxa) should be tested in several geographically different areas and modified, if necessary. To establish the criteria validity, patient data (symptoms included in hospital records, symptoms reported via internet-based tools, Google Trends, etc.), wherever available, should be taken into consideration and assessed in parallel, as Pfaar et al. (2017) clearly suggested. To this end, the recently published findings that Google Trends data reflect the pollen season, as resulting from the EAACI definition, for birch and grass in the Berlin–Brandenburg area (Karatzas et al. 2018c) provide useful methodological support. Given the need for timely and accurate information on pollen levels and PS features, operational pollen forecasting models are required, and computational intelligence may help in responding to this requirement (Voukantis et al. 2010; Kassandros et al. 2018).

5 Conclusions

The analysis of a 30-year series of airborne pollen concentrations for cypress, olive, and grasses, from Thessaloniki, Greece, an area with Mediterranean-type climate, aimed at defining PS start and end dates, according to the new EAACI criteria for PS definition, and in examining the yearly trends of these PS features. Application of these criteria defined PS for grasses for all years of study but not for olive (Oleaceae), for most of the period examined, neither for cypress (Cupressaceae) to a less extent. Nevertheless, the latter taxa have very allergenic pollen and

have always been among the most abundant in the area. Pfaar et al. (2017) clearly stated that the definitions given should be regarded as tentative and for critical evaluation. Application of the EAACI thresholds to data series of Thessaloniki shows the need for an accurate analysis and revision of the symptom thresholds for olive and cypress pollen in the Mediterranean area. This is a prerequisite for any revisions of the proposed thresholds for the start and end date in the EAACI definitions. Given that the pollen season, as defined after the EAACI criteria, corresponds to a part of the entire pollen season, the one inducing allergy symptoms, it would be better to use a different term to describe it. We propose ‘allergy symptom-inducing pollen season’ (ASIPS) meaning the time when pollen-induced allergy symptoms are manifested as opposed to pollen season (PS) meaning the time when pollen is present in the air, so as to avoid confusion. In doing so, it becomes clear that different criteria could be applied for studies of the pollen season depending on the focus of the research. When it comes to a definition that will be used for the support of medical praxis, information on allergy symptoms and on the influence of medication on allergic subjects is additionally required.

Authors’ contribution KK initiated the study, made data analysis, and drafted the manuscript; AT made data analysis and PS calculations; AC, AD, and DV provided pollen data and expert support. All authors contributed to the writing of the manuscript and DV concluded the final version.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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