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ORIGINAL ARTICLE

Rhinitis, Sinusitis, and Upper Airway Disease

"Whole" vs. "fragmented" approach to EAACI pollen season definitions: A multicenter study in six Southern European cities

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Abbreviations: AIA, associazione Italiana di aerobiologia; AIT, allergen immunotherapy; ARPACal, agenzia regionale per la protezione dell´ambiente della Calabria; EAACI, European academy of allergy and clinical immunology; EAN, European aerobiology network; FHS, fragmented high season; FPS, fragmented pollen season; HD, high days; RIMA-AIA, rete Italiana di monitoraggio in aerobiologia – associazione Italiana di aerobiologia; RNSA, réseau national de surveillance aérobiologique; SAR, seasonal allergic rhinitis; WHS, whole high season; WPS, whole pollen season.

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Abstract

Background: The adequate definition of pollen seasons is essential to facilitate a correct diagnosis, treatment choice, and outcome assessment in patients with seasonal allergic rhinitis. A position paper by the European Academy of Allergy and Clinical Immunology (EAACI) proposed season definitions for Northern and Middle Europe.

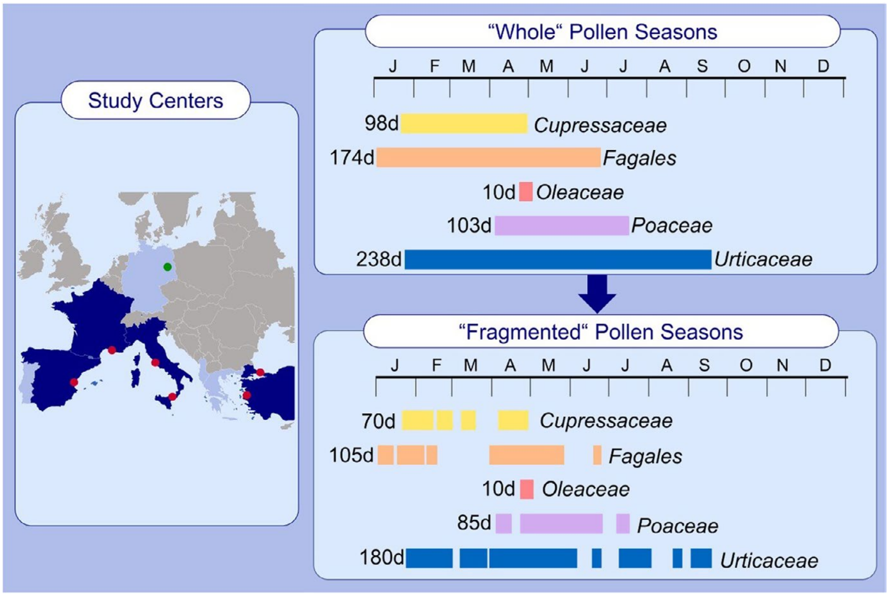
Objective: To test the pollen season definitions proposed by EAACI in six Mediterranean cities for seven pollen *taxa*.

Methods: As part of the @IT.2020 multi-center study, pollen counts for Poaceae, Oleaceae, Fagales, Cupressaceae, Urticaceae (*Parietaria* spp.), and Compositae (*Ambrosia* spp., *Artemisia* spp.) were collected from January 1 to December 31, 2018. Based on these data, pollen seasons were identified according to EAACI criteria. A unified monitoring period for patients in AIT trials was created and assessed for feasibility.

Results: The analysis revealed a great heterogeneity between the different locations in terms of pattern and length of the examined pollen seasons. Further, we found a fragmentation of pollen seasons in several segments (max. 8) separated by periods of low pollen counts (intercurrent periods). Potential monitoring periods included often many recording days with low pollen exposure (max. 341 days).

Conclusion: The Mediterranean climate leads to challenging pollen exposure times. Monitoring periods for AIT trials based on existing definitions may include many intermittent days with low pollen concentrations. Therefore, it is necessary to find an adapted pollen season definition as individual solution for each pollen and geographical area.

KEYWORDS
EAACI, Mediterranean, pollen allergy, season definitions, seasonal allergic rhinitis



GRAPHICAL ABSTRACT

Depending on the criteria used, the length of a pollen season can vary dramatically. EAACI criteria generate very extended pollen seasons in Southern Europe, which also include many days/weeks with no/low pollen counts. The exclusion of “intercurrent periods” with no/low pollen counts (“fragmented pollen season” method) improves the accuracy of EAACI criteria.

1 | INTRODUCTION

Seasonal allergic rhinitis (SAR) poses a great socioeconomic burden affecting between 10% and 30% of the world population.[1] Symptoms occur in seasons during which pollen are abundant in the outdoor air. Depending on the geographic location and local climate, the timeframes of high pollen exposure may vary significantly. Thus, a correct and precise definition of the start and end of allergenic pollen seasons is crucial for an adequate diagnostic approach.[2,3] In Southern European countries, pollen calendars, based on the daily monitoring of pollen concentrations in the local air, have been established as a widely used methodology to report pollination periods throughout the year.[4-7] In their most frequent version, these calendars are based on data from a minimum of 5 years and structured in 36 sections of 10 days, usually color coded, for example, to represent absent (white), low (yellow), intermediate (orange), or high (red) pollen concentrations in the atmosphere. Pollen calendars are then used by allergists to establish a cause-effect association between allergic sensitization to a given pollen, demonstrated by IgE tests or skin prick tests, and symptoms occurring during exposure to that pollen.[5,8,9] However, trends of climate change are progressively modifying the pollination periods of many allergenic species and—in parallel—the seasonality of the allergic symptoms triggered by these pollens.[10,11] Therefore, the reliability and precision of historically acquired pollen data (pollen calendars) as a tool to predict and define future and current pollen seasons are increasingly questioned.[12,13]

Given this premise, current monitoring of pollen counts is increasingly needed to define, year after year, the start, course, and end of a pollen season. Several criteria for pollen season definitions have been proposed in the last decades.[13-20] These definitions rely on percentages, thresholds, and trend analyses as well as inter-regional comparisons. However, thresholds vary from study to study, even if performed in the same climatic area and there remains a lack of harmonization and validation of one (gold)standard as demanded by academia and regulatory authorities.[21-23] To overcome this need, a task force of the European Academy of Allergy and Clinical Immunology (EAACI) recently published a position paper providing pollen exposure time definitions for middle and northern European countries, which are easily applicable thanks to clear methodological instructions.[24] This consensus acquired special relevance for clinical trials of allergen immunotherapy (AIT), for which the clinical endpoints are defined according to predefined seasonal time periods based on pollen concentrations.[24]

However, the inter-regional geographic and climatic influences on the vegetation may make the adoption of the same standardized thresholds difficult in Southern European countries. The typical vegetation in the Mediterranean zone is abundant in Urticaceae (*Parietaria* spp. and *Urtica* spp.), Oleaceae, Cupressaceae, Poaceae (Graminaceae), Compositae (Asteraceae), and many other allergenic species, with internal variations by region and country.[25] Therefore, the Mediterranean region, as established climatic and vegetation zone, shows different characteristics not only when compared to Northern and Middle Europe, but also within its own northern or southern, urban, or rural territories.[6,25,26] This heterogeneity is further complicated by the fact that pollen seasons widely overlap, making a diagnostic use of pollen calendars very difficult for the allergist, especially in the case of polysensitized patients.[25,27]

So far, the EAACI criteria have been confirmed in retrospective analyses on the Poaceae pollen seasons between 2012 and 2016 in Germany.[28] Another study compared two definitions (EAACI vs European Aerobiology Network [EAN]) of several pollen seasons (birch, hazel, alder, grass, ragweed, mugwort) in Austria (Vienna) in 2018.[29] We have targeted the present study to define the seasonality of seven pollen taxa during 2018 using the EAACI definitions in six cities of four Southern European countries, namely Rome, Messina (Italy), Marseille (France), Valencia (Spain), Istanbul, and Izmir (Turkey).

2 | METHODS

2.1 | Study centers

The present study was conducted as part of the "@IT.2020" multicenter project, an observational longitudinal study on the combined impact of molecular IgE tests and mobile health technology on the diagnosis and allergen immunotherapy prescription for seasonal allergic rhinitis in Southern European countries. From January 1 to December 31, 2018, aerobiological monitoring was performed in six Southern European cities: Valencia, Marseille, Rome, Messina, Istanbul, and Izmir. The Mediterranean sub-tropical climate is overall characterized by mild winters, opposed by long and dry summer periods. While Valencia, Marseille, Messina, Istanbul, and Izmir are located directly next to the sea, Rome is situated at 30km inland from the coast. Moreover, several other meteorological differences among the six cities are known (Table S1).

2.2 | Pollen data

The pollen of Cupressaceae (Cupressaceae and Taxaceae), Fagales (Fagaceae, and Betulaceae), Oleaceae, Poaceae, Urticaceae (*Parietaria* spp.), *Ambrosia* spp., and *Artemisia* spp. (Compositae) were monitored. Being a clinical study, Fagaceae, and Betulaceae were considered as a single group belonging to the Fagales botanical order as they all contain cross-reactive PR-10-like proteins. The most relevant sources of allergenic pollen from this order, such as Betula verrucosa (Birch), Alnus glutinosa (Alder), Carpinus betulus (Hornbeam), Corylus avellana (Hazel), Quercus alba (Oak), Castanea sativa (Chestnut), and Fagus sylvatica (Beech), are represented within the monitored plants of the Fagales order. Pollen counts were recorded with validated methodologies [30] by using a volumetric Hirst type sampler (Burkard or VPPS Lanzoni) with a suction flow of 10 L of air per minute, which allows a continuous sampling for up to 7 days (Table S1). The trap's surface for collecting pollen grains is a 7-day transparent Melinex tape with silicon (polydimethylsiloxane) for Lanzoni traps and Vaseline for Burkard traps. The samples have been prepared as glass slides for the microscopic analysis, using Fuchsine as coloring medium. The reading of the slides was performed by experienced (5-29 years of experience) and locally trained aerobiologists with an optical microscope at a magnification of 400×. Daily pollen concentrations are expressed as pollen grains per cubic meter air (pollen grains/m^3) as previously recommended.[29,31] In order to obtain the concentration value from the pollen data, the count is multiplied by a conversion factor specific to the microscope and lens combination that were used.[32] The pollen monitoring and reading were carried out according to the minimum requirement criteria for pollen monitoring networks.[33] The aerobiological centers in Marseille, Rome, and Messina belong to established aerobiological monitoring networks, namely RNSA, RIMA-AIA, and ARPACal, respectively. Further, linear interpolation of data gaps has been performed following the computational tool "AeRobiology" of the software R which has been designed specifically to calculate aerobiological data.[34]

2.3 | Season definitions

The season definition criteria adopted in this study are those proposed by a recent EAACI position paper.[24] Briefly, EAACI criteria define for each pollen (*Betula* spp., Poaceae, *Cupressus* spp., *Olea* spp., *Ambrosia* spp.) a start and a stop signal based on daily and cumulative pollen counts within a short sequence of days.[24] For example, the Fagales (*Betula spp.*) pollen season starts with the 1st of 5 days (out of seven consecutive days) with ≥10 pollen grains/m^3, when the cumulative pollen concentration of these 5 days is ≥100 pollen grains/m^3. The season ends after the last day respecting the same conditions, that is, the last of 5 days (out of seven consecutive days) with ≥10 pollen grains/m^3, when the sum of these 5 days is ≥100 pollen grains/m^3. Throughout the text, these conditions will be termed "start signal" and "stop signal" accordingly. For other pollen taxa, the daily concentrations and cumulative thresholds have been adapted (Table 1). These EAACI criteria generate seasons composed by a single, continuous period for each pollen species, by defining the very first and the very last day of a season. By adjusting the established thresholds, the EAACI criteria define two types of seasons, a "pollen season" (longer) and "high season" (shorter), by using lower and higher thresholds, respectively (Table 1).[24] As these definitions do not take into account stop signals occurring during the season, they generate continuous seasons without interruption. These seasons will be termed "Whole Pollen Season (WPS)" and "Whole High Season (WHS)," respectively, throughout the manuscript. In our study, we have further established two additional season definitions by taking into account stop signals according to EAACI definition occurring during a season. These stop signals (eg, for Fagales the last of five days [out of seven consecutive days] with ≥10 pollen grains/m^3, when the sum of these 5 days is ≥100 pollen grains/m^3) create an interruption of the season which is later continued as further start signals occur, creating an intercurrent period of days not fulfilling the season criteria. Considering the resulting fragments, additional season definitions have been termed "fragmented pollen season" (FPS) and "fragmented high season" (FHS). In this second representation, a season might be characterized by several fragments/periods interrupted by out-of-season periods. The EAACI criteria also define "high days (HD)" as those days with pollen counts exceeding a given threshold. This EAACI HD threshold is fixed for Cupressaceae, Oleaceae, and Fagales at ≥100 pollen grains/m^3,

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TABLE 1 Overview of season definition criteria in accordance with the EAACI position paper [24]. Thresholds[a] for whole pollen season (WPS), whole high season (WHS), and high days (HD) are presented

	Season start			Season end	
	Pollen season	High season	High days	Pollen season	High season
Pollen	1st of 5/7 d (sum 5 d)	1st of 3 d		Last of 5/7 d (sum 5 d)	Last of 3 d
Cupressaceae, Oleaceae	20 (200)	100	100	20 (200)	100
Betula spp., Fagales (except *Betula* spp.)[b]	10 (100)	100	100	10 (100)	100
Poaceae, Urticaceae[b], *Ambrosia* spp., *Artemisia* spp.[b]	3 (30)	50	50	3 (30)	50

[a]Equal or higher than the indicated value in pollen grains/m^{3}.

[b]Adaptation of the EAACI criteria for the present study as these pollen species had not been included in the EAACI position paper.

while for Poaceae and *Ambrosia* spp., it is fixed at ≥50 pollen grains/m^3 (Table 1). We have adopted the EAACI thresholds and criteria for Cupressaceae, *Betula* spp., Poaceae, Oleaceae, and *Ambrosia* spp. For Fagales (Betulaceae except *Betula* spp., and Fagaceae) we adopted the EAACI criteria for *Betula* spp., while for Urticaceae, and *Artemisia spp.*, which were not considered in the original paper,[24] we have adopted the criteria established by EAACI for the less abundant pollen (Poaceae),[24] as previously proposed (Table 1).[29] For defined thresholds for the individual pollen seasons, please see Appendix S1.

2.4 | Meteorological data

To assess the influence on pollen concentrations and season length, meteorological data from all six centers were analyzed, see Table S3.

3 | RESULTS

3.1 | Pollen count courses, by city

The count courses of all seven examined pollens showed similarities and differences among the six included cities. (Figure 1, Figure S1) In Valencia, relevant peaks of Cupressaceae were registered in February and March, while May and June were dominated by Oleaceae, Poaceae, and Urticaceae. In Marseille, Cupressaceae were observed throughout the whole year, reaching extremely high peaks in February and appearing consistently until end of April. Fagales also had high peaks in April. Oleaceae were observed in April and May and Poaceae mostly in May, June, and July. In Rome, Cupressaceae and Fagales were observed from January to April and both reached consistent levels. Urticaceae

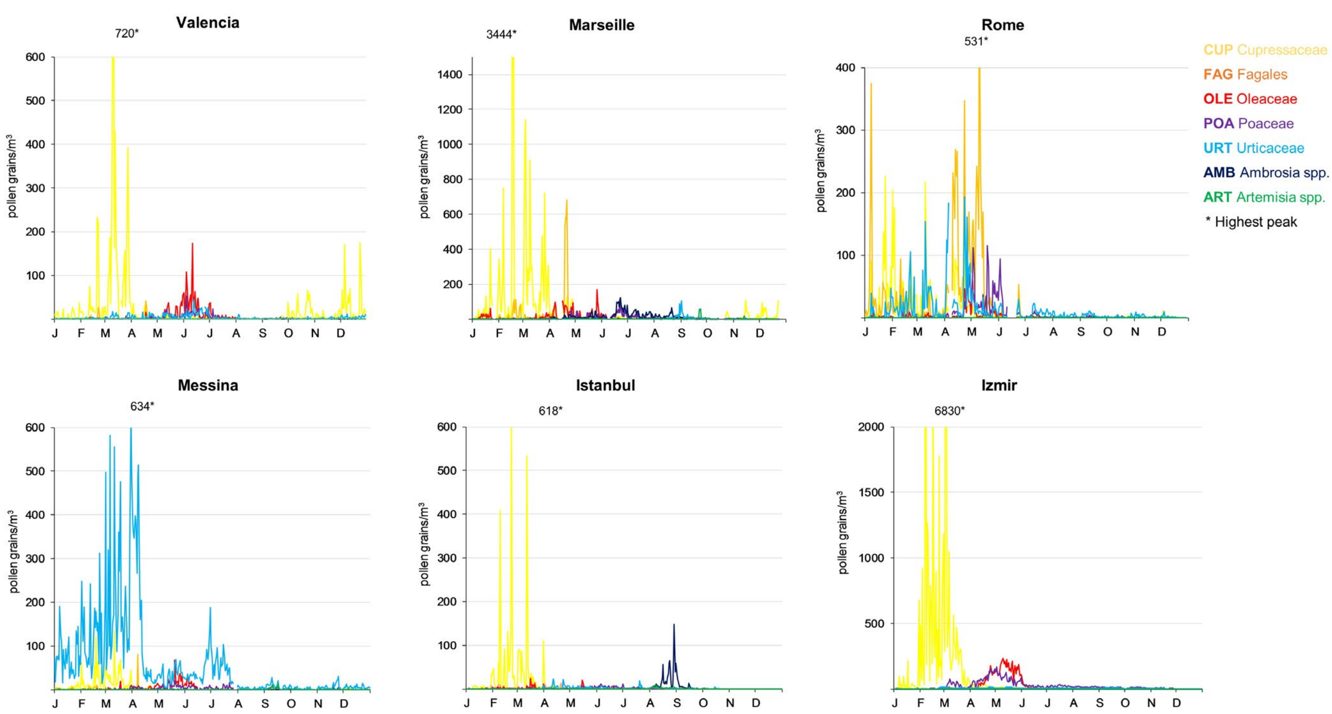
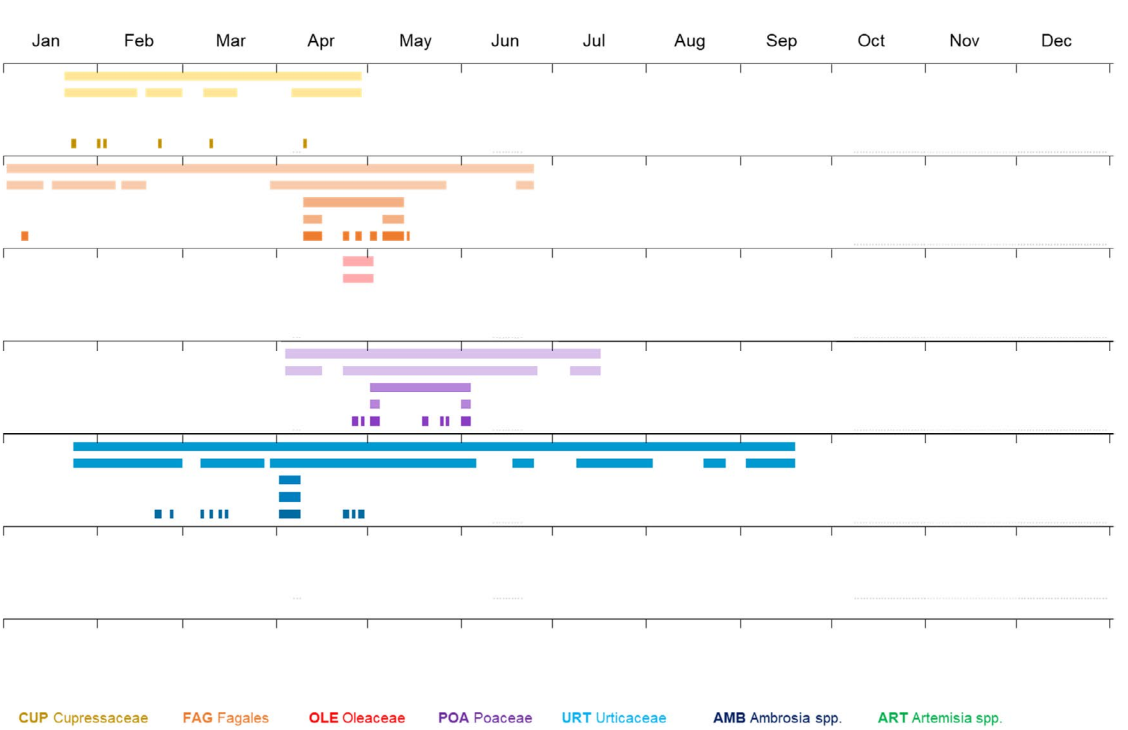


FIGURE 1 Daily pollen concentration (pollen grains/m^3) of Cupressaceae, Fagales, Oleaceae, Poaceae, Urticaceae, *Ambrosia* spp., and *Artemisia* spp. in six Southern European/Mediterranean cities in 2018



		days (n)	segments (n)
CUP	WPS	98	1
	FPS	70	4
	WHS	-	-
	FHS	-	-
	HD	7	-
FAG	WPS	174	1
	FPS	105	5
	WHS	33	1
	FHS	13	2
	HD	22	-
OLE	WPS	10	1
	FPS	10	1
	WHS	-	-
	FHS	-	-
	HD	-	-
POA	WPS	103	1
	FPS	85	3
	WHS	33	1
	FHS	6	2
	HD	13	-
URT	WPS	238	1
	FPS	180	7
	WHS	7	1
	FHS	7	1
	HD	19	-
AMB	WPS	-	-
	FPS	-	-
	WHS	-	-
	FHS	-	-
	HD	-	-
ART	WPS	-	-
	FPS	-	-
	WHS	-	-
	FHS	-	-
	HD	-	-

FIGURE 2 Whole pollen season (WPS), fragmented pollen season (FPS), whole high season (WHS), fragmented high season (FHS), and high days (HD) for the year 2018 in Rome as defined on the basis of the EAACI criteria[24] with further adaptation (see methods). On the left side, the length of each season (number of days) and number of segments are given

were observed from January to September, with its highest peak in April. Oleaceae was observed in April and May, while Poaceae was mostly observed in May and June. In Messina, Urticaceae was the dominating pollen throughout the whole year, with its highest peaks in March and April and a second, smaller wave of peaks in June and July. Also, Cupressaceae was observed mostly in February and March, but at much lower levels than Urticaceae. Relatively low peaks of Poaceae were observed in between April and July. In this center, the pollen counts of Oleaceae were rather low and those of Fagales irrelevant. In Istanbul, Cupressaceae were observed at high peaks in February and March. Fagales and Oleaceae were observed at very low levels only in a few days in February and in March and May, respectively, while *Artemisia* spp. appeared with a small cluster of peaks in August and September. In Izmir, Cupressaceae were observed at extremely high peaks in February and March. Oleaceae appeared at much lower peaks in April and May, while Poaceae and Urticaceae were registered for long periods, from the end of March up to the summer.

3.2 | Pollen seasons in Rome

We arbitrarily chose Rome as the prototype database to start our analytical exercise. By applying the EAACI criteria (Table 1) to the pollen counts registered in that city throughout 2018 (Figure 2), we first identified WPS for five pollens, including Cupressaceae, Fagales, Oleaceae, Poaceae, and Urticaceae, but not for *Artemisia* spp. and *Ambrosia* spp. (Figure 2 and Table 2). The WPS was very short for Oleaceae (only 10 days), long for Fagales (174 days), Poaceae (103 days), and Cupressaceae (98 days) and extremely long for Urticaceae (238 days). After exclusion of intercurrent periods with low pollen counts, causing an interruption of the season, shorter fragments of pollen seasons, consequently named here “fragmented pollen season” (FPS), were generated, with the number of days decreasing for Fagales (from 174 to 105 days), Poaceae (from 103 to 85 days), Cupressaceae (from 98 to 70 days), and Urticaceae (from 238 to 180 days). Interestingly, FPS was split into three periods for Poaceae (range 10-64 days), four periods for Cupressaceae (range 11-24 days), five periods for Fagales (range 6-58 days), and into seven periods for Urticaceae (range 7-68 days). Similarly, we first identified WHS for Fagales (33 days), Poaceae (33 days), and Urticaceae (7 days only) which were shortened into fragmented high seasons (FHS) by excluding the intercurrent periods. This resulted in FHS of only 13 and 6 days for Fagales and Poaceae, respectively, with two periods each. The number of High Days (HD) ranged from 0 for Oleaceae to 22 for Fagales (Figure 2 and Table 2).

3.3 | WPS and FPS in the six centers

We then applied the same methodology to all the other centers (Figure 3, Tables S2 and 3). We identified WPS for all seven pollen in Marseille, but only for three pollen (Cupressaceae, Poaceae, and Urticaceae) in Messina, and for four pollen for the remaining four centers. The length of the WPS ranged from a minimum of 8 days for *Artemisia* spp. in Istanbul to a maximum of 363 days for Urticaceae in Messina. In some cases (Cupressaceae in Izmir; Poaceae and *Artemisia* spp. in Istanbul; Oleaceae in Valencia), the WPS and the FPS coincided perfectly. In others (Cupressaceae in Marseille; Cupressaceae and Urticaceae in Valencia), significant reductions in the season length were observed when the intercurrent periods were excluded (Figure S2). The most fragmented pollen season was that of Urticaceae in Valencia, with eight different segments (ranging from 6 to 28 days) while the longest interruption period occurred during the Cupressaceae WPS in Valencia (201 days).

TABLE 2 Start, end, and length (days) of pollen seasons according to EAACI criteria in Rome during 2018

	WPS			FPS				WHS			FHS				HD
	Date			Segments[a]				Date			Segments				
Pollen	Start	End	Days	No	Shortest	Longest	Days	Start	End	Days	No	Shortest	Longest	Days	Days
Cupressaceae	*21 Jan*	*28 Apr*	98	4	11	24	70	-	-	-	-	-	-	-	7
Fagales	*2 Jan*	*24 Jun*	174	5	6	58	105	*10 Apr*	*12 May*	33	2	6	7	13	22
Oleaceae	*23 Apr*	*2 May*	10	1	10	10	10	-	-	-	-	-	-	-	-
Poaceae	*5 Apr*	*16 Jul*	103	3	10	64	85	*2 May*	*3 Jun*	33	2	3	3	6	13
Urticaceae	*14 Jan*	*18 Sep*	238	7	7	68	180	*2 Apr*	*8 Apr*	7	1	7	7	7	19
Ambrosia spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Artemisia spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Abbreviations: FHS, fragmented high season; FPS, fragmented pollen season; HD, high days; WHS, whole high season; WPS, whole pollen season.

[a]Season is divided into >2 periods considering all the stop and start signals observed between the first start and the last stop signal – the respective intermediate periods (bridged gaps) are then excluded from computation. The number of segments as well as the length of the shortest and longest segment in days are indicated.

3.4 | WHS, FHS, and HD in the six centers (Appendix S1)

In contrast to seven WPS, only three WHS (Cupressaceae, Fagales, and Urticaceae) were identified in Marseille (Figure 3, Tables S2, 3). Similarly, less WHS than WPS were identified in all the other centers, with the extreme case of Istanbul, where no WHS could be identified for any pollen. The length of the WHS was always much shorter than that of the WPS for all pollen in all centers.

With regard to the context of potential clinical trials on allergen immunotherapy, especially in a multicenter setting, establishing a unified symptom monitoring period for participating patients in individual centers is crucial.[22,24] On the basis of our data, we therefore tried to establish a monitoring period, based on the WPS and the WHS definitions, capable of unifying and covering the pollen seasons of all six centers for the pollen taxa with the highest relevance for allergen immunotherapy in Southern Europe (Cupressaceae, Poaceae, Urticaceae) (Figure S3). We then compared the resulting scenario with that generated by the alternative strategy, based on the adaptation of the monitoring period in each city to the local conditions (Figure S3). The comparison clearly showed, for all three pollen, that in the first "unified monitoring period"—solution the number of monitoring days is considerably (up to 341 days) higher than with the localized or flexible solution (Table 4). Moreover, the proportion of no/low pollen days during which patients would have been monitored is, in the "unified monitoring period"—approach up to 47% for the WPS and up to 49% for the WHS (Table 4).

3.5 | Influence of rainfall on season length

See Table S3.

4 | DISCUSSION

We investigated the recently established EAACI pollen season definitions [24] and criteria for seven pollen taxa in six Southern European/Mediterranean cities. We found (a) a great heterogeneity

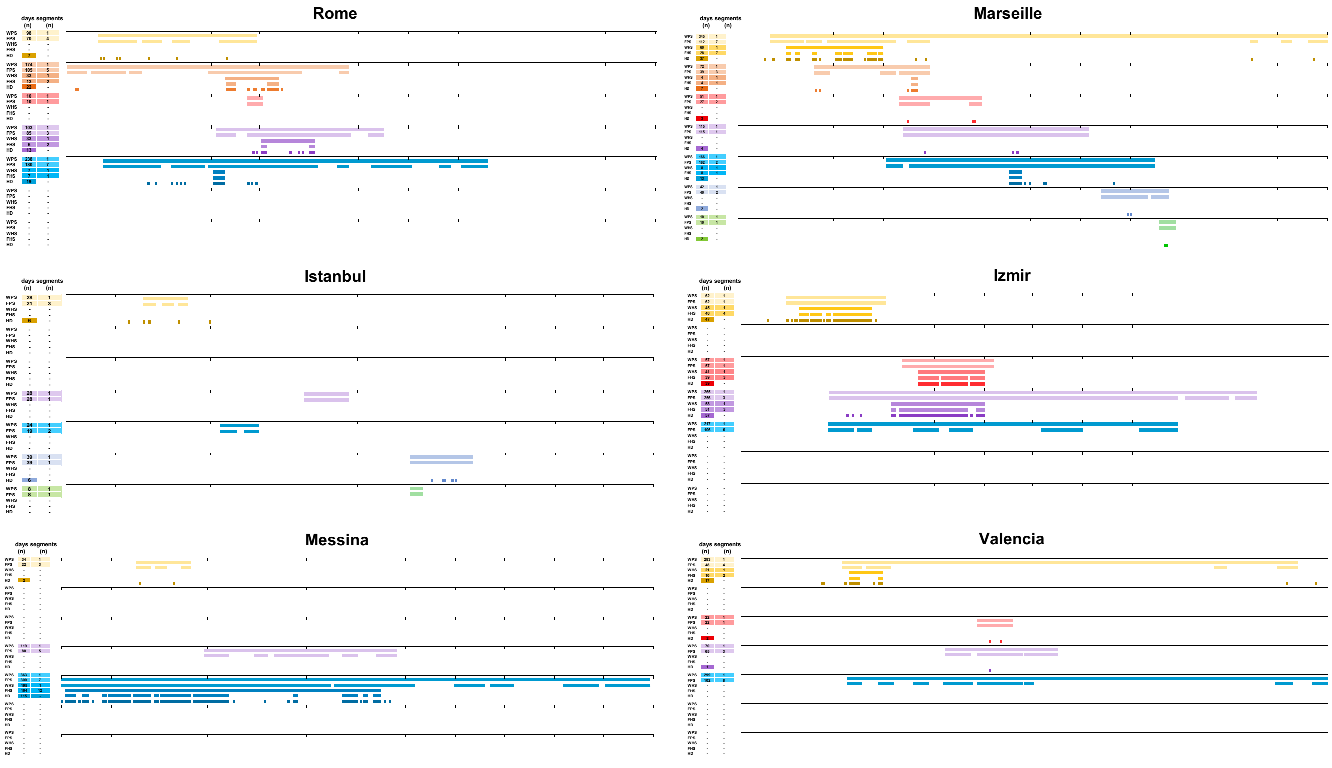


FIGURE 3 Pollen seasons of Cupressaceae, Fagales, Oleaceae, Urticaceae, Poaceae, *Ambrosia* spp., and *Artemisia* spp. in six Southern European/Mediterranean cities in 2018. Graphical representation of the days included in the whole pollen season (WPS), fragmented pollen season (FPS), whole high season (WHS), fragmented high season (FHS), and high days (HD) as defined on the basis of EAACI criteria[24] with further adaptation (see methods). On the left side, the length of each season (number of days) and number of segments are given

among the six cities in terms of pattern and length of the examined pollen seasons, showing especially long WPS and WHS in some centers; (b) a fragmentation of pollen seasons in several segments separated by periods of low pollen counts (intercurrent periods) and therefore suggest a new definition of "*fragmented pollen season*"; (c) no viable option for a unified pollen calendar for multicenter AIT trials in Southern European or Mediterranean countries. As such, our analysis adds to the recent confirmation of the relevance of the EAACI criteria in Germany [28] and other European countries through a multicenter approach in several Southern European countries.

4.1 | Aerobiological heterogeneity

Firstly, our analysis focused on the high level of aerobiological heterogeneity. All seven investigated pollen *taxa* could be identified on at least one day in each of the six cities, with the exception of *Ambrosia* spp. in Valencia and Izmir. However, the pollen concentration of *Ambrosia* spp. and *Artemisia* spp. in the atmosphere in most cities was neither high nor continuous enough to generate "seasons" according to the EAACI definitions. The six species of *Ambrosia* spp. have different distribution patterns in the Mediterranean basin.[35] As expected, season length was regulated by the threshold and criteria for definition, so that the pollen seasons (WPS, FPS) were always longer than the high seasons (WHS, FHS). The pattern of pollen seasons was very heterogeneous in different centers. For example, Urticaceae WPS and FPS could be defined in all the centers, while Urticaceae WHS and FHS could be defined only in Messina, Rome, and Marseille. Similarly, Poaceae WPS and FPS could be identified in all six centers, but their length was limited to 28 days in Istanbul and reached up to 265 days in Izmir. Accordingly, WHS were identified in Izmir and Rome but not in the other cities. Indeed, a great heterogeneity in the pollen seasons was found even within countries, as demonstrated by the comparison between the Italian cities of Rome and Messina or the Turkish cities of Istanbul and Izmir. Altogether, this aerobiological scenario prevents the formulation of a unified "Southern European Pollen Calendar" and has an impact on the design of multicenter trials of AIT for pollen allergies in Southern European countries (see below).

4.2 | Fragmented pollen seasons

Another important finding is the comparison of "whole" vs "fragmented" pollen season data. The pollen seasons defined in six cities by applying the EAACI criteria were almost invariably fragmented in many segments.

Given the observed fragmentation, we split each definition into two categories:

- The first category, that is, "WPS" and "WHS", takes into account only the first of the start-days and the last of the stop-days within

TABLE 3 Start, end, and length of defined pollen seasons according to EAACI criteria in six European centers, 2018

		WPS		FPS Periods			WHS		FHS Periods			HD	
	Center	Start [a]	End [a]	(n)	Shortest	Longest	Start [a]	End [a]	(n)	Shortest	longest	(n)	Peak [b]
Cupressaceae	Valencia	*5 Mar*	*12 Dec*	4	8	15	*9 Mar*	*29 Mar*	2	3	7	17	720
	Marseille	*21 Jan*	*31 Dec*	7	5	43	*31 Jan*	*31 Mar*	7	3	6	37	3444
	Rome	*21 Jan*	*28 Apr*	4	11	24	-	-	-	-	-	7	226
	Messina	*16 Feb*	*21 Mar*	3	6	9	-	-	-	-	-	2	134
	Istanbul	*18 Feb*	*17 Mar*	3	6	8	-	-	-	-	-	6	618
	Izmir	*29 Jan*	*31 Mar*	1	62	62	*6 Feb*	*22 Mar*	4	3	24	47	6830
Fagales	Valencia	-	-	-	-	-	-	-	-	-	-	-	42
	Marseille	*17 Feb*	*29 Apr*	3	10	19	-	-	-	-	-	7	679
	Rome	*2 Jan*	*24 Jun*	5	6	58	*10 Apr*	*12 May*	2	6	7	22	531
	Messina	-	-	-	-	-	-	-	-	-	-	-	81
	Istanbul	-	-	-	-	-	-	-	-	-	-	-	12
	Izmir	-	-	-	-	-	-	-	-	-	-	-	17
Oleaceae	Valencia	*28 May*	*18 Jul*	1	22	22	-	-	-	-	-	2	174
	Marseille	*16 Apr*	*31 May*	2	8	14	-	-	-	-	-	3	168
	Rome	*23 Apr*	*2 May*	1	10	10	-	-	-	-	-	-	78
	Messina	-	-	-	-	-	-	-	-	-	-	-	67
	Istanbul	-	-	-	-	-	-	-	-	-	-	-	25
	Izmir	*11 Apr*	*6 Jun*	1	57	57	*21 Apr*	*31 May*	3	9	17	39	237
Poaceae	Valencia	*8 May*	*16 Jul*	3	16	28	-	-	-	-	-	1	53
	Marseille	*13 Apr*	*5 Aug*	1	115	115	-	-	-	-	-	4	113
	Rome	*5 Apr*	*16 Jul*	3	10	64	*2 May*	*3 Jun*	2	3	3	13	115
	Messina	*30 Mar*	*26 Jul*	5	8	34	-	-	-	-	-	-	20
	Istanbul	*29 May*	*25 Jun*	1	28	28	-	-	-	-	-	-	12
	Izmir	*25 Feb*	*16 Nov*	3	3	13	*4 Apr*	*31 May*	3	3	43	57	167
Urticaeae	Valencia	*8 Mar*	*31 Dec*	8	6	28	-	-	-	-	-	-	45
	Marseille	*3 Apr*	*15 Sep*	2	10	152	*18 Jun*	*25 Jun*	-	-	-	13	121
	Rome	*14 Jan*	*18 Sep*	7	7	68	*2 Apr*	*8 Apr*	1	7	7	19	193
	Messina	*1 Jan*	*29 Dec*	6	7	166	*3 Jan*	*16 Jul*	12	3	22	115	634
	Istanbul	*7 Apr*	*30 Apr*	2	9	10	-	-	-	-	-	-	23
	Izmir	*24 Feb*	*28 Sep*	6	9	26	-	-	-	-	-	-	14
Ambrosia spp.	Valencia	-	-	-	-	-	-	-	-	-	-	-	0
	Marseille	*14 Aug*	*24 Sep*	2	11	29	-	-	-	-	-	2	105
	Rome	-	-	-	-	-	-	-	-	-	-	-	1
	Messina	-	-	-	-	-	-	-	-	-	-	-	1
	Istanbul	*3 Aug*	*10 Sep*	1	39	39	-	-	-	-	-	6	147
	Izmir	-	-	-	-	-	-	-	-	-	-	-	0
Artemisia spp.	Valencia	-	-	-	-	-	-	-	-	-	-	-	5
	Marseille	*19 Sep*	*28 Sep*	-	-	-	-	-	-	-	-	2	57
	Rome	-	-	-	-	-	-	-	-	-	-	-	2
	Messina	-	-	-	-	-	-	-	-	-	-	-	14
	Istanbul	*3 Aug*	*10 Aug*	1	8	8	-	-	-	-	-	-	8
	Izmir	-	-	-	-	-	-	-	-	-	-	-	-

Abbreviations: FHS fragmented high season; FPS, fragmented pollen season; HD, high days; WHS, whole high season; WPS, whole pollen season.

[a]Start and end date of the defined season.

[b]Pollen concentration in pollen grains/m^3.

TABLE 4 Monitoring period for three relevant pollens in comparison with proportion of no/low pollen days[a] in six Southern European cities

Pollen		Monitoring period			Extra days with low pollen[a]					
		Start	End	Days	VAL	MAR	ROM	MES	IST	IZM
Cupressaceae	WPS	*21 Jan*	*31 Dec*	345	43	0	228	292	298	264
	WHS	*31 Jan*	*31 Mar*	60	39	0	-	-	-	15
Poaceae	WPS	*25 Feb*	*16 Nov*	265	195	150	162	146	237	0
	WHS	*4 Apr*	*3 Jun*	61	-	-	28	-	-	3
Urticaceae	WPS	*1 Jan*	*31 Dec*	365	66	199	127	2	341	148
	WHS	*3 Jan*	*16 Jul*	195	-	187	188	0	-	-

[a]Number of days with pollen concentration below given thresholds.
"-" season definition not applicable.

the calendar year 2018. This definition produced in some cases very long WPS (eg, 363 days for Urticaceae in Messina) and long WHS including intercurrent periods of more than 30 days of low pollen counts (eg, Cupressaceae in Marseille; Urticaceae in Messina).

- The second category, that is, "fragmented pollen season" (FPS) and "fragmented high season" (FHS) produced shorter, more precise, multiple segments, which were well separated by the excluded, low pollen count periods.

This *"fragmentation method"* may be more relevant and useful at least in three circumstances: (a) when pollens with a bimodal distribution are considered (eg, Urticaceae in Valencia); (b) when a shorter unimodal pollen season is artefactually made bimodal by our calendar definition of the year (January 1st-December 31st) (eg, Cupressaceae in Marseille and Valencia); and (c) when we need to integrate pollen seasons of multiple species of the same family, pollinating in the same territory but with intercurrent periods and sharing cross-reacting major allergenic molecules (such as PR-10 from Fagales in Rome, Ole e 1-like protein family from Oleaceae in Marseille).[36] As we have seen for Cupressaceae and Urticaceae in Valencia, the fragmentation method can solve these issues only partially or the calendar definition of the year would need, for an individual pollen, another start and end date (eg, from November/October until March/April for Cupressaceae pollen in Valencia).

4.3 | Relevance for AIT trials

On the basis of the analysis of pollen seasons in Southern Europe, the relevance for AIT trials with pollen aeroallergens should be further elaborated. A unified pollen season (based on 2018 data) for our six cities should be retrospectively designed starting with the earliest start-day and ending with the latest end-day of each geographic area where the patients are recruited. We found that a unified grass pollen season for a multicenter trial of AIT in our six cities would last for 9.5 months, starting at the end of February and finishing at mid-November (Izmir). By excluding Izmir from our calculations, the grass pollen season would last four months, starting at the end of March (Messina) and finishing at the beginning of August (Marseille). However, even with this restriction, patients in Istanbul and in Valencia would be unnecessarily monitored for more than 50% of the study period. A logical consequence of our results is therefore that the monitoring periods of multicenter trials should be precisely differentiated to meet the local conditions and pollen seasons of each Southern European/Mediterranean area. This implies that in some cases a decision on which definition is used will strongly modify the outcome (diagnostic, therapeutic).[37] Concepts on the evaluation of personal pollen exposure, for example, via electronic devices could be possibly an additional tool in this given situation, as recently investigated.[19,29,38]

4.4 | Limitations

We have to consider a few limitations of our study. First, we had a percentage (although very limited) of missing pollen data in each of the centers. Second, our study is limited to six centers in four Mediterranean countries, and any conclusion we draw may not perfectly apply to all regions of such a broad and diverse geographic area. However, we believe that the inclusion of more centers, by increasing the heterogeneity of our results, would have reinforced our conclusions. Third, the study was performed on the basis of 2018 pollen data only, and the concept of fragmented season would need further confirmation with data from other years. Fourth, the height level of pollen traps is recommended on rooftops, which could lead to a possible underestimation of herbaceous pollen.[39] Last, periods of low pollen counts measured in the air can be also influenced by meteorological conditions; nonetheless, the impact on the clinical situation remains questionable since recent studies showed a nonlinear correlation between the amount of allergens and low pollen concentrations due to humidity.[40,41] However, we believe that the conclusions reached by our study are relatively independent from the year of dataset generation. Fifth, by summarizing the counts of multiple Fagales species, the local and individual impact per species cannot be distinguished and there are little data on the clinical relevance of some plants like Castanea or Fagus. Nevertheless, there is a high degree of sequence homology and sub-sequential cross-reactivity within the group of birch-homologous species. Therefore, for example, the European medicine agency (EMA) groups the following plants into one "Fagales group" for regulatory purposes of allergen products: Betula verrucosa (Birch), Alnus glutinosa (Alder), Carpinus betulus Hornbeam, Corylus avellana (Hazel), Quercus alba (Oak), Castanea sativa (Chestnut), and Fagus sylvatica (Beech).[42,43] Due to a strong cross-reactivity caused by PR-10 molecules, the majority of Fagales species will induce symptoms in patients sensitized and allergic to a source belonging to the birch-homologous group.[44]

4.5 | Conclusions

Seasons of major allergenic pollen are highly heterogeneous in terms of pattern, length, and periodicity in Southern European countries. A unifying pollen calendar or season cannot be established in such a climatically and aerobiologically complex geographic area. When applying the EAACI season criteria, validated in Central and Northern European countries, to Southern European regions, this results in very long seasons, which also include many days/weeks with no/low pollen counts. By excluding these "intercurrent periods" with no/low pollen counts ("fragmented pollen season" method), our results are offering a more precise use of the same EAACI methodology. The approach we propose appears to generate shorter, more specific, and accurate although fragmented pollen seasons in Southern European countries. Whether this approach will have a similar useful impact also in Central and Northern Europe remains to be tested.

5 | GLOSSARY

Whole pollen season (WPS) and whole high season (WHS)

Pollen season definitions according to EAACI criteria.[24]

Fragmented pollen season (FPS) and fragmented high season (FHS)

Pollen seasons defined according to EAACI criteria, but resulting in multiple segments (fragments), after the application of start and stop signals also *within* the season and not only at its start and end.

Intercurrent period

A period of low pollen concentrations interrupting a WPS or a WHS. It is generated by a stop signal (eg, for Fagales the last of 5 days (out of seven consecutive days) with ≥10 pollen grains/m^3, when the sum of these 5 days is ≥100 pollen grains/m^3) followed by a start signal (eg, for Fagales the first of 5 days (out of seven consecutive days) with ≥10 pollen grains/m^3, when the sum of these 5 days is ≥100 pollen grains/m^3).

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTION

Hoffmann TM, ACAR Sahin A, Aggelidis X, Arasi S, Barbalace A, Bourgoin A, Bregu B, Caglayan Sosmen S, Caminiti L, Charpin D, Couto M, Delgado L, Di Rienzo Businco A, Dimier C, Dimou M, Fonseca JA, Goksel O, Hernandez D, Jang DT, Kalpakioglu F, Lame B, Llusar R, Makris MP, Mazon A, Mesonjesi E, Nieto A, Öztürk A, Pahus L, Pajno GP, Panasiti I, Papadopoulos NG, Pelosi S, Pereira

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Brighetti A, Caeiro E, Dimou M, Guvensen A, Lame B, Pellegrini E, Pinar M, Suarez J, Thibaudon M, Travaglini A contributed to the aerobiological data collection. Panetta V contributed to the data analysis. Pfaar O contributed to the writing of the manuscript and provided expert revisions. All authors contributed to the writing and revisions of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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