

**“Whole” vs. “Fragmented” approach to EAACI Pollen Season Definitions:
A Multicenter Study in Six Southern European Cities**

Short title: Pollen Seasons in Southern European Countries

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Abbreviations

A.I.A.	Associazione Italiana di Aerobiologia
AIT	Allergen Immunotherapy
ARPACal	Agenzia Regionale per la Protezione dell'Ambiente della Calabria
EAN	European Aerobiology Network
EAACI	European Academy of Allergy and Clinical Immunology
FHS	Fragmented High Season
FPS	Fragmented Pollen Season
HD	High Days
R.I.M.A.-A.I.A.	Rete Italiana di Monitoraggio in Aerobiologia – Associazione Italiana di Aerobiologia
R.N.S.A.	Réseau National de Surveillance Aérobiologique
SAR	Seasonal Allergic Rhinitis
WHS	Whole High Season
WPS	Whole Pollen Season

Abstract

Background: The adequate definition of pollen seasons is essential to facilitate a correct diagnosis, treatment choice and outcomes 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 @IT2020 multi-center study, pollen counts for Poaceae, Oleaceae, Fagales, Cupressaceae, Urticaceae (*Parietaria* spp.) and Compositae (*Ambrosia* spp., *Artemisia* spp.) were collected from 1st January to 31st December 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 tested 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. 298 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.

Words: 243

Key words- pollen season, pollen allergy, seasonal allergic rhinitis, pollination period, Southern Europe

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 pollens 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 five years and structured in 36 sections of 10 days, usually color coded, e.g. 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 is 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 varied 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 the background pollen concentration.(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 evidence 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).

Methods

Study centers. The present study was conducted as part of the “@IT-2020” multi-center project, an observational longitudinal study on the combined impact of molecular IgE tests and mobile health technology in the diagnosis and allergen immunotherapy prescription for seasonal allergic rhinitis in Southern European countries. From 1st January to 31st December 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 e1].

Pollen Data. The pollens of Cupressaceae (Cupressaceae and Taxaceae), Fagales (Corylaceae, Fagaceae and Betulaceae), Oleaceae, Poaceae, Urticaceae, *Ambrosia* spp. and *Artemisia* spp. (Compositae) were monitored. Being a clinical study, Corylaceae, Fagaceae and Betulaceae were considered as a single group belonging to the Fagales botanical order as they all contain cross-reactive PR-10 allergens. 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), *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 liters of air per minute, which allows a continuous sampling for up to 7 days [Table e1]. The trap's surface for collecting pollen grains is a 7-days 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 Fuchsin 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 400x. Daily pollen concentrations are expressed as pollen grains per cubic meter air (pollen grains/m³) 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 was 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 R.N.S.A., R.I.M.A.-A.I.A., 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)

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 day of 5 days (out of 7 consecutive days) with ≥ 10 pollen grains/m³, when the cumulative pollen concentration of these 5 days is ≥ 100 pollen grains/m³. The season ends after the last day respecting the same conditions, i.e. the last of five days (out of 7 consecutive days) with ≥ 10 pollen grains/m³, when the sum of these 5 days is ≥ 100 pollen grains/m³. 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 (e.g. for Fagales the last of five days (out of 7 consecutive days) with ≥ 10 pollen grains/m³, when the sum of these 5 days is ≥ 100 pollen grains/m³) 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/m³ while for Poaceae and *Ambrosia* spp. it is fixed at ≥ 50 pollen grains/m³ [Table 1]. We have adopted the EAACI thresholds and criteria for Cupressaceae, Fagales, Poaceae, Oleaceae, Urticaceae, *Ambrosia* spp., and *Artemisia* spp. For Fagales (Betulaceae except *Betula* spp., Corylaceae and Fagaceae), 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)

Defined thresholds for the individual pollen seasons: see electronic repository.

Meteorological data: To assess the influence on pollen concentrations and season length, meteorological data from all six centers was analyzed: see electronic repository.

Results

Pollen count courses, by city – The count courses of all seven examined pollens showed similarities and differences among the six included cities. [Figure 1] 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 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.

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 **Whole Pollen Seasons** (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, from 23.04. to 02.05.), long for Fagales (174 days, from 02.01. to 24.06.), Poaceae (103 days, from 05.04. to 16.07.) and Cupressaceae (98 days, from 21.01. to 28.04.) and extremely long for Urticaceae (238 days, from 14.01. to 18.09.). After exclusion of intercurrent periods with low pollen counts, causing an interruption of the season, shorter fragments of **Pollen Seasons** (PS), consequently named here “**Fragmented Pollen Season**” (FPS), were generated, with 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** were split into 3 periods for Poaceae (range 10 to 64 days), into 4 periods for Cupressaceae (range 11 to 24 days), into 5 periods for Fagales (range 6 to 58 days), and into 7 periods for Urticaceae (range 7 to 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 2 periods each. The number of **High Days** (HD) ranged from 0 for Oleaceae to 22 for Fagales [Figure 2 and Table 2].

WPS and FPS in the six centers – We then applied the same methodology to all the other centers [Figure 3, Table 2 and 3]. We identified **WPS** for all seven pollens in Marseille, but only for three pollens (Cupressaceae, Poaceae, Urticaceae) in Messina, and for four pollens 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 e2]. The most FPS was that of Urticaceae in Valencia, with 8 different segments (ranging from 6 to 28 days) while the longest interruption period occurred during the Cupressaceae WPS in Valencia (201 days).

WHS, FHS and HD in the six centers (see electronic repository) - In contrast to seven WPS, only three WHS (Cupressaceae, Fagales, Urticaceae) were identified in Marseille [Figure 3, Table e2 and 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 pollens. 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 multi-center 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 e3]. 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 e3]. The comparison clearly showed, for all three pollens, that in the first “unified monitoring period”- solution the number of monitoring days is considerably (up to 298 days) [Table 4] higher than with the localized or flexible solution. Moreover, the proportion of no/low pollen days during which patients would have been monitored is, in the “unified monitoring period”- solution up to 47% for the Whole Pollen Season and up to 49% for the Whole High Season [Table 4].

Influence of rainfall on season length: see electronic repository

Discussion

We investigated the recently established EAACI definitions (24) and criteria for seven pollen taxa in six Southern European/Mediterranean cities. We found: 1) a great heterogeneity among the six cities in terms of pattern and length of the examined pollen seasons, showing especially long WPS and WHS in some centers; 2) 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*”; 3) 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.

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 **PS** 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).

Fragmented pollen season - 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 in two categories:

- The first category, i.e. “Whole Pollen Season” (WPS) and “Whole High Season” (WHS), takes into account only the first of the start-days and the last of the stop-days within the calendar year 2018. This definition produced in some cases very long **WPS** (e.g. 363 days for Urticaceae in Messina) and long **WHS** including intercurrent periods of more than 30 days of low pollen counts (e.g. Cupressaceae in Marseille; Urticaceae in Messina).
- The second category, i.e. “Fragmented Pollen Season” (**FPS**) and “Fragmented High Season” (**FHS**) produced shorter, more precise, multiple segments, which were well separated by the excluded, low-pollen counts periods.

This “*fragmentation method*” may be more relevant and useful at least in three circumstances: 1) when pollens with a bimodal distribution are considered (Urticaceae in Valencia); 2) when a shorter unimodal pollen season is artefactually made “bimodal” by our calendar definition of the year (Jan 1st to Dec 31st) (Cupressaceae in Marseille and Valencia); 3) 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 (e.g. from November/October until March/April for Cupressaceae pollen in Valencia).

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 areas 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 e.g. via electronic gadgets could be possibly an additional tool in this given situation, as recently investigated (19,29,38).

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, 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 non-linear 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 is little data on the clinical relevance of some plants like Castanea or Fagus. Nevertheless, there's 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 satavia (Chestnut), 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).

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.

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 Whole Pollen Season or a Whole High Season. It is generated by a stop signal (e.g. for Fagales the last of five days (out of 7 consecutive days) with ≥ 10 pollen grains/m³, when the sum of these 5 days is ≥ 100 pollen grains/m³) followed by a start signal (e.g. for Fagales the first of five days (out of 7 consecutive days) with ≥ 10 pollen grains/m³, when the sum of these 5 days is ≥ 100 pollen grains/m³).

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Legends to Figures

Figure 1 - Daily pollen concentration (pollen grains/m³) of Cupressaceae, Fagales, Oleaceae, Poaceae, Urticaceae, *Ambrosia* spp., and *Artemisia* spp. in six Southern European/Mediterranean cities in 2018.

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.

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 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.

Table 1 - Overview of seasons definition criteria in accordance to the EAACI position paper [24]. Thresholds[°] for Whole Pollen Season (WPS), Whole High Pollen Season (WHS) and High Days (HD) are presented.

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

[°] Equal or higher than the indicated value in pollen grains/m³

[#] Adaptation of the EAACI Criteria for the present study as these pollen species had not been included in the EAACI Position Paper

Table 2- Start, end and length (days) of pollen seasons according to EAACI criteria in Rome during 2018.[°]

Pollen	WPS			FPS				WHS			FHS				HD
	date			segments [#]				date			segments [#]				days
	start	end	days	n	shortest	longest	days	start	end	days	n	shortest	longest	days	
Cupressaceae	21.1	28.4	98	4	11	24	70	-	-	-	-	-	-	-	7
Fagales	2.1	24.6	174	5	6	58	105	10.4	12.5	33	2	6	7	13	22
Oleaceae	23.4	2.5	10	1	10	10	10	-	-	-	-	-	-	-	-
Poaceae	5.4	16.7	103	3	10	64	85	2.5	3.6	33	2	3	3	6	13
Urticaceae	14.1	18.9	238	7	7	68	180	2.4	8.4	7	1	7	7	7	19
<i>Ambrosia</i> spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Artemisia</i> spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[°] WPS = whole pollen season including *bridged gaps*, PS = pollen season, WHS = high pollen season including *bridged gaps*, HD = *high days*.

[#] Season is divided in >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

Table 3 - Start, end and length of defined pollen seasons according to EAACI criteria in six European centers, 2018.*

	Center	WPS		FPS Periods			WHS		FHS Periods			HD	
		start [§]	end [§]	(n)	shortest	longest	start	end [§]	(n)	shortest	longest	(n)	peak*
Cupressaceae	Valencia	5.3	12.12	4	8	15	9.3	29.3	2	3	7	17	720
	Marseille	21.1	31.12	7	5	43	31.1	31.3	7	3	6	37	3444
	Rome	21.1	28.4	4	11	24	-	-	-	-	-	7	226
	Messina	16.2	21.3	3	6	9	-	-	-	-	-	2	134
	Istanbul	18.2	17.3	3	6	8	-	-	-	-	-	6	618
	Izmir	29.1	31.3	1	62	62	6.2	22.3	4	3	24	47	6830
Fagales	Valencia	-	-	-	-	-	-	-	-	-	-	-	42
	Marseille	17.2	29.4	3	10	19	-	-	-	-	-	7	679
	Rome	2.1	24.6	5	6	58	10.4	12.5	2	6	7	22	531
	Messina	-	-	-	-	-	-	-	-	-	-	-	81
	Istanbul	-	-	-	-	-	-	-	-	-	-	-	12
	Izmir	-	-	-	-	-	-	-	-	-	-	-	17
Oleaceae	Valencia	28.5	18.7	1	22	22	-	-	-	-	-	2	174
	Marseille	16.4	31.5	2	8	14	-	-	-	-	-	3	168
	Rome	23.4	2.5	1	10	10	-	-	-	-	-	-	78
	Messina	-	-	-	-	-	-	-	-	-	-	-	67
	Istanbul	-	-	-	-	-	-	-	-	-	-	-	25
	Izmir	11.4	6.6	1	57	57	21.4	31.5	3	9	17	39	237
Poaceae	Valencia	8.5	16.7	3	16	28	-	-	-	-	-	1	53
	Marseille	13.4	5.8	1	115	115	-	-	-	-	-	4	113
	Rome	5.4	16.7	3	10	64	2.5	3.6	2	3	3	13	115
	Messina	30.3	26.7	5	8	34	-	-	-	-	-	-	20
	Istanbul	29.5	25.6	1	28	28	-	-	-	-	-	-	12
	Izmir	25.2	16.11	3	3	13	4.4	31.5	3	3	43	57	167
Urticaceae	Valencia	8.3	31.12	8	6	28	-	-	-	-	-	-	45
	Marseille	3.4	15.9	2	10	152	18.6	25.6	-	-	-	13	121
	Rome	14.1	18.9	7	7	68	2.4	8.4	1	7	7	19	193
	Messina	1.1	29.12	6	7	166	3.1	16.7	12	3	22	115	634
	Istanbul	7.4	30.4	2	9	10	-	-	-	-	-	-	23
	Izmir	24.2	28.9	6	9	26	-	-	-	-	-	-	14
Ambrosia spp.	Valencia	-	-	-	-	-	-	-	-	-	-	-	0
	Marseille	14.8	24.9	2	11	29	-	-	-	-	-	2	105
	Rome	-	-	-	-	-	-	-	-	-	-	-	1
	Messina	-	-	-	-	-	-	-	-	-	-	-	1
	Istanbul	3.8	10.9	1	39	39	-	-	-	-	-	6	147
	Izmir	-	-	-	-	-	-	-	-	-	-	-	0
Artemisia spp.	Valencia	-	-	-	-	-	-	-	-	-	-	-	5
	Marseille	19.9	28.9	-	-	-	-	-	-	-	-	2	57
	Rome	-	-	-	-	-	-	-	-	-	-	-	2
	Messina	-	-	-	-	-	-	-	-	-	-	-	14
	Istanbul	3.8	10.8	1	8	8	-	-	-	-	-	-	8
	Izmir	-	-	-	-	-	-	-	-	-	-	-	4

* WPS = whole pollen season with bridged gaps, PS = pollen season, WHS = high pollen season with bridged gaps, HD = high days.

[§] start and end date of the defined season

* pollen concentration in pollen grains/m³

Table 4 - Monitoring period for three relevant pollen in comparison to proportion of no/low pollen days* in six Southern Europe cities.

Pollen		monitoring period			extra days with low pollen*°					
		start	end	days	VAL	MAR	ROM	MES	IST	IZM
Cupressaceae	WPS	21.1	31.12	345	43	0	228	292	298	264
	WHS	31.1	31.3	60	39	0	-	-	-	15
Poaceae	WPS	25.2	16.11	265	195	150	162	146	237	0
	WHS	4.4	3.6	61	-	-	28	-	-	3
Urticaceae	WPS	1.1	31.12	365	66	199	127	2	341	148
	WHS	3.1	16.7	195	-	187	188	0	-	-

* number of days with pollen concentration below given thresholds

° "-" no season definition applicable

Figure 1

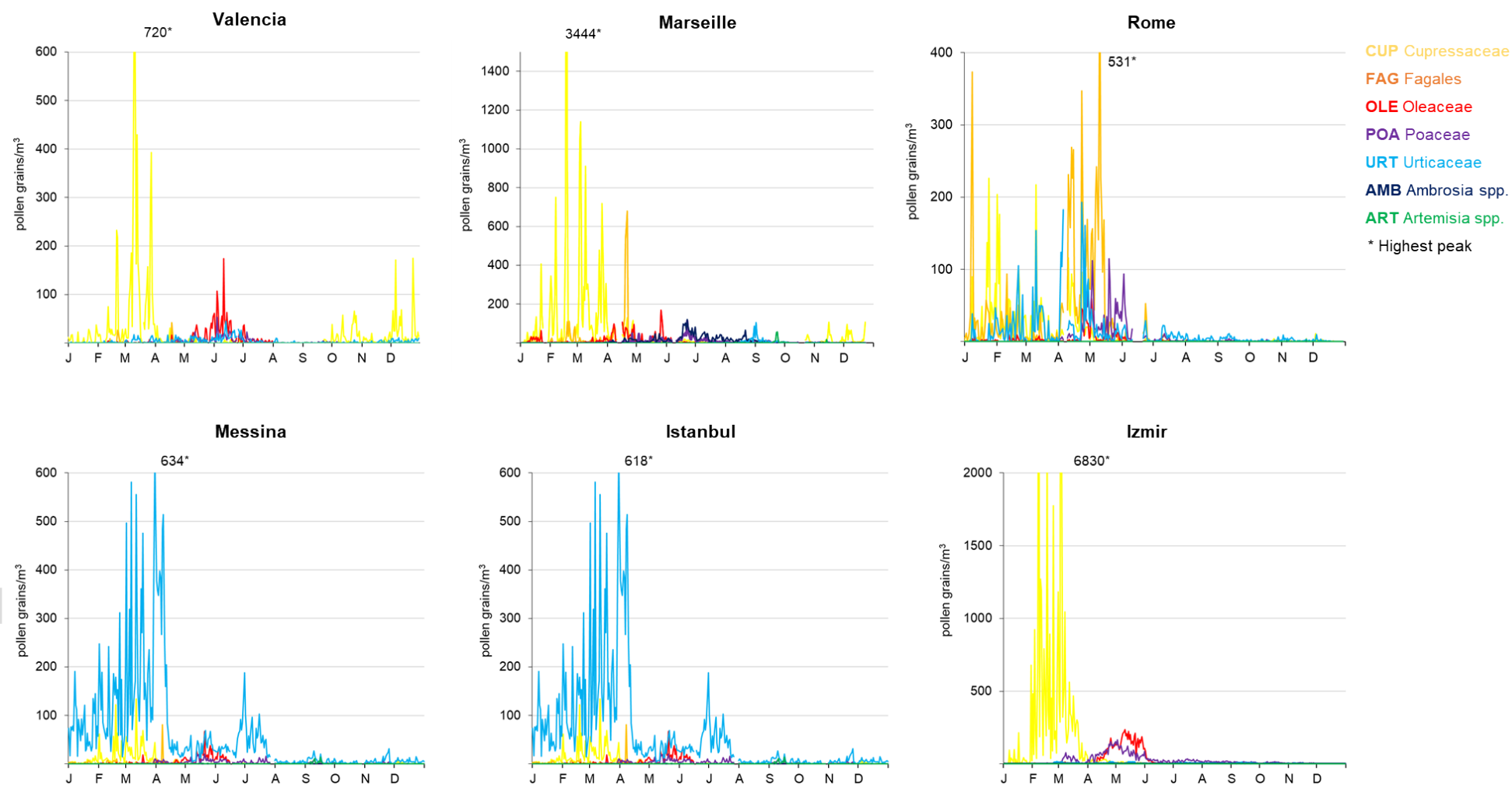
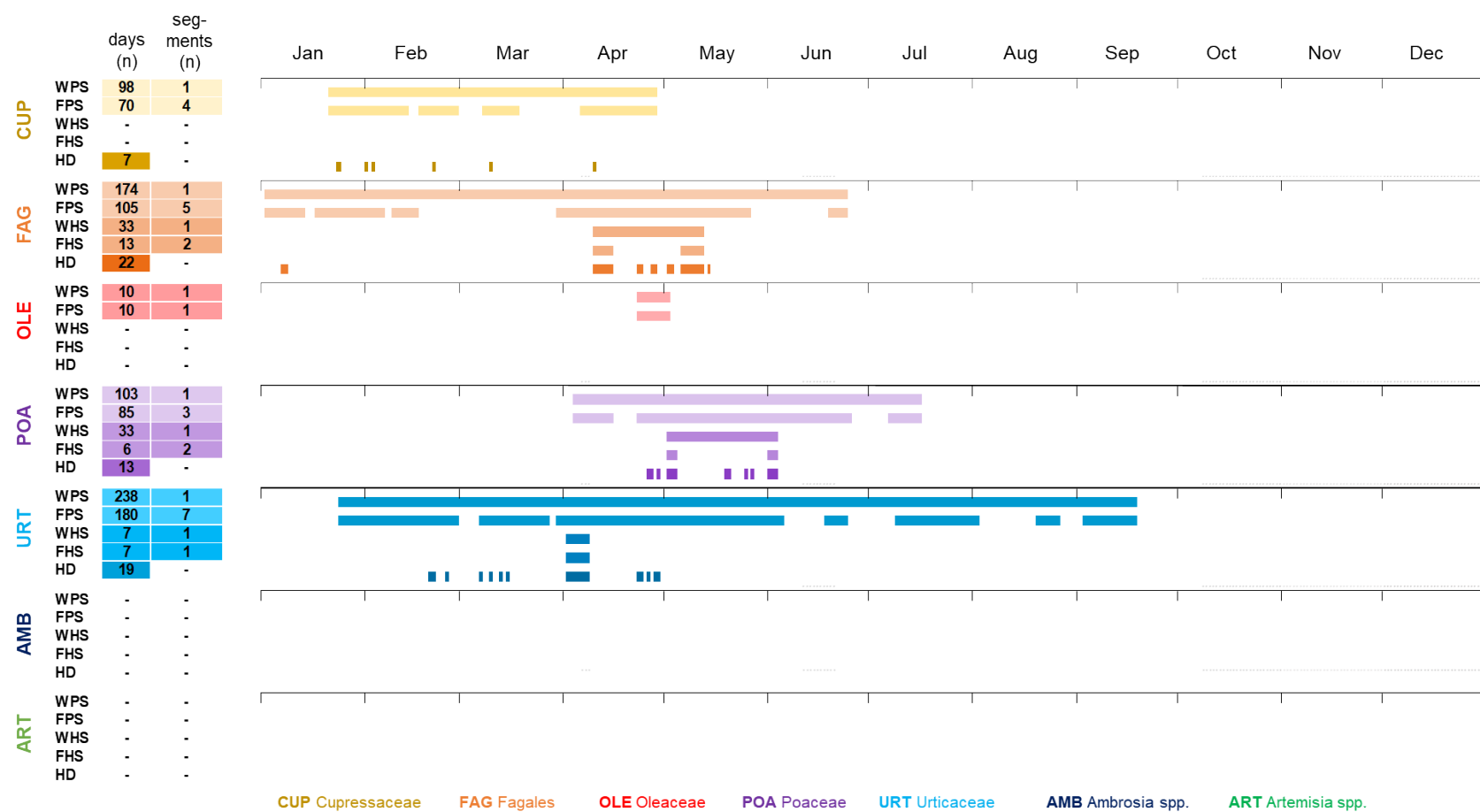


Figure 2



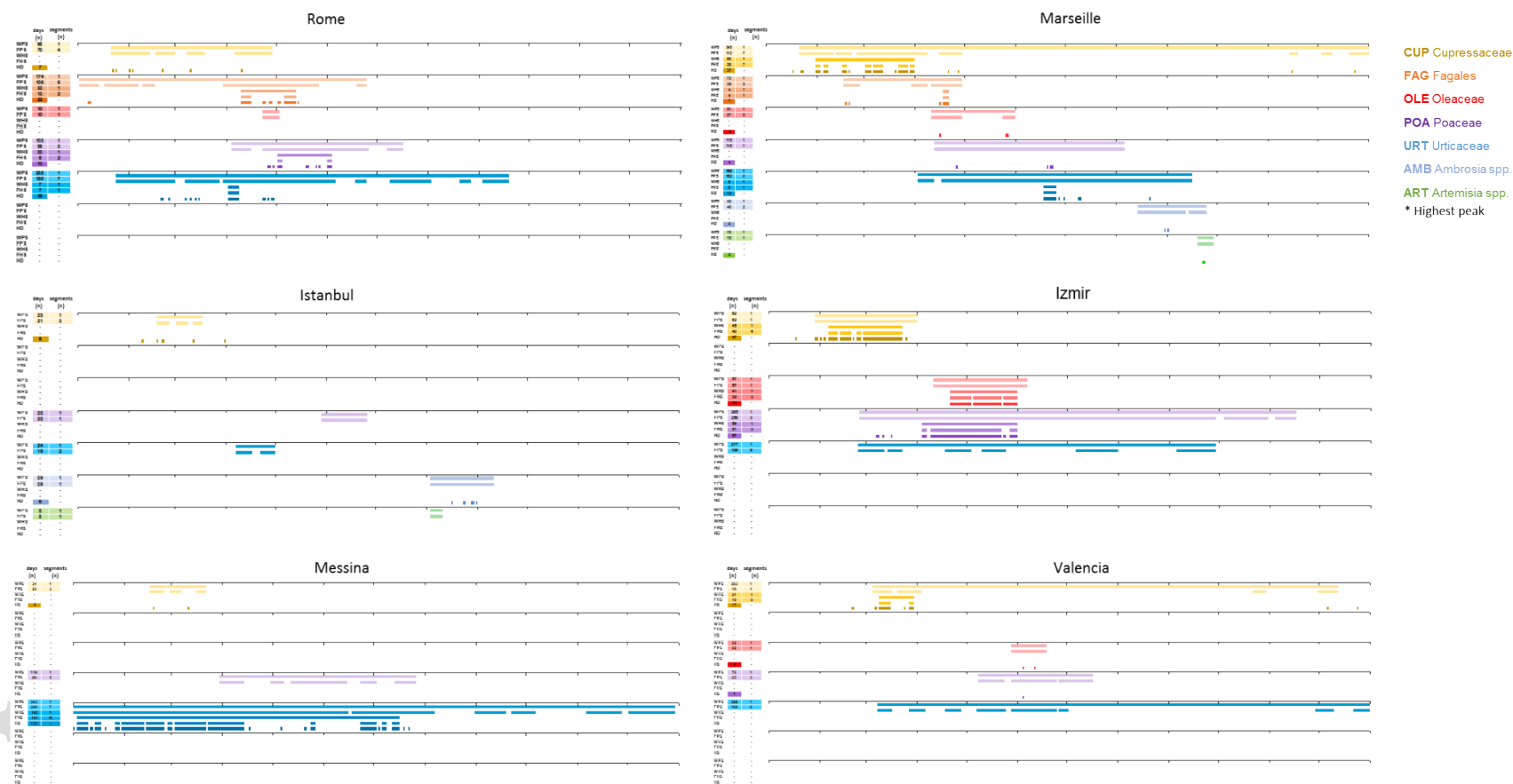


Figure 3

**“Whole” vs. “Fragmented” approach to EAACI Pollen Season Definitions:
A Multicenter Study in Six Southern European Cities**

Short title: Pollen Seasons in Southern European Countries

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Methods

Meteorological Data

In order to assess the influence of rainfall on pollen concentrations and therefore season length, we acquired meteorological data from all six centers. The daily amounts of rainfall in mm were provided by experienced and established meteorological centers, namely the Valencia Institute of Agricultural Investigation, Meteo France, the Meteorological Station of University “Tor Vergata” Rome, Centro Funzionale Multirischi ARPACAL, Bornova/Izmir meteorological station and the Turkish State Meteorological Service. Chi square test was used to calculate p-values, $p < 0.05$ was considered statistically relevant.

Results

WHS, HS and HD in the six centers - After exclusion of the intercurrent periods, most **High Seasons** (HS) could still be identified. The strongest impact of the exclusion method was observed in the case of Urticaceae in Messina: the length dropped from 195 (**WHS**) to 104 days (**FHS**), 12 different **FHS** segments (range from 3 to 22 days) were identified, with two intercurrent periods with 27 and 40 days. In contrast, the impact of the exclusion of the intercurrent periods was much smaller for other pollen and centers [**Figure e2**]. Overall, the seasons (**WPS** and **WHS**) generated by the EAACI criteria are extremely long in some centers. Last, the number and distribution of **HD** throughout the year strongly reflected the distribution of pollen peaks [**Figure 1**]. We observed sporadic high days in Istanbul, in the case of Cupressaceae and *Ambrosia* spp., and in Marseille for Oleaceae, Poaceae, *Ambrosia* spp., and *Artemisia* spp. [**Figure 3**]. In contrast, long sequences of **HD** could be observed in Messina for Urticaceae, where each segment of the **FHS** presented only high days [**Figure 3**].

Influence of rainfall on season length - The meteorological data show a higher number of rainy days as well as higher average amounts of rainfall per day among the excluded days (intercurrent period) than the days included in the fragmented pollen seasons. The numbers represent the expected phenomenon of lower pollen counts during rainy days. This difference is statistically significant for single periods and in five of the six centers [**Table e3**].

Discussion

Characteristics of pollen abundancy in Southern European countries (see electronic repository)

For example, birch pollen seasons are relatively uniform in Scandinavian countries (e2) and grass or birch pollen seasons are also less heterogenous across Germany (e3) than across Italy, although in

Italy the presence of birch is mainly concentrated in the northern region. A similar heterogeneity can be observed for the other Fagaceae, e.g. oak, not only in Italy, but in the Mediterranean basin.(e4-e6) However, this heterogeneity in pollen seasons is reflecting the great complexity and diversity of the climate and vegetation in the six cities.(e4) For example, Urticaceae favor a damp climate with mild winter periods and are well adapted to the material of wall constructions of Southern Italy. This is reflected in our results, confirming its perennial presence in the air (see Messina). (e7-e10) In contrast, Urticaceae only appears in low abundance in Izmir's atmosphere, reflecting aerobiological heterogeneity in Mediterranean countries. (e11) Cupressaceae have been abundantly cultivated in Marseille over centuries (e2,e12) and also increasingly in Rome (e2,e13). In the whole Mediterranean basin *Cupressus* is distributed. (e14) This tree is commonly planted and cultivated in urban areas, parks, and utilized as windbreakers in the countryside.(e15)

Fragmented pollen season - Segments were generated by multiple "start-days" and "stop-days" (i.e. days with the characteristics required to start or end a season). This fragmentation was not registered in the previous studies performed in Northern or Central European countries, where pollen seasons are shorter and continuous, so that only one start-day and one stop-day could be identified.(2) In our study, this phenomenon may be due to several factors. For example, meteorological factors (e.g. persistent or prolonged rains during spring) may have interrupted long pollen seasons by lowering the amount of pollen in the atmosphere for many days.

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186

187 **Legends to figures**

188 **Figure e1** - Daily pollen concentration (pollen grains/m³) of Cupressaceae, Fagales, Oleaceae,
189 Poaceae, Urticaceae, *Ambrosia* spp., and *Artemisia* spp. in six Southern European/Mediterranean
190 cities in 2018.

191 **Figure e2** - Difference in length (number of days) between pollen seasons of Cupressaceae, Fagales,
192 Oleaceae, Poaceae, Urticaceae, *Ambrosia* spp., and *Artemisia* spp. defined as a Whole Pollen Season
193 (WPS) vs. Fragmented Pollen Season (FPS) in (a) and Whole High Season (WHS) vs. Fragmented High
194 Season (FHS) in (b) in six Southern European/Mediterranean cities in 2018.

195 **Figure e3** - Comparison between a “unified” (monitoring period) vs. “localized” season approach to
196 definition of pollen seasons of Cupressaceae, Poaceae, and Urticaceae in six Southern
197 European/Mediterranean cities for participating patients in potential AIT trials. The overall length of
198 the monitoring periods (red bars representing number of days during the month) is reported. (a)
199 showing the potential Whole Pollen Season monitoring period (WPS MP) in comparison to the
200 individual Whole Pollen Season (WPS) length in the centers and (b) comparing the Whole High
201 Season monitoring period (WHS MP) and Whole High Season (WHS) length.

Centers	Climate		Aerobiologists			Pollen Traps			Missing days	
	mean temp (°C)	annual rainfall (mm)	affiliation	experience (y)^	individually counted pollen types (n)	pollen trap	altitude (m)	coordinates	n	longest period (n)^#
Istanbul Turkey	15.6	803	Ankara University	8-29	> 50	Burkard	73	41°01'26.11" N 28°55'02.34" E	0	0
Izmir Turkey	19.6	565	Ege University	13	> 50	Lanzoni	27	38°27'32.9" N 270°13'18.6" E	0	0
Marseille France	15.5	515	R.N.S.A.	29	> 75	Lanzoni	28	43°17'12.48" N 05°22'44.76" E	29	7
Messina Italy	18.1	547	ARPACal	5	44	Burkard	85	38°10'8.375" N 15°40'5.25" E	8	4
Rome Italy	16.6	1007	R.I.M.A.-A.I.A.	21	70	Lanzoni	80	41°51'16" N 12°36'19" E	14	10
Valencia Spain	18.2	475	University of Oviedo	21	50	Burkard	61	39°28'44" N 0°21'42" W	4	1

* pollen counts collected from 01.01 to 31.12 2018 (365 days); all readings were done with a continuous stripe, field diameter 0.5mm, magnification 400x

^ aerobiological and pollen monitoring experience of the correspondent aerobiologist (years)

number of consecutive missing days

Table e2- Length (days) and mean pollen count (pollen grains/m³) of pollen seasons, defined according to EAACI criteria in six cities throughout 2018.*

<i>Pollen Seasons</i>		<i>Valencia</i>		<i>Marseille</i>		<i>Rome</i>		<i>Messina</i>		<i>Istanbul</i>		<i>Izmir</i>	
		days	mean	days	mean	days	mean	days	mean	days	mean	days	mean
Cupressaceae	WPS	283	21	345	58	98	40	34	34	28	79	62	593
	FPS	48	110	112	170	70	52	22	38	21	103	62	593
	WHS	21	186	60	263	-	-	-	-	-	-	45	751
	FHS	10	332	28	516	-	-	-	-	-	-	40	827
	HD	17	259	37	428	7	168	2	128	6	320	47	770
Fagales	WPS	-	-	72	52	174	45	-	-	-	-	-	-
	FPS	-	-	39	89	105	71	-	-	-	-	-	-
	WHS	-	-	4	487	33	150	-	-	-	-	-	-
	FHS	-	-	4	487	13	236	-	-	-	-	-	-
	HD	-	-	7	324	22	222	-	-	-	-	-	-
Oleaceae	WPS	22	48	51	35	10	33	-	-	-	-	57	121
	FPS	22	48	27	55	10	33	-	-	-	-	57	121
	WHS	-	-	-	-	-	-	-	-	-	-	41	151
	FHS	-	-	-	-	-	-	-	-	-	-	39	155
	HD	2	141	3	125	-	-	-	-	-	-	39	155
Poaceae	WPS	70	9	115	13	103	19	119	5	28	5	265	31
	FPS	65	9	115	13	85	23	80	6	28	5	256	32
	WHS	-	-	-	-	33	38	-	-	-	-	58	84
	FHS	-	-	-	-	6	74	-	-	-	-	51	90
	HD	1	53	4	69	13	72	-	-	-	-	57	87
Urticaceae	WPS	299	4	166	20	238	17	363	60	24	6	217	4
	FPS	102	10	162	21	180	22	300	72	19	8	106	6
	WHS	-	-	8	85	7	21	195	105	-	-	-	-
	FHS	-	-	8	85	7	21	104	170	-	-	-	-
	HD	-	-	13	77	19	7	115	160	-	-	-	-
Ambrosia spp.	WPS	-	-	42	14	-	-	-	-	39	21	-	-
	FPS	-	-	40	15	-	-	-	-	39	21	-	-
	WHS	-	-	-	-	-	-	-	-	-	-	-	-
	FHS	-	-	-	-	-	-	-	-	-	-	-	-
	HD	-	-	2	96	-	-	-	-	6	75	-	-
Artemisia spp.	WPS	-	-	10	16	-	-	-	-	8	4	-	-
	FPS	-	-	10	16	-	-	-	-	8	4	-	-
	WHS	-	-	-	-	-	-	-	-	-	-	-	-
	FHS	-	-	-	-	-	-	-	-	-	-	-	-
	HD	-	-	2	56	-	-	-	-	-	-	-	-

* WPS = whole pollen season with bridged gaps, PS = pollen season, WHS = high pollen season with bridged gaps, HD = high days.

Table e3 - Rainy days (>3mm per day) in- and excluded from the fragmented pollen season in six centers.

				days incl in FPS				days excl in FPS				
						mm rain				mm rain		
		incl	excl	n	%	mean/d	cum	n	%	mean/d	cum	p value*
Valencia	CUP	48	235	1	2.1	0.4	17.4	22	9.4	2.1	496.4	0.144
	FAG	0	0	0	-	-	-	0	-	-	-	n/a
	OLE	22	0	2	9.1	1.7	36.8	0	-	-	-	n/a
	GRA	65	5	2	3.1	0.6	36.8	1	20.0	1.3	6.3	0.202
	PAR	102	197	2	2.0	0.4	36.8	22	11.2	2.5	485.2	0.005
Marseille	CUP	112	233	8	7.1	0.5	61.1	45	19.3	2.4	570.4	0.003
	FAG	39	33	3	7.7	1.0	39.0	6	18.2	1.1	37.8	0.285
	OLE	27	24	5	18.5	1.9	50.0	10	41.7	2.2	53.3	0.285
	GRA	115	0	16	13.9	0.8	95.8	0	-	-	-	n/a
	PAR	162	4	21	13.0	0.9	147.4	0	-	-	-	n/a
Rome	CUP	70	28	14	20.0	2.7	188.1	11	39.3	4.2	116.6	0.048
	FAG	105	69	18	17.1	1.8	189.2	24	34.8	3.7	254.8	0.008
	OLE	10	0	0	-	-	-	0	-	-	-	n/a
	GRA	85	18	16	18.8	2.1	175.2	0	-	-	-	n/a
	PAR	180	58	35	19.4	2.2	404.8	15	25.9	2.9	165.8	0.354
Messina	CUP	22	12	6	27.3	2.5	54.2	3	25.0	2.9	35.0	0.999
	FAG	0	0	0	-	-	-	0	-	-	-	n/a
	OLE	0	0	0	-	-	-	0	-	-	-	n/a
	GRA	80	39	2	2.5	0.3	24.2	3	7.7	3.3	129.8	0.329
	PAR	300	63	37	12.3	1.4	433.8	18	28.6	7.1	444.2	0.001
Istanbul	CUP	21	7	7	33.3	2.4	51.4	6	85.7	7.1	49.6	0.029
	FAG	0	0	0	-	-	-	0	-	-	-	n/a
	OLE	0	0	0	-	-	-	0	-	-	-	n/a
	GRA	28	0	5	17.9	1.8	51.2	0	-	-	-	n/a
	PAR	19	5	0	-	-	-	0	-	-	-	n/a
Izmir	CUP	62	0	16	25.8	2.8	171.1	0	-	-	-	n/a
	FAG	0	0	0	-	-	-	0	-	-	-	n/a
	OLE	57	0	3	5.3	1.1	60.5	0	-	-	-	n/a
	GRA	256	9	14	5.5	0.8	194.1	2	22.2	4.2	37.6	0.096
	PAR	106	111	7	6.6	1.0	110.8	7	6.3	0.8	83.3	1.000

* p values for comparison of %_{incl} and %_{excl}, a p value of <0.05 was considered statistically significant

