Aus der Klinik für Pädiatrie m.S. Pneumologie, Immunologie und Intensivmedizin
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Pollen Season Definitions in the Context of Seasonal Pollen Induced Allergic Rhinitis in Southern European Countries

Pollensaisondefinitionen im Kontext der saisonalen polleninduzierten allergischen Rhinitis in südeuropäischen Ländern

zur Erlangung des akademischen Grades
Doctor medicinae (Dr. med.)

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Abbreviations

@IT.2020	AIT (allergen-specific immunotherapy).2020 (year) study acronym
AIT	allergen-specific immunotherapy
ARIA	Allergic Rhinitis and its Impact on Asthma
CDSS	clinical decision support system
CRD	component-resolved diagnostics
e-Diary	electronic diary
EMA	European Medicine Agency
ENT	ear, nose and throat
ESEP test	Euroline Southern European Profile test from EUROIMMUN Lübeck, Germany
FHS	fragmented high season
FPS	fragmented pollen season
GINA	Global Initiative for Asthma
IUIS	International Union of Immunological Societies
mHealth	mobile health
PHD	patient's hayfever diary
sIgE	specific immunoglobulin E
SMP	symptom-monitoring period
SPT	skin prick tests
RTSS	Rhinoconjunctivitis Total Symptom Score
WHO	World Health Organization
WHS	whole high season
WPS	whole pollen season

Definitions

Fragmented Pollen Season [*Fragmentierung der Pollensaisons; Übersetzung durch die Autorin*]
'*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*.'[1]

Intercurrent period [*Zwischenperioden; Übersetzung durch die Autorin*]
'*A period of low pollen concentrations interrupting a WPS or a WHS. It is generated by a stop signal (e.g., 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 (e.g., 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*.'[1]

Abstrakt (Deutsch)

HINTERGRUND:

Bei poly-sensibilisierten Patienten mit pollen-induzierter allergischer Rhinitis stehen Mediziner vor der diagnostischen Herausforderung, das klinisch relevante Hauptallergen zu identifizieren, um eine adäquate allergenspezifische Immuntherapie (AIT) einzuleiten. Insbesondere in mediterranen Ländern erschweren überlappende Pollenflugzeiten und Kreuzsensibilisierungen die Diagnostik. In diesem Kontext wurde ein klinisches Entscheidungsunterstützungssystem (CDSS) entwickelt, dessen Einzelkomponenten im Rahmen der multizentrischen @IT.2020 Studie untersucht wurden. Ein Positionspapier der European Academy of Allergy and Clinical Immunology (EAACI) schlägt Kriterien für Pollensaisondefinitionen vor, insbesondere für die standardisierte Überwachung von symptomatischen Patienten in klinischen AIT-Studien.

ZIELSETZUNG:

Das Ziel dieser Arbeit ist die Untersuchung der im EAACI Positionspapier empfohlenen Pollensaisondefinitionen in vier Ländern Südeuropas für sieben Pollenarten.

METHODIK:

Im Rahmen der @IT.2020 Studie wurden in sechs Städten (Valencia, Marseille, Rom, Messina, Istanbul, Izmir) vom 1. Januar bis 31. Dezember 2018 tägliche Pollenkonzentrationen für Poaceae, Oleaceae, Fagales, Cupressaceae, Urticaceae (*Parietaria* spp.) und Compositae (*Ambrosia* spp., *Artemisia* spp.) erhoben. Basierend auf diesen Daten wurden die jeweiligen Pollensaisons nach EAACI-Kriterien identifiziert. Für Urticaceae, Poaceae und Cupressaceae wurde ein potenzieller einheitlicher Überwachungszeitraum für Patienten in AIT-Studien erstellt.

ERGEBNISSE:

Die Untersuchung ergab eine ausgeprägte Heterogenität hinsichtlich Muster und Länge der untersuchten Pollensaisons, sowohl zwischen als auch innerhalb der Länder. Durch Hinzufügen eines auf EAACI-Kriterien basierenden Stoppsignals, wurden Perioden mit geringerer Pollenkonzentration („Zwischenperioden") ausgeschlossen. Daraus resultierte eine Fragmentierung in maximal 8 Segmente für die Gesamt-Pollensaison und in maximal 12 Segmente für die Hochsaison. Potenzielle Überwachungszeiträume für AIT-

Studien umfassten bis zu 341 Tage mit geringer Pollenbelastung (unterhalb der EAACI-Grenzwerte). Der Ausschluss von „Zwischenperioden" („Fragmentierte Pollensaison"-Methode) verbessert die Genauigkeit der EAACI-Kriterien.

SCHLUSSFOLGERUNG:

Auf Anwendung der EAACI-Kriterien basierende Symptomüberwachungszeiträume in AIT Studien beinhalten teils lange Zeiträume mit niedrigen Pollenkonzentrationen. Dies ist insbesondere mit Blick auf die Beurteilung von Therapieerfolgen und Studien-Outcomes problematisch, denn diese basieren häufig auf der Symptombewertung innerhalb festgelegter Expositionszeiträume. Um eine mögliche Verzerrung zu minimieren, wird die Verwendung fragmentierter, auf EAACI-Kriterien basierender, Pollensaisons für Südeuropa empfohlen. Dieser Ansatz sollte in weiteren Studien untersucht werden.

Abstract (English)

BACKGROUND:

The identification of the causative pollen in polysensitized patients with seasonal allergic rhinitis (SAR) is of particular challenge to healthcare professionals. As this diagnostic step is essential for the prescription of allergen-specific immunotherapy (AIT), the only disease-modifying treatment of SAR, a clinical decision support system (CDSS) was designed in the @IT.2020 multicenter study to assist professionals with this task. The diagnostic decision process is complicated by overlapping pollination periods, which are particularly prominent in regions with Mediterranean climate such as Southern Europe. A position paper by the European Academy of Allergy and Clinical Immunology (EAACI) suggests criteria to define pollen seasons for medical purposes, especially for standardized monitoring of symptomatic patients in clinical trials testing AIT.

OBJECTIVE:

This present thesis aims to test definitions of pollen seasons proposed by EAACI in four Mediterranean countries for seven pollen taxa.

METHODS:

From January 1 to December 31, 2018, pollen data was collected daily in six cities (namely Valencia, Marseille, Rome, Messina, Istanbul and Izmir) for Poaceae, Oleaceae, Fagales, Cupressaceae, Urticaceae (*Parietaria* spp.) and Compositae (*Ambrosia* spp., *Artemisia* spp.) as part of the @IT.2020 multicenter study. EAACI criteria were applied to the collected data and pollen seasons were identified. On the basis of these seasonal definitions, a potential unified monitoring period for patients sensitized to Urticaceae, Poaceae and Cupressaceae in AIT trials was determined.

RESULTS

The analysis revealed a wide variance in the patterns and lengths of the investigated pollen seasons, both within and between countries. By adding a stop signal according to the EAACI criteria and, consequently, excluding periods of lower pollen concentration (i.e., intercurrent periods), a fragmentation of the seasons was observed. A fragmentation into eight and twelve segments was found for the fragmented pollen seasons and the fragmented high seasons, respectively. Potential monitoring periods for AIT trials included up to 341 days with low pollen exposure (i.e., below EAACI thresholds). The removal of intercurrent periods with no or low pollen counts (i.e., by the fragmented pollen season method) improved the precision of EAACI criteria.

CONCLUSION

Potential symptom-monitoring periods based on EAACI criteria include intermittent periods with low pollen concentrations. This is particularly problematic regarding the assessment of AIT effectiveness as it is based on the evaluation of that patients' symptoms within the defined pollen exposure periods. To improve the precision of exposure-related outcomes, fragmented pollen seasons based on EAACI criteria are recommended for Southern Europe. This approach should be further investigated.

Manteltext

Der Manteltext stellt im Folgenden eine Vertiefung des Studienhintergrundes, des derzeitigen Forschungsstandes, der Methodik, der Ergebnisse, sowie der Diskussion dieser zugrundeliegenden Publikation mit dem Titel: „"Whole" vs. "fragmented" approach to EAACI pollen season definitions: A multicenter study in six Southern European cities.", dar. Die Ergebnisse, Teile der Methodik, Teile der Diskussion, sowie Teile der Abbildungen und Tabellen der vorliegenden Arbeit wurden veröffentlicht in:

Hoffmann TM, Acar Şahin A, Aggelidis X, Arasi S, Barbalace A, Bourgoin A, Bregu B, Brighetti MA, Caeiro E, Caglayan Sozmen S, Caminiti L, Charpin D, Couto M, Delgado L, Di Rienzo Businco A, Dimier C, Dimou MV, Fonseca JA, Goksel O, Guvensen A, Hernandez D, Jang DT, Kalpaklioglu F, Lame B, Llusar R, Makris MP, Mazon A, Mesonjesi E, Nieto A, Öztürk A, Pahus L, Pajno GB, Panasiti I, Panetta V, Papadopoulos NG, Pellegrini E, Pelosi S, Pereira AM, Pereira M, Pinar M, Pfaar O, Potapova E, Priftanji A, Psarros F, Sackesen C, Sfika I, Suarez J, Thibaudon M, Travaglini A, Tripodi S, Verdier V, Villella V, Xepapadaki P, Yazici D, Matricardi PM, Dramburg S. "Whole" vs. "fragmented" approach to EAACI pollen season definitions: A multicenter study in six Southern European cities. Allergy. 2020;75:1659-1671.

Eine ausführliche Anteilserklärung zu dieser Publikation und weiteren Arbeiten im Rahmen der Promotion finden sich unter dem Punkt: Anteilserklärung (*Seite 84*).

1 Introduction

1.1 Seasonal pollen-induced allergic rhinitis in Southern Europe

Allergic rhinitis is a chronic condition of great socio-economic impact on 10 – 30% of the world's population [2], affecting the patients' work performance [3] and quality of sleep [4]. This immunoglobulin E (IgE)-mediated non-communicable disease is characterized by symptoms such as nasal congestion, sneezing and rhinorrhea [5]. While it's etiology is multifactorial, symptoms are mainly caused by exposure to eliciting allergens, such as the flowering of allergenic plants. During the flowering season, mature pollen grains are emitted in the air and patients are exposed through their respiratory, ocular or oral mucosa. The moistness of these mucosa results in hydration of the pollen grains, followed by a release of soluble allergenic and non-allergenic molecules, causing type-2 inflammatory reactions in allergic patients [6,7].

Available treatment options for patients with allergic rhinitis can be divided into (a) symptom-relieving drugs and (b) allergen-specific immunotherapy (AIT), the only disease-modifying therapy with potential long-term effects [8]. Adherence to AIT remains low and results in treatment failure [9]. Moreover, its efficacy depends on the precise identification of the major eliciting allergen [10].

Unfortunately, the identification of elicitors is often difficult in Southern European countries, since patients are often polysensitized to multiple, cross-reactive allergenic sources with overlapping flowering periods, making the precise diagnosis and treatment for those patients a challenge [11–13].

1.2 The @IT.2020 multicenter study: precision medicine for patients suffering from seasonal allergic rhinitis

Precision medicine in allergology can facilitate the diagnosis for patients with complex conditions, as described in the previous section. The aim of precision medicine is to comprehensively assess the patient's condition, based on optimized diagnostic procedures adequate for the individual patient and resulting in a successful treatment [14]. An attempt has been made to develop asthma phenotypes to capture and categorize the complexity of this chronic disease. This attempt enables the provision of appropriate therapeutic management for each individual phenotype [15].

Figure 1 summarizes possible means to implement precision medicine. For example, a widely investigated approach is the integration of mobile health (mHealth) tools in diagnosis and treatment of allergic rhinitis [16]. They can facilitate the transition to participatory medicine, allowing the patient's needs to be foregrounded [14]. Further, molecular biomarkers are widely investigated for the identification of patient endotypes, known as component-resolved diagnostics (CRD) [17,18]. Accordingly, the @IT.2020 multicenter study is designed to meet this need and to investigate this precision medicine approach for patients with seasonal allergic rhinitis.

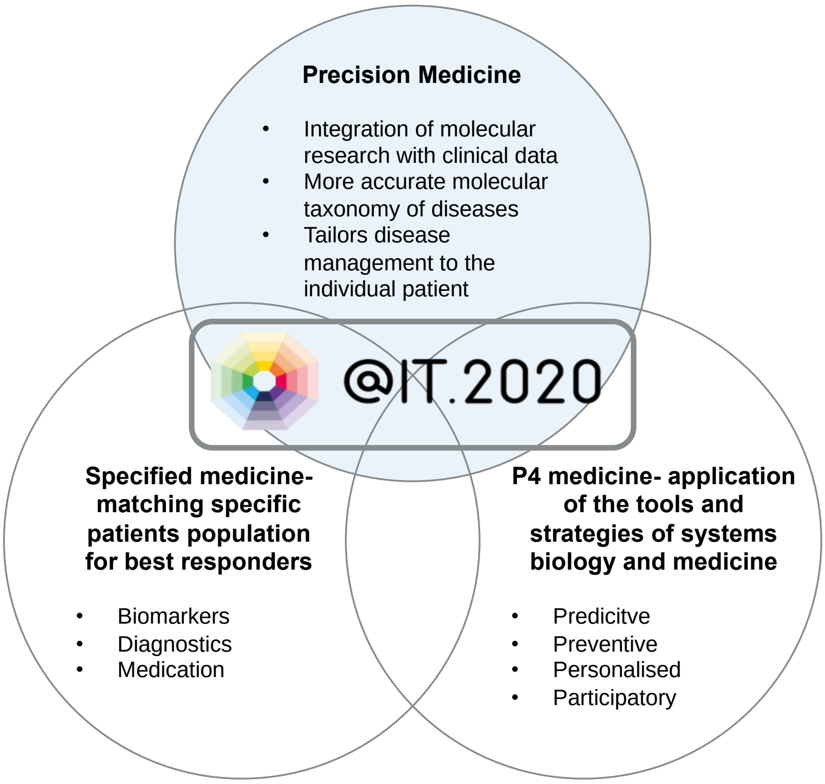

Figure 1. *Individual-oriented medicine in practice. Adapted from Pfaar et al. [14] with kind permission from Wiley.*

The @IT.2020 study aims to assess a new clinical decision support system (CDSS), supporting health care professionals, especially doctors, in the precise decision of AIT prescription in patients affected by seasonal pollen and mold-induced allergic rhinitis in Southern Europe [19]. In addition to the established diagnostical approach, including clinical history, serum IgE tests (sIgE), and skin prick test (SPT), this study incorporates component-resolved diagnostics (CRD) and an mHealth approach, here an electronical diary (e-Diary), offering a higher level of diagnostic precision (Figure 2). In detail, CRD is used to identify the patient's molecular sensitization profile [20,21], and the e-Diary is used to collect patient's symptom data and match them with pollen count data [22].

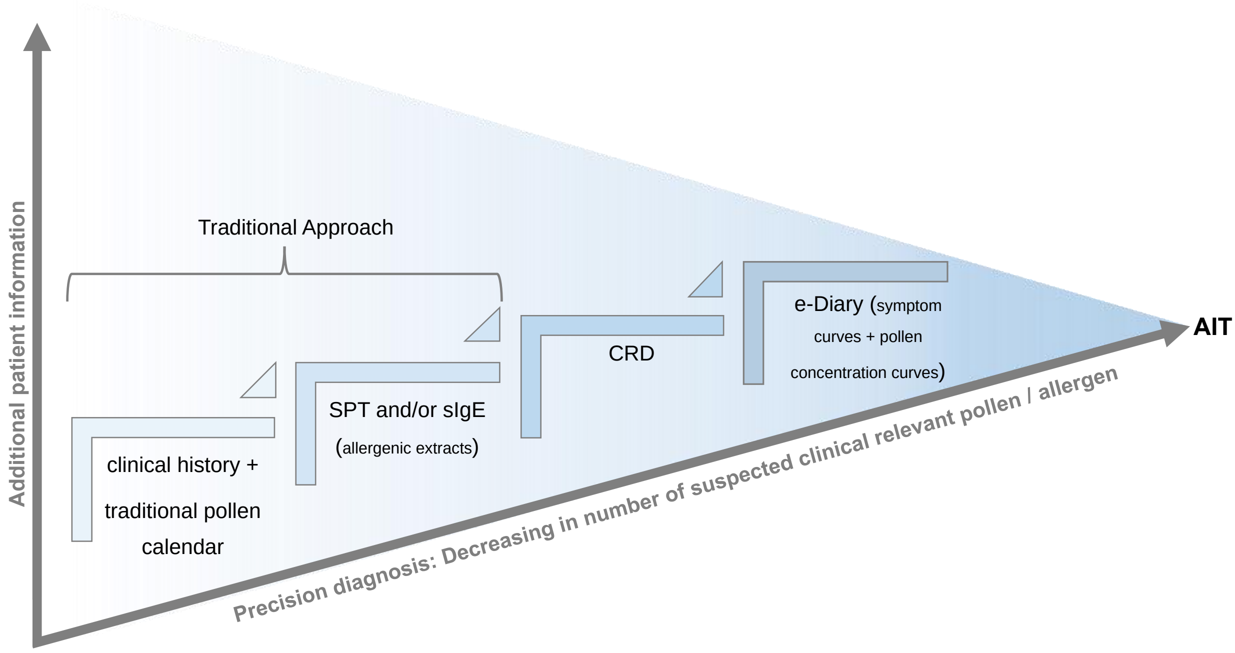

Figure 2. *General concept of the clinical decision support system (CDSS) for seasonal allergic rhinitis tested in the @IT.2020 multicenter study. The traditional approach reveals several potential allergens causing the patient's symptoms. With successive diagnostic steps (SPT – skin prick test, sIgE – serum immunoglobulin E, CRD – component resolved diagnostics, e-Diary – electronic diary) the choice of allergens may be limited to very few or even one clinically relevant allergen [23]. This specification enables the performance of patient-tailored allergen-specific immunotherapy (AIT). Adapted from Matricardi et al. [23], with kind permission from Wiley.*

A pilot study for introducing the @IT.2020 concept was conducted in Italy, including 101 children and 93 adult patients suffering from allergic rhinitis [24]. The @IT.2020 multicenter study is meant as validation study of the findings from the @IT.2020 pilot study and was performed in nine Southern European study centers. The major findings of the study are expected to be published soon, including

(A) a description of the molecular and clinical phenotype of patients suffering from seasonal allergic rhinitis in Southern European countries and

(B) a summary of the impact of CRD and e-Diaries on health care professionals' AIT prescription decisions.

The following secondary findings have already been published:

(a) descriptive analysis of pollen food syndrome occurring in patients with seasonal allergic rhinitis in Southern European countries by Lipp et al. [25].

(b) cumulative pollen concentration curves for pollen allergy diagnosis by Hoffmann et al. [26].

(c) testing the pollen season definitions proposed by EAACI by Hoffmann et al. [1].

1.3 Relevance of pollen counts in the diagnosis of seasonal pollen-induced allergic rhinitis

Since pollen are the allergenic source of seasonal pollen-induced allergic rhinitis, the use of pollen counts in a clinical context is crucial, and modes of application are constantly probed and developed over time. This section provides examples of these applications.

Traditionally, pollen calendars are a diagnostic tool recognized to support the graphic demonstration of the flowering times of allergenic plants. They are based on daily pollen counts, collected throughout the year. In most cases, they include a five-year period and are divided into 36 sections of 10 days each. In addition, the pollen counts are color-coded to indicate different levels of pollen exposure [27–29]. An example for the German area [27] is depicted in Figure 3.

Figure 3. *Example of a traditional pollen calendar for Northern Germany, dating from December to November based on pollen counts from different allergenic sources, 2011 to 2016. Red bars indicate the main flowering season: orange, early and late flowering season; And yellow bars indicate potential occurrence. From Werchan et al. [27]. Figure not shown here for copyright reasons.*

Health care professionals incorporate pollen calendars in their clinical routine to search for a cause-and-effect relationship between allergic sensitization to a particular pollen (e.g., as suggested by an SPT) and the symptoms that occur during exposure to the same source [21,29,30]. Climate change and increasing air pollution [31], among other factors, increasingly influence pollen seasons and, thus, the seasonality of allergic symptoms in susceptible individuals [32,33]. Since pollen calendars are based on retrospectively collected data, their reliability and robustness in the diagnosis of patients with pollen-induced allergic rhinitis is under increasing scrutiny [34,35].

As mHealth applications on smartphones are becoming more readily accessible, a new possibility is discernible. Patients can enter their daily symptoms via e-Diary and match them with individual pollen concentration curves. Investigations widely proved this potential benefit and findings [22,36] seem to indicate this digital approach as promising alternative to pollen calendars. Through automated real-time pollen monitoring [37], the deployment of e-Diaries can be improved by matching the symptoms with current daily pollen counts rather than with retrospective pollen counts. This trend is evolving to new dimensions, such as asking whether crowd-sourced data may reflect pollen seasons even without pollen monitoring [38]. Two recent studies showed that symptom curves increased before the start of, for example, the grass and birch pollen season [38,39]. These findings raise the question of whether crowd-sourced symptom data could on its own support viable pollen forecasting.

In terms of the graphical presentation of pollen data, a study [26] conducted in the @IT.2020 context has revealed that representing the annual pollen load index based on cumulative pollen concentrations for the whole year is informative and perceived as potentially helpful by clinicians for the management and diagnosis of pollen-allergic patients. Due to overlapping pollen-exposure times in Mediterranean regions, the pollen concentration curves of multiple species are often difficult to distinguish in a single graphical representation [26]. Figures 4 A and B exemplify such a graphic. Cumulative pollen concentrations are described in aerobiology [40], but have not been investigated in a clinical context. Consequently, this study’s objective was to test whether doctors acquire additional information from pollen concentration graphs, including cumulative values and absolute values. In total, 80 of 112 (71.4%) of the participants have suggested that the combined use of daily and cumulative pollen concentrations may be clinically relevant in this given situation [26].

Finally, the importance of pollen season definitions in a clinical context is portrayed in the next section.

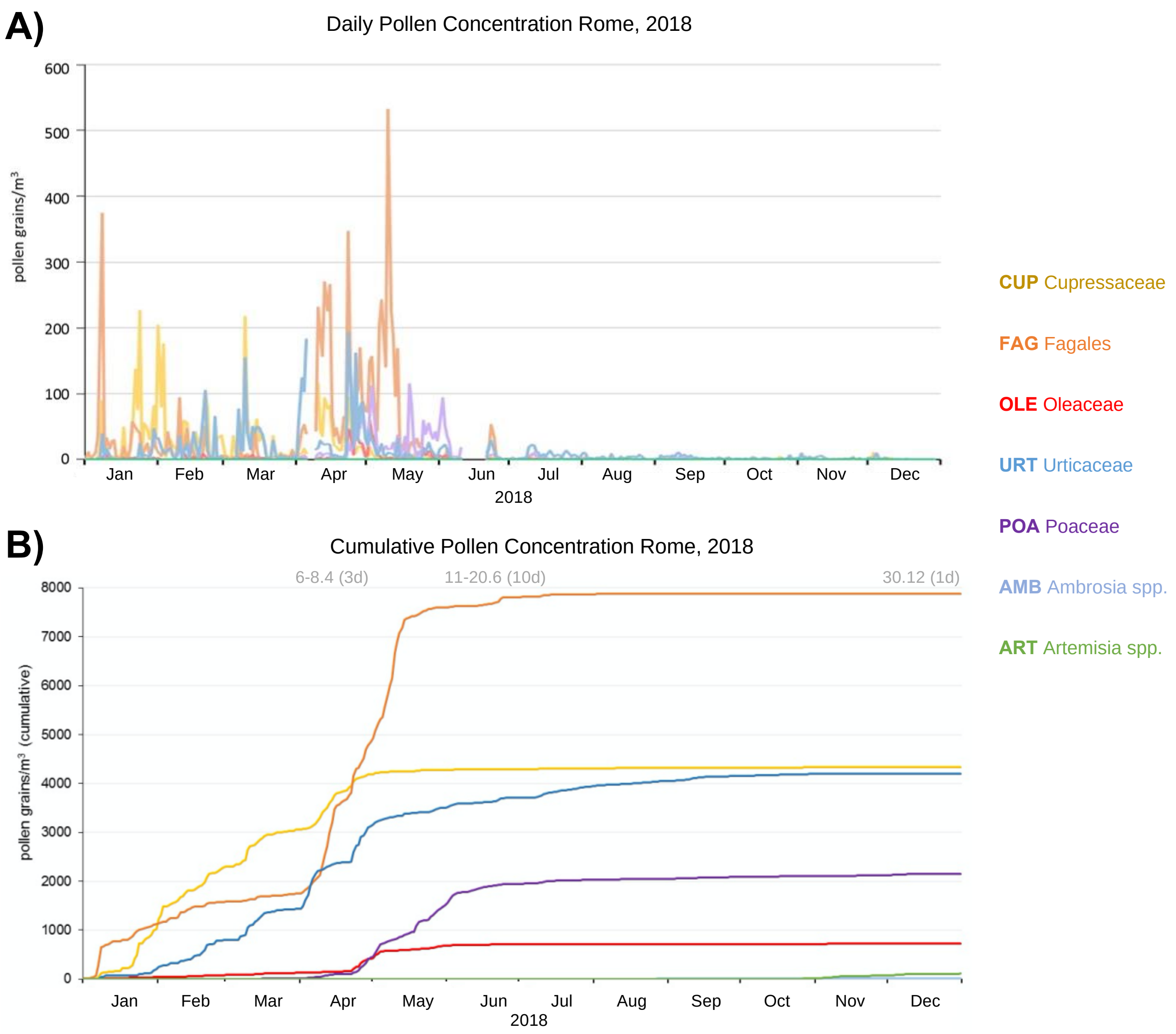

***Figure 4. (A)** Example of the graphical challenge demonstrating pollen concentrations (pollen grains/m^3) for multiple pollen taxa in one graph, in contrast to **(B)** the graphical demonstration of cumulative values, for Rome 2018. Grey numbers indicate periods of missing values. From Hoffmann et al. [26] with kind permission from JIACI.*

1.4 The evolution of pollen season definitions

As previously mentioned, flowering periods are influenced by external factors such as weather conditions and air pollution [41]. Those conditions further impact pollen allergenicity: One study showed that increasing birch pollen allergenicity correlated significantly with ozone. The allergenicity increase was due to elevated allergen content and a shift in the arrangement of pollen-associated lipid mediators inducing immunomodulation and stimulation [42]. Other findings have demonstrated that the increase of carbon dioxide in the atmosphere leads to more ragweed (Ambrosia spp.)

seeds [31]. Additionally, internal factors, such as individual levels of allergic reactions in patients with seasonal allergic rhinitis, influence the process of setting pollen season thresholds for pollen season definitions [43]. A scheme is shown in Figure 5.

Figure 5. *Scheme depicting influences on pollen thresholds for symptom occurrence (grey) [44]. Time of the season, marked in darker blue, is the main factor for this thesis. Note that the factors air pollution and weather conditions impact the other factors, according to the indicating black arrows. Adapted from De Weger et al. [44]. Figure not shown here for copyright reasons.*

From a more local perspective, high pollen exposures naturally depend on local geographic and climatic conditions [45]. However, to ensure adequate diagnosis for patients with pollen-induced allergic rhinitis, it is of utmost importance to define both the beginning and the end of an allergenic pollen season as precisely as possible. Regarding AIT studies, efficacy analysis also depends on the validity of these definitions [45,46]. Accordingly, such a definition is difficult to find, as is reflected in the number and type of pollen season definitions over time [40,46,47].

Given this situation, pollen-exposure times for clinical purposes are increasingly required to follow standardized definitions, as demanded by researchers and regulatory authorities [48–50]. As aforementioned, in recent decades various pollen season definitions have been proposed, relying mostly either on percentages or thresholds [40,46,47]. New methods are also under consideration, such as crowd-sourced system data as a kind of clinical pollen season indicator [38] or trend analysis for pollen forecasts [51]. Percentages are recommended for retrospective pollen season analysis, whereas pollen concentrations thresholds are suggested for clinical trials [46]. However, thresholds tend to differ from study to study, despite that the same climatic circumstances are considered.

This variation complicates comparisons of the efficacy and outcomes of AIT trials [49,50,52]. To cover the need for a (gold-)standard, a task force from the European Academy of Allergy and Clinical Immunology (EAACI) published a position paper proposing definitions for pollen-exposure times for European countries. The consensus particularly addresses the circumstances of clinical AIT studies. In these studies, outcome analyses of clinical endpoints are oriented by predefined pollen (peak) seasons [45]. Typically, the Mediterranean zone is rich mainly in Urticaceae (*Parietaria* spp. and *Urtica* spp.), Oleaceae, Cupressaceae, Poaceae (Graminaceae), Compositae (Asteraceae) with internal variations depending on the region [13]. Thus, the Mediterranean region is not only distinguished from the Northern and Central European region but is also characterized by these internal differences [53,54]. This stratification can hamper the application of pollen season definitions with standardized thresholds for Mediterranean countries that contain such a wide range of vegetation. Accordingly, the EAACI taskforce which has published the position paper further states that, depending on the examined region, the criteria might need adaptation [45].

1.5 Objective

In some retrospective studies on the application and robustness of the EAACI criteria [45] for the investigation of pollen seasons, certain pollen species have been included especially in Central and Northern European countries, apart from France [39,46,55–57]. Table 1 presents an overview of these studies. This thesis aims primarily to test the seasonality of seven pollen taxa for 2018 using the EAACI criteria in six cities: Valencia, Marseille, Rome, Messina, Istanbul and Izmir. Data was collected as part of the @IT.2020 multicenter study. Accordingly, regarding the clinical context, a uniform symptom-monitoring period (SMP) for the three most clinically relevant pollen to the @IT.2020 study (Cupressaceae, Poaceae and Urticaceae [13]) was attempted for the patients included in a potential AIT trial conducted in Southern European cities in 2018.

Table 1. *Overview of recently conducted studies on the applicability of European Academy of Allergy and Clinical Immunology (EAACI) criteria [45] on pollen seasons. Source: Own table.*

authors	study purpose	pollen	countries	sites monitored°	time frame	key results*
Bastl et al (2018) [46]	review, testing two different pollen season definitions (1) EAN standard definition [46] (2) EAACI definition [45], matching with symptom loads	birch, hazel, alder, grass, mugwort, ragweed	Austria	not indicated	2018	working well in prospective design, useful when continuous exposure to aeroallergen has to be assured, applicability for mugwort, hazel, alder
Karatzas et al (2018) [57]	EAACI criteria [45] validation, showing that they can mirror symptom loads for pollen-induced allergic rhinitis	birch, grass	Germany	4	2014-2016	confirmed validity
Karatzas et al (2018) [56]	testing the robustness and sensitivity of the EAACI criteria [45]	Poaceae	Germany	up to 40	2012-2016	confirmation of robustness and sensitivity
Pfaar et al (2020) [39]	confirmation of EAACI criteria feasibility [45], correlation with TNSMS#	grass, birch	Austria, Finland, France	23	2014-2016	identification of all pollen seasons and peak pollen seasons for all countries, significant Pearson correlation of TNSMS and pollen levels, exceptions: grass in Finland 2016/France 2016, birch in Austria/Finland 2015
Bergmann et al (2020) [55]	analysis of time changes in birch pollen season by using the EAACI criteria for season definitions [45]	birch	Germany	1	1988-2018 (except 1995)	appropiate for demonstration of change in birch pollen seasons across time (earlier start, significant increase in days with high pollen counts

* regarding the applicability of EAACI criteria [45]

° number of sites monitored

\# Total Nasal Symptom Medication Score [39]

2 Methods

2.1 The @IT.2020 multicenter study

This section briefly summarizes the key aspects of the @IT.2020 multicenter study on which the data of this thesis is based. Aerobiological aspects are excluded from this section to avoid redundant information.

Study design

The @IT.2020 multicenter study was designed as an observational longitudinal clinical multicenter study, investigating the combined impact of CRD and e-Diary on the doctor's decision on AIT prescription. The study was conducted in 9 clinical centers (Porto, Valencia, Marseille, Rome, Messina, Tirana, Athens, Istanbul, Izmir) and recruited 815 patients with a diagnosis of seasonal allergic rhinitis (467 adults and 348 children). In summary, the data collection process for the study followed this structure: time "0" visit (T_0), patient monitoring, time "1" visit (T_1) and workshops. Figure 6 depicts the study timeline, an overview of the study centers and the numbers of recruited patients.

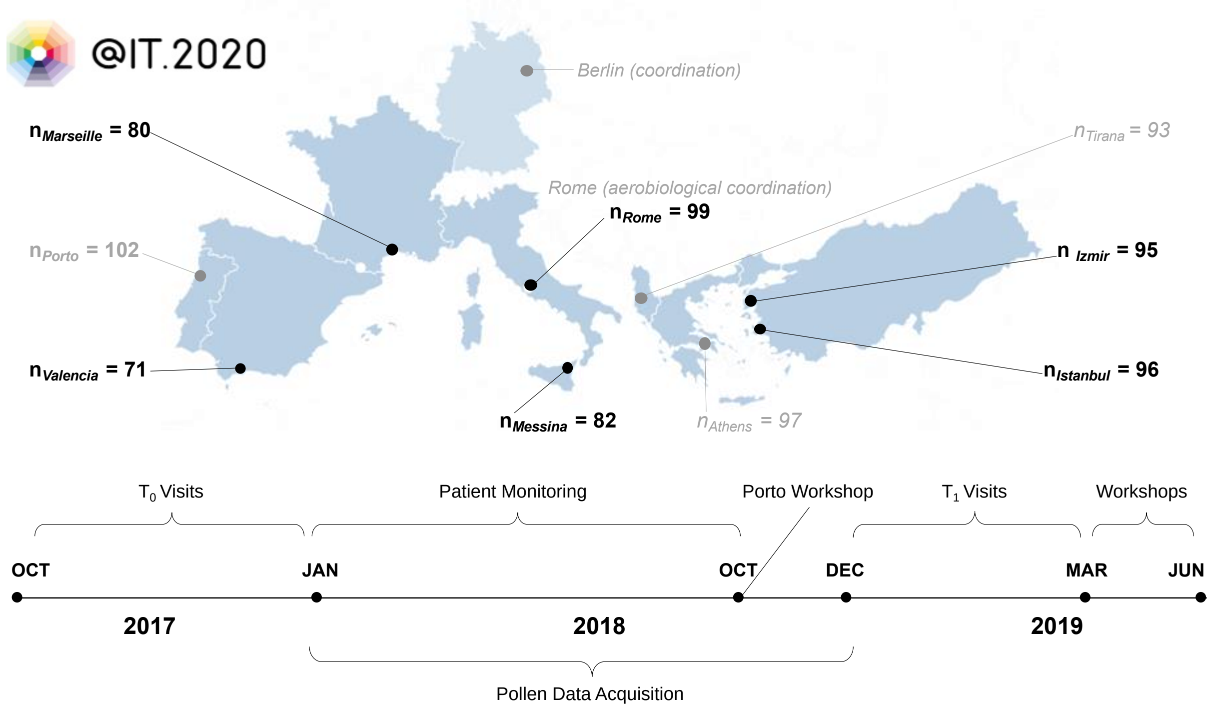

Figure 6. *Data acquisition timeline of the @IT.2020 multicenter study and overview of the study centers with patient recruitment numbers. Centers marked in black participated in both clinical and aerobiological data acquisition. Centers marked in grey exclusively collected clinical data (patient recruitment). Source: Own illustration.*

To be included in the study, a patient had to match the following inclusion criteria:

a) doctor's diagnosis of seasonal allergic rhinitis;
b) age 10-18 years (for pediatric patients) or 19-60 years (for adult patients);
c) solid understanding of the national language of the respective country in which their data was collected;
d) access to a smartphone;
e) written informed consent.

Subsequently, patients were excluded if they

a) had previously received pollen-AIT;
b) had any other severe chronic disease (e.g., chronic sinusitis, immunodeficiencies); or
c) lived more than 30km away from the local pollen trap assessing pollen concentrations for the @IT.2020 study.

The nine clinical centers recruited a total of 815 patients between October 1, 2017, and January 15, 2018 (Figure 6). Participants were informed in detail about the study protocol and all procedures. Subsequently, each participant (or their legal guardian) signed an informed consent form. A study identification number was created for each participant for pseudonymization purposes. The study protocol was approved by the respective local ethics committee for every study center.

T_0 visit – During the T_0 visit, the patients or their parents/legal guardians completed a questionnaire on personal characteristics, clinical history and family history. Validated questionnaires were used to assess the current severity of allergic rhinitis (Allergic Rhinitis and its Impact on Asthma (ARIA) questionnaire [58]) and asthma (GINA guidelines [59]). Skin prick tests (SPT) using a standard panel of seasonal and perennial respiratory allergens [25] and blood drawing were performed. For the e-Diary, the local version of the free smartphone app AllergyMonitor® (Technology, Project and Software production s.r.l., Rome, Italy) was installed on the patient's phone. This app is based on international validated symptom scores, such as the rhinoconjunctivitis total symptom score (RTSS) [60], and is available in different languages [22]. Based on the retrieved information, the doctor decided upon a first etiologic diagnosis for the patient: Cypress, Fagales, Olive, Grass, Pellitory, Ragweed, Mugwort, Alternaria (spore) or no AIT. An appointment for T_1 was set, and based on the diagnosis, a monitoring period was specified for the patient. Sera samples were sent to the laboratory of the coordination team in Berlin. For performance of the CRD, based on the Euroline Southern European Profile (ESEP; EUROIMMUN, Lübeck, Germany) [61], sera were sent further to Luebeck.

Monitoring period – The monitoring period followed the relevant pollen seasons based on previous pollen calendars in the specific region for the individual patient. The local aerobiological center provided pollen counts in pollen grains/m^3 daily. During the monitoring period, patients entered their individual symptoms by completing a daily questionnaire in the app. The patient's individual daily symptom score was matched with pollen concentration curves (e.g., see Figure 4). The mobile application (front end) relates to a website with secured user access for the doctor (back end) [22]. Thus, the doctor could follow the data entry of the study participants. Example screenshots from the mobile application and website are given in Figures 7 and 8.

Figure 7. *Screenshots of the mobile application AllergyMonitor® by Technology, Project and Software production s.r.l., Rome, Italy. The examples show the daily symptoms questionnaire together with a visual analogue scale (VAS) [22]. From Tripodi et al. [22]. Figure not shown here for copyright reasons.*

Figure 8. *Screenshots of the corresponding doctor's back end of the mobile application AllergyMonitor® called AllergyCard® by Technology, Project and Software production s.r.l., Rome, Italy [22]. The examples offer a hypothetical patient overview (left upper corner), patient symptom registration (right upper corner), symptom scores matched with pollen concentration data (left lower corner) and intake of symptomatic drugs (right lower corner). From Tripodi et al. [22]. Figure not shown here for copyright reasons.*

T_1 visits – At the end of the pollen seasons, patients were recalled for a final T_1 visit to discuss their IgE and e-Diary results.

Workshops – The @IT.2020 workshops, to evaluate individual components of the CDSS, took place in Tirana, Rome, Ankara, Marseille and Porto between 15 March and 12 June, 2019. Participating doctors were either specialized allergists with ≥5 years of clinical experience in prescribing AIT, or residents in allergology with no degree of specialization yet. In a standardized educational module, information on the individual components of the CDSS (clinical history, SPT, (molecular) IgE, e-Diary and pollen data) were presented, before working on 10 clinical cases from the local study cohort. In three steps, doctors were asked to decide on the theoretical prescription of AIT based on sub-sets of information representing the different modules of the CDSS (first: clinical history and SPT, second: the previous plus IgE results; third: the previous plus e-Diary and pollen data). In addition, usability and acceptance of a digital CDSS for pollen allergy were evaluated. Another goal of the workshops was, to evaluate whether health care professionals are able to adopt an innovative graphical representation of cumulative pollen concentration data in their clinical routine. Following an introduction, which established an understanding of cumulative values, a test questionnaire and subsequent validation questionnaire were given to participants. Example questionnaires used for the workshop in Rome are listed in the Appendix of this thesis.

Financial support

This study has been supported by an unrestricted grant from the EUROIMMUN company (grant number 118583). The ESEP essay has also been provided by the EUROIMMUN company. The smartphone app AllergyMonitor® and the back-end platform AllergyCard® have been provided by Technology, Project and Software production s.r.l., Rome, Italy.

Ethical considerations

The study was performed according to the Helsinki declaration, the European laws and the rules of Good Clinical Practice. The study was approved by the local ethics committees. The study protocol (including documents concerning the power-calculation, privacy, data handling and data protection, and informed consent) was submitted to all local ethics comittees.

2.2 Aerobiological study centers

For the analysis of pollen season definitions, aerobiological monitoring was performed in six Southern European aerobiological centers: Valencia (Spain), Marseille (France), Rome, Messina (both Italy), Istanbul and Izmir (both Turkey) from 1 January to 31 December, 2018. Overall, the Mediterranean climate of subtropical geographical latitudes, from 38°N (Izmir and Messina) to 43°N (Marseille), is marked by mild winters and long, dry summer periods [62]. The mean temperatures of the examined centers varied between 15.5°C in Marseille to 19.6°C in Izmir (Table 2). The aerobiological centers have a maritime influence, as they are situated near the sea; the exception is Rome, which is located 30 km inland from the coast. The 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 inter-center variations can be retrieved from Table 2; this data was provided by the aerobiologists of each center.

Table 2. *Characteristics of aerobiological monitoring* in all six participating centers. From Hoffmann et al. [1].*

	Climate		Aerobiologists			Pollen Traps			Missing days	
Centers	**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°40'05.25" 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 continuos stripe, field diameter 0.5mm, magnification 400x
° aerobiological and pollen monitoring experience of the correspondent aerobiologist (years)
number of consecutive missing days

2.3 Acquisition of pollen data

Central to this study was the collection of pollen from Cupressaceae (Cupressaceae and Taxaceae), Fagales (Corylaceae, Fagaceae and Betulaceae), Oleaceae, Poaceae, Urticaceae, *Ambrosia* spp. and *Artemisia* spp. (Compositae).

Aerobiological monitoring was embedded in the clinical @IT.2020 study, thus considering both botanical origin (Figure 9A) and the clinically relevant grouping of allergenic plant molecules based on their biological function (Figure 9B). Analysis of allergen epitopes of Fagales species indicated that Fagaceae allergies are putatively induced due to antibody cross-reactivity against allergenic molecules (Bet v 1) of the Betulaceae family [63]. Thus, the plant families of the Fagales order were also considered as one group for this study, since they contain homologous pathogenesis-related (PR)-10 allergens, also known as the Bet v 1-like family [64]. In regulation of allergenic products, the European Medicine Agency (EMA) groups the following plants into one "Fagales group": *Betula verrucosa* (Birch), *Alnus glutinosa* (Alder), *Carpinus betulus* (Hornbeam), *Corylus avellana* (Hazel), *Quercus alba* (Oak), *Castanea satavia* (Chestnut), *Fagus sylvatica* (Beech) [65,66]. Fagales are also acknowledged as plant order, containing allergenic species by the World Health Organization (WHO) and International Union of Immunological Societies (IUIS) *Allergen Nomenclature* [67].

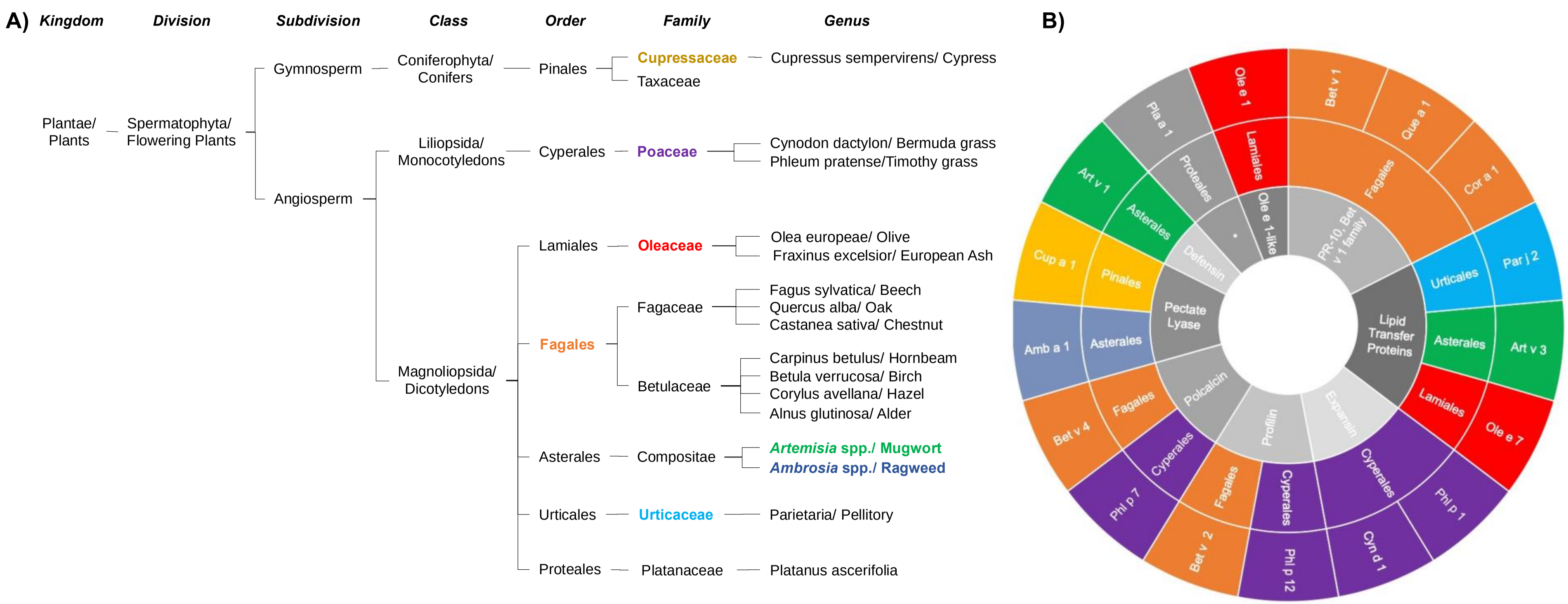

Figure 9 (A) *Phylogenetic tree of plant orders relevant for this thesis, based on the United Stated Department of Agriculture Classification [68]. Taxa used for pollen season analysis are colored according to the figures in the results section of this thesis, namely Cupressaceae (yellow), Poaceae (purple), Oleaceae (red), Fagales (orange), Artemisia spp. (green), Ambrosia spp. (dark blue) and Urticaceae (light blue). Source: Own illustration.* ***(B)*** *Scheme of molecules contained in the ESEP test, relevant for this thesis. The molecules were clustered regarding their biological function and colored matching the analyzed plant family as depicted in (A). Pla a 1 (grey) is contained in the ESEP test, but not part of the pollen season analysis and marked with * since it is a putative invertase inhibitor [69]. Figure (B) is adapted from Asam et al. [70], Wiley open access article.*

The pollen monitoring and the reading of the collected pollen was conducted in alignment with the minimum-requirement criteria for pollen-monitoring networks [71]. The aerobiological centers recorded the pollen counts by using volumetric Hirst type samplers, Burkard or VPPS Lanzoni (Figure 10) with a suction-flow of 10 L of air per minute. Pollen traps were located on roof tops, following validated methodologies [72].
Via the intake orifice and induced suction-flow of the pump placed in the pollen trap, pollen grains are collected on a transparent Melinex tape wrapped around the drum of the pollen trap. The drum is installed on a clock, so after a rotation with a duration of 7-days, the Melinex tape is changed.

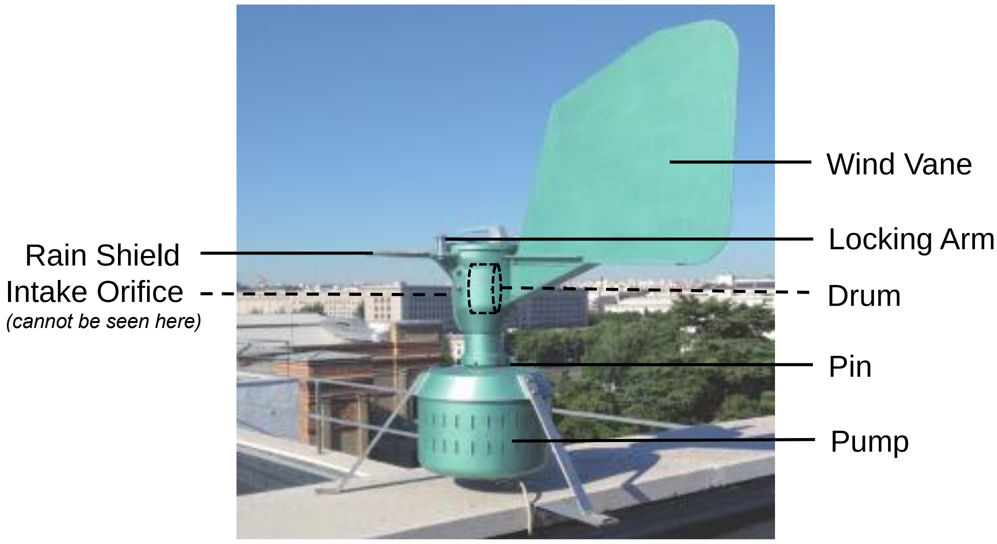

Figure 10. *Structure of a Hirst-type pollen sampler. Adapted from Núñez et al. [73], Springer open access article.*

For fixation of the pollen grains, the tape's surface was covered with silicon (polydimethylsiloxane) for Lanzoni samplers and vaseline for Burkard samplers. The samples have been prepared as glass slides for further microscopic analysis, using Fuchsine stain. Locally trained aerobiologists with 5–29 years of experience carried out the microscopic analysis at a microscopic magnification of 400×. As recommended by Galán et al. [74], daily pollen concentrations are expressed as pollen grains per cubic meter of air (pollen grains/m^3) [74]. To determine the pollen concentration from the pollen count, it is subsequently multiplied by a conversion factor specific to the microscope and objective combination used [75]. The pollen count refers to the raw data of the aerobiological analysis and gives an integer quantity that cannot be compared; therefore, the conversion factor is needed [74].

According to the package AeRobiology [76] for the statistical program R, a linear interpolation is executed by default for a maximum of 30 consecutive days [76]. It was decided arbitrarily to admit a maximum of only 10 consecutive days to be interpolated for the linear method. Accordingly, missing pollen concentration values were implemented by means of linear interpolation, as depicted by the following formula:

$$y_i = y_1 + \frac{y_2 - y_1}{x_2 - x_1}(x_i - x_1).$$

The value to be interpolated is represented by y_i at the date point x_i. Values y_2, y_1 represent the pollen concentration one day before and after day(s) with missing value(s) (data gap), while $x_2 - x_1$ stand for the indefinite number of days of the data gap.

2.4 Meteorological data

To assess whether rainfall affects pollen concentrations in the centers and, consequently, season length, meteorological data from all six centers was acquired. The aerobiologists provided openly available rain data from local meteorological centers: the Valencia Institute of Agricultural Investigation, Meteo France, the Meteorological Station of University “Tor Vergata” Rome, Centro Funzionale Multirischi, ARPACAL, the Bornova/Izmir meteorological station and the Turkish State Meteorological Service. Rain data were expressed in millimeters. A rainy day with rainfall of at least >3 mm was deemed relevant. For statistical analysis, the chi-square test of independence was used to calculate *p*-values, where $p < 0.05$ was considered statistically relevant. The hypothesis H_0 was defined as follows: “The percentage of included rainy days in the fragmented pollen season is not associated with the percentage of excluded rainy days (days with no rain) in the fragmented pollen season”; H_1 as “The percentage of included rainy days in the fragmented pollen season is associated with the percentage of excluded rainy days (days with no rain) in the fragmented pollen season.”

2.5 Analysis of season definitions

The EAACI position paper challenged the definition of pollen seasonality using pollen concentration thresholds as criteria [45]. These criteria are briefly summarized and adapted for this study in Table 3.

Table 3. *Overview of pollen season definition criteria in accordance with the European Academy of Allergy and Clinical Immunology (EAACI) position paper [45]. Thresholds° for the whole pollen season (WPS), the whole high season (WHS) and high days (HD) are demonstrated. From Hoffmann et al. [1].*

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

° Equal or higher than the indicated value in pollen grains/m^3

Adaptation of the EAACI criteria for the present study as these pollen species had not been included in the EAACI Position Paper

The EAACI criteria have been adjusted for *Betula* spp., Poaceae, *Cupressus* spp., *Olea* spp. and *Ambrosia* spp. According to the EAACI definition, a season start, a season end and cumulative signal were set for the pollen-exposure times of the listed plants [45]. The cumulative signal is indicative of the successive addition of previous daily pollen concentrations for a defined period of time. For instance, the start of the *Betula spp*. pollen season is defined by the 1st day of 5 variably chosen days out of 7 consecutive days with ≥10 pollen grains/m^3 for each of the 5 days and when the cumulative sum of these 5 days reached ≥100 pollen grains/m^3 pollen. The season ends after the last day, respecting the same conditions. By adjusting the established thresholds, the EAACI criteria define two types of seasons, a *pollen season* (longer) and *peak pollen period/high season* (shorter), by using lower and higher thresholds, respectively [45].

The thresholds proposed by the EAACI position paper were adopted for Cupressaceae (Cupressaceae and Taxaceae), Fagales (Corylaceae, Fagaceae and Betulaceae), Oleaceae, Poaceae, Urticaceae, *Ambrosia* spp. and *Artemisia* spp. (Compositae). Since *Betula* spp. is a genus of the Fagales order, the given EAACI thresholds were adopted for the Fagales order. Urticaceae and *Artemisia* spp. were also tested taxa in this study and were not considered in the position paper; therefore, the daily concentrations and cumulative thresholds have been adopted (Table 3) according to those for Poaceae [45]. These thresholds were selected because Urticaceae, *Artemisia* spp. and Poaceae all represent herbaceous flowering plants, as previously proposed by Bastl et al. (for Mugwort) [46].

The EAACI criteria create a continuous period for each pollen-exposure time regarding the very first and the very last defined days of a season. Throughout this thesis, these conditions are referred to as start signal and stop signal, accordingly. The continuous seasons are defined as the *whole pollen season* (WPS) (originally, pollen season [45]) and *whole high season* (WHS) (originally, peak pollen period [45]).

For the purpose of this study, two additional season definitions were established, taking into account stop signals according to the EAACI definition, with the exception of any occurring during a season. These additional stop signals can create an interruption of the season, causing more start signals to occur and creating an intercurrent period of days not fulfilling the season criteria. To explicitly describe these adapted stop signals for Fagales as an example, according to the EAACI criteria it would be the last of 5 days (of 7 consecutive days) with ≥10 pollen grains/m^3, when the sum of these 5 days is ≥100 pollen grains/m^3, however found within the season. Given the resulting fragments, further season definitions have been defined: the *fragmented pollen season* (FPS) and *fragmented high season* (FHS). In the light of this second representation, a season might be characterized by several fragments or periods interrupted by out-of-season periods: *intercurrent periods*.

Moreover, the EAACI position paper also proposes the definition of *high days* (HD) as 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, while for Poaceae and *Ambrosia* spp., it is fixed at ≥50 pollen grains/m^3 (Table 3) [45].

3 Results

3.1 Pollen concentrations by center

The pollen concentrations of all seven investigated pollen demonstrated similarities and differences among the included aerobiological centers. An overview is shown in Figure 11. In Valencia, Cupressaceae reached the highest concentration in comparison to other pollen and peaked in February and March (max. 720 pollen grains/m^3); lower peaks (below 200 pollen grains/m^3) were observed in October and December. The early summer months, mainly May, June and part of July, were dominated by Oleaceae, Poaceae and Urticaceae. In Marseille, extremely high peaks for Cupressaceae pollen concentration (max. 3444 pollen grains/m^3) were registered in February and appeared consistently until end of April. The Fagales pollen concentration had its highest annual peak in April as well. Oleaceae were observed in April and May, whereas Poaceae pollen concentration was registered mostly in May and in the summer months June to August. In Rome, Cupressaceae were observed from January to April, similar to Fagales, although peaks were registered until May (max. 531 pollen grains/m^3). The pollen concentration of Oleaceae remained below a mark of 100 pollen grains/m^3 from March to May. Poaceae pollen were mostly observed in May and June. In contrast to the other centers, Messina registered Urticaceae as the dominating abundant pollen in the air throughout the whole year, with its highest peaks in March and April (max. 634 pollen grains/m^3) and a second period of peaks in June and July. Cupressaceae peaks were observed mostly in February and March and were the lowest among those in all cities. In this aerobiological center, the pollen concentration of Oleaceae was rather low, and Fagales not plentiful in the air; apart from a single peak in April. In Istanbul, the pollen concentration of Cupressaceae peaked in February (max. 618 pollen grains/m^3) and March. Fagales and Oleaceae were observed at very low levels only in a few days in February, and in March and May, respectively. The pollen concentration of *Ambrosia* spp. reached above 100 pollen grains/m^3 in August/September; *Ambrosia* spp. was the only taxa to have a peak this high, apart from Cupressaceae. In Izmir, the pollen concentration of Cupressaceae reached the highest peaks of all cities (max. 6830 pollen grains/m^3) in February and March. Oleaceae and Poaceae showed overlapping pollen concentration peaks from April to May.

An inter-city comparison for each pollen taxa is demonstrated in Figure 12. Izmir stands out, with the highest overall pollen concentration compared to other centers for

Cupressaceae, Oleaceae and Poaceae. Marseille and Istanbul share a similar distribution and level of *Ambrosia* spp. pollen concentration.

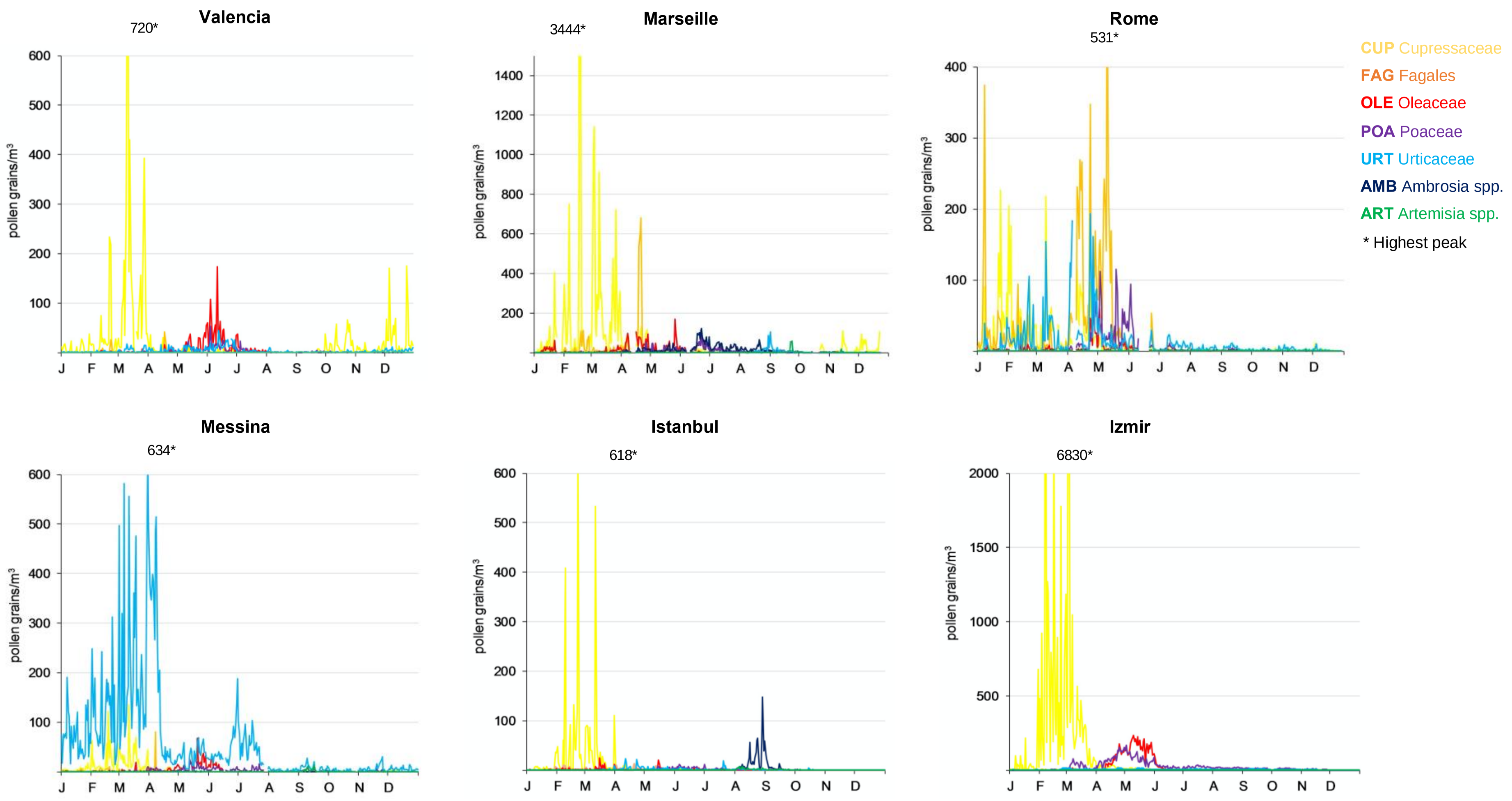

Figure 11. *Pollen concentrations for all seven investigated taxa depicted in pollen grains/m^3 by center for 2018. Months are abbreviated with the respective first letter. Numbers marked with * indicate the highest peaks exceeding the y axis. From Hoffmann et al. [1].*

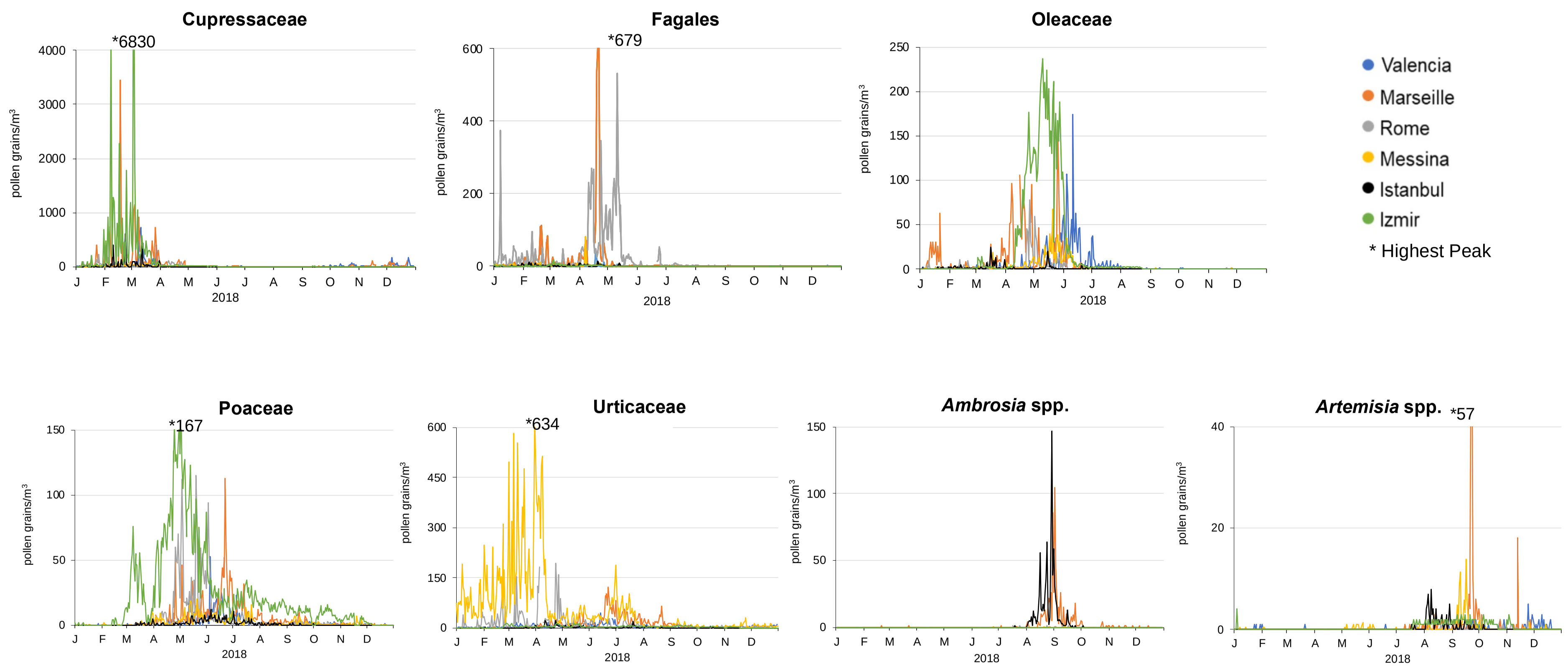

Figure 12. *Pollen concentrations for all seven investigated taxa in the different cities depicted in pollen grains/m^3 by taxa for 2018. Months are abbreviated with the respective first letter. Numbers marked with * indicate the highest peaks exceeding the y axis. The aerobiological centers are colored matching to Figure 16. Adapted from Hoffmann et al. [1].*

3.2 Pollen seasons

3.2.1 Pollen seasons in Rome

The aerobiological center in Rome was chosen as an arbitrary case by which to gain a better understanding. First, the proposed EAACI criteria were applied, as described in the Methods, to the registered pollen concentration for the whole year of 2018 in Rome. The WPS for five pollens, including Cupressaceae, Fagales, Oleaceae, Poaceae and Urticaceae – except *Artemisia* spp. and *Ambrosia* spp. – were identified (Figure 13 and Table 4).

Table 4. *Start, end and duration (in days) of pollen seasons according to European Academy of Allergy and Clinical Immunology (EAACI) criteria in Rome during 2018.° From Hoffmann et al. [1].*

	WPS			FPS				WHS			FHS				HD
	date			segments#				date			segments#				
Pollen	start	end	day	n	shortest	longest	day	start	end	day	n	shortest	longest	day	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
Ambrosia spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Artemisia 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

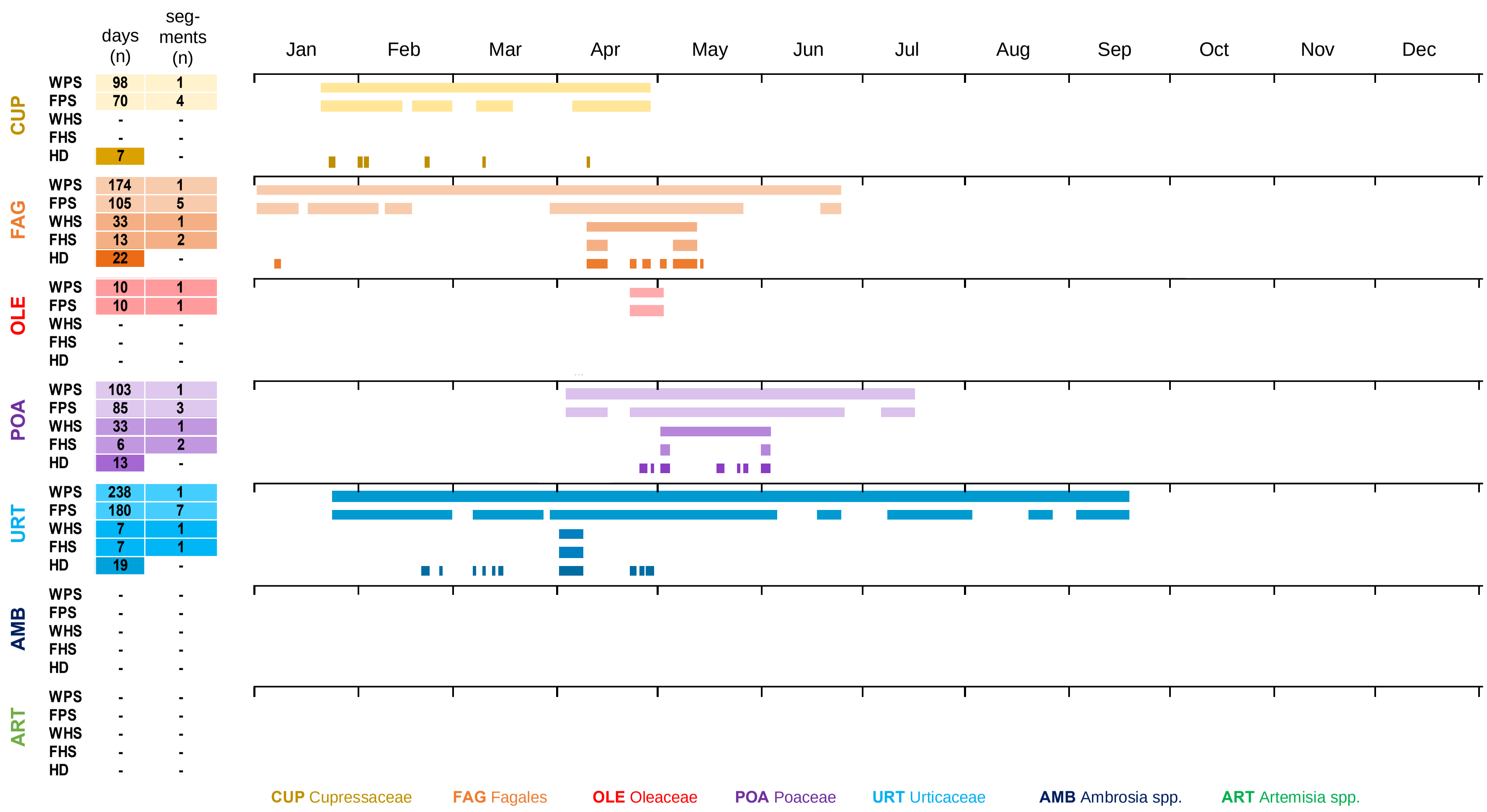

Figure 13. *Pollen season analysis based on the adapted European Academy of Allergy and Clinical (EAACI) criteria [45] for Rome (see Methods), including whole pollen season (WPS), fragmented pollen season (FPS), whole high season (WHS), fragmented high season (FHS) and high days (HD) for 2018. On the left side the number of segments is shown, and season length is indicated by number of days for each season definition. From Hoffmann et al. [1].*

Overall, the WPS lasted less than two weeks for Oleaceae (only 10 days, from April 23 to May 2), 47.7% of the year (365 days) for Fagales (174 days, from January 2 to June 24), less for Poaceae (103 days, from April 5 to July 16) and Cupressaceae (98 days, from January 21 to April 28) and with a duration of 65.2% of the year for Urticaceae (238 days, from January 14 to September 18). Intercurrent periods with low pollen concentrations causing an interruption of the season were excluded, as described in the Methods. After generating the FPS, the number of days decreased 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). FPS were split into three segments for Poaceae (range 10 to 64 days), into four segments for Cupressaceae (range 11 to 24 days), into five segments for Fagales (range 6 to 58 days) and into seven segments for Urticaceae (range 7 to 68 days).

Similarly, the WHS was generated for Fagales (33 days), Poaceae (33 days) and Urticaceae (7 days only), which were then transferred into FHS by exclusion of the intercurrent periods. This transfer resulted in an FHS with two segments each, of only 13 and 6 days for Fagales and Poaceae, respectively.

3.2.2 Whole pollen seasons and fragmented pollen seasons in the six centers

The previous detailed example (Rome) for the methodology is now applied to all other centers. The results are summarized in Tables 5, 6 and Figures 14, 15 and 16. WPS were found for all seven examined taxa in Marseille. WPS for Cupressaceae, Poaceae, Urticaceae (only these three) were identified in all centers, except Marseille and Messina. Overall, the WPS length ranged from a minimum of 8 days for *Artemisia* spp. in Istanbul to a maximum of 363 days for Urticaceae in Messina.

Table 5. *Start, end and length of defined pollen seasons according to European Academy of Allergy and Clinical Immunology (EAACI) criteria in six European centers, 2018.° From Hoffmann et al. [1].*

		WPS		FPS Periods			WHS		FHS Periods			HD	
	Center	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
Urticaeae	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/m^3

Table 6. *Length (in number of days) and mean pollen concentration (in pollen grains/m³) of adapted and adopted European Academy of Allergy and Clinical Immunology (EAACI) pollen season definitions in six Mediterranean centers for 2018.° From Hoffmann et al. [1].*

Pollen		Valencia		Marseille		Rome		Messina		Istanbul		Izmir	
		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.

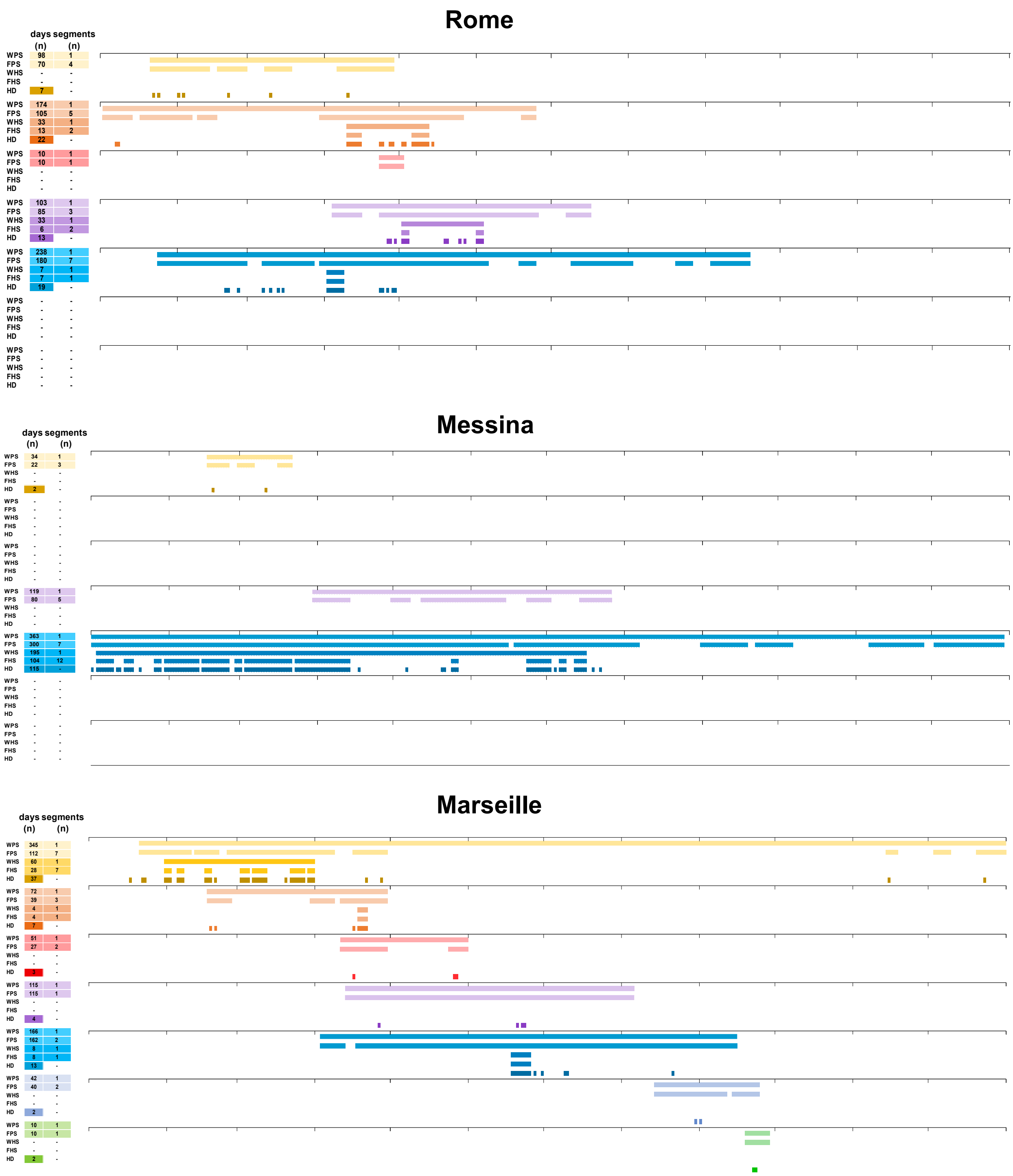

Figure 14. *Representation of all defined pollen seasons of Cupressaceae (yellow), Fagales (orange), Oleaceae (red), Poaceae (purple), Urticaceae (blue), Ambrosia spp. (paler blue) and Artemisia spp. (green) for Rome, Messina and Marseille in 2018. Included are the whole pollen season (WPS), the fragmented pollen season (FPS), the whole high season (WHS), the fragmented high season (FHS) and high days (HD) for the year 2018. On the left side, the length is indicated by number of days for each season definition and the numbers of season segments is given. From Hoffmann et al. [1].*

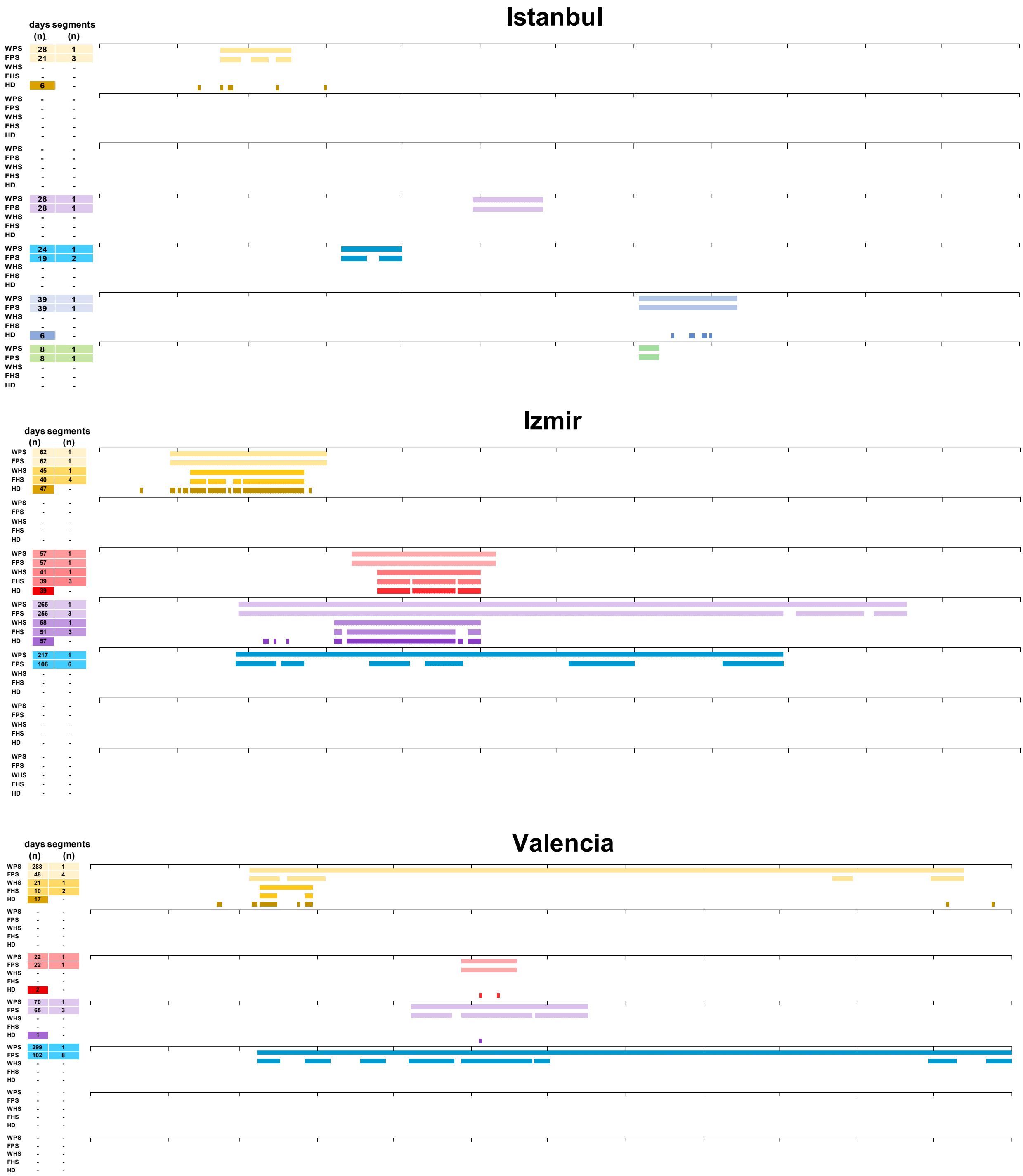

Figure 15. *Representation of all defined pollen seasons of Cupressaceae (yellow), Fagales (orange), Oleaceae (red), Poaceae (purple), Urticaceae (blue), Ambrosia spp. (paler blue) and Artemisia spp. (green) for Istanbul, Izmir and Valencia in 2018. Included are the whole pollen season (WPS), the fragmented pollen season (FPS), the whole high season (WHS), the fragmented high season (FHS) and high days (HD) for the year 2018. On the left side, the length is indicated by number of days for each season definition and the numbers of season segments is given. From Hoffmann et al. [1].*

In more detail, the minimum and maximum number of WPS days are distributed among the pollen, as follows (Tables 5,6): Cupressaceae shows a variation in WPS length from 28 days in Istanbul to 345 days in Marseille, although the highest mean pollen concentration of 593 pollen grains/m^3 was found in Izmir (WPS_{Izmir} = 62 days). For Fagales, the WPS was observed in Marseille and Rome on only 72 days and 174 days, respectively. The WPS for Oleaceae had a rather small range, from 10 days in Rome to 57 in Izmir. The Poaceae WPS lasted from 28 days in Istanbul to 265 days in Izmir. For Urticaceae, the broadest difference of WPS length (339 days) among the centers was noticed (24 days in Istanbul to 363 days in Messina). The WPS for this pollen exceeded a mark of 200 days for four centers, with Istanbul and Marseille as an exception. In comparison to the other centers, these generated WPS were rather long, apart from Cupressaceae in Valencia and Marseille, and the mean pollen concentration barely exceeded the 3 pollen grains/m^3 threshold set for the $WPS_{Urticaceae}$ (4 pollen grains/m^3 in Valencia and Izmir). Similar to the WPS for Fagales, the two WPS for *Ambrosia* spp. and *Artemisia* spp. each were identified on 39 days in Istanbul and 42 days in Marseille, and 8 days in Istanbul to 10 days in Marseille, respectively.

In 8 of 42 cases (namely Cupressaceae in Izmir; Poaceae in Marseille and Istanbul; *Ambrosia* spp., *Artemisia* spp. in Istanbul; Oleaceae in Valencia, Rome and Izmir), the WPS and the FPS coincided perfectly. In three cases, major reductions in the season length were registered when the intercurrent periods were excluded (Figures 13,14,15): The FPS for Cupressaceae in Marseille was reduced from 345 days (WPS) to 32.5% (112 days), with similar results for Urticaceae in Valencia to 34.1% (from 299 days, WPS, to 102 days, FPS). The strongest reduction from WPS length to FPS was 83.0% from 283 days to 48 days, respectively, for Cupressaceae in Valencia.

The most segments found during the FPS were observed for Urticaceae in Valencia, with 8 segments (ranging from 6 to 28 days) (Table 5).

3.2.3 Whole high season, fragmented high season and high days in the six centers

Generally, less WHS than WPS were generated for all centers, Istanbul excluded since no WHS were found for any examined taxa. The identified WPS length always exceeded the WHS length for all pollen in all centers (Table 6 and Figures 14,15).

The longest WHS was distinguished for Urticaceae in Messina (195 days), which goes along with the finding of the longest WPS in this case. For Cupressaceae, WHS were found in Valencia (21 days), Marseille (60 days) and Izmir (45 days). Urticaceae was equally observed in three centers (Marseille, Rome, Messina). Fagales and Poaceae WHS were identified in only two centers each, in Marseille and Rome, and in Rome and Izmir, respectively. Subsequently, the WHS of Oleaceae registered only in Izmir (41 days). No WHSs were found for *Ambrosia* spp. and *Artemisia* spp. in any of the centers. By excluding intercurrent periods of low pollen concentrations from the WHS, the FHS were generated. Overall, the mean pollen concentration of the FHS was higher than that of the WHS, with few exceptions, namely Fagales in Marseille (4 days, 487 pollen grains/m^3), Urticaceae in Marseille (8 days, 85 pollen grains/m^3) and Rome (7 days, 21 pollen grains/m^3). For these exceptions, the WHS and FHS lengths were equal. In contrast to the reduction of season length between WPS and FPS, the strongest impact of the exclusion method retrieved was for Poaceae in Rome (81.8%, from 33 days to 6 days) and for Urticaceae in Messina (46.7%, from 195 days to 104 days) (Table 5).

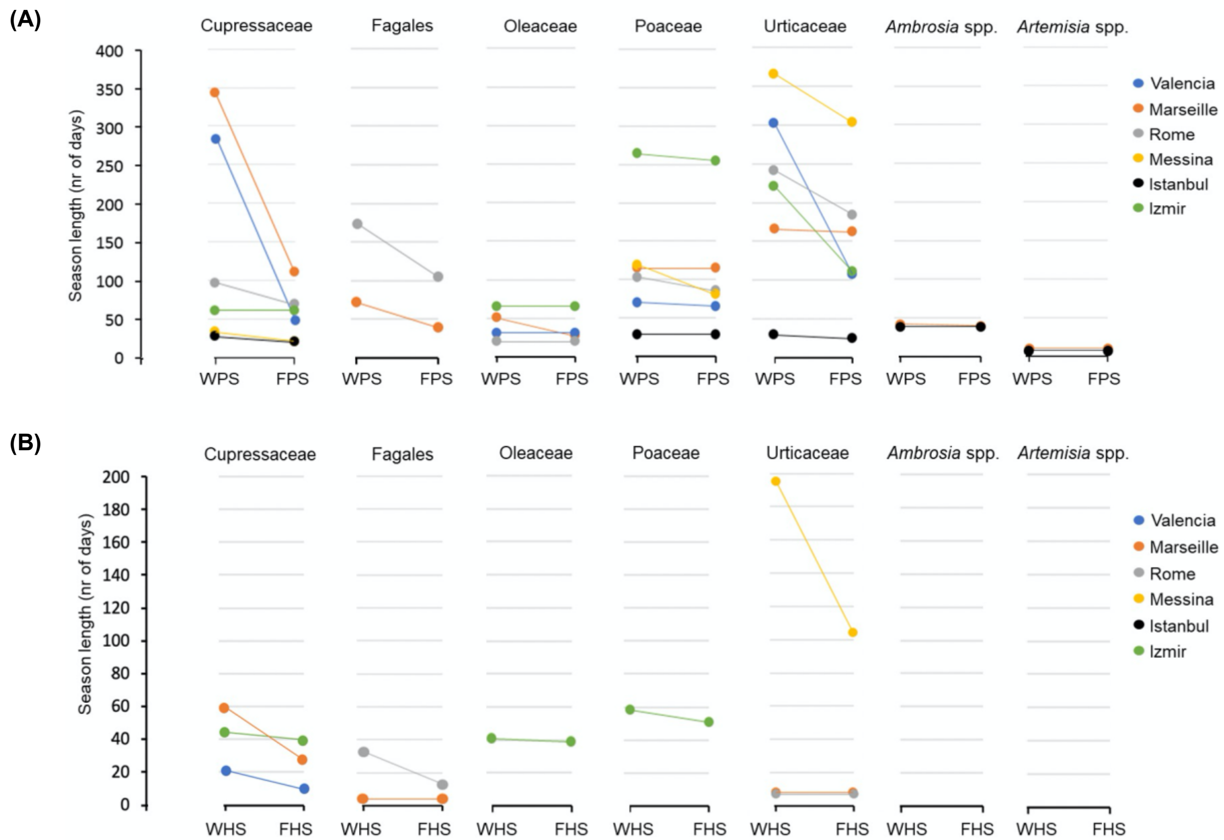

Figure 16. ***(A)*** *Graphical demonstration of season length difference between the European Academy of Allergy and Clinical (EAACI) definition [45] of the whole pollen seasons (WPSs) and the adapted fragmented seasons approach (FPSs),* ***(B)*** *as well as for whole high season (WHS) and the fragmented high season (FHS) for seven pollen in six Southern European centers. From Hoffmann et al. [1].*

Last, the number and distribution of HD throughout the year strongly reflected the distribution of pollen peaks. Sparse HD outside the WHS were identified for Rome (Cupressaceae, Fagales, Poaceae, Urticaceae), for Messina (Cupressaceae, Urticaceae), for Marseille (all pollen), for Istanbul (Cupressaceae, *Ambrosia* spp.), for Izmir (Cupressaceae, Poaceae) and for Valencia (Cupressaceae, Oleaceae, Poaceae) (Table 5). Some of these sparse days were observed in isolation from the WPS (Cupressaceae in Valencia, Marseille, Istanbul, Izmir). Segments of the FHS were represented by long sequences of HD in some centers for some of the investigated pollen (e.g., Fagales and Poaceae FHS in Rome) (Figure 14,15).

3.3 Unified symptom monitoring period based on whole pollen season und whole high season

Within the framework of potential clinical trials on AIT, the establishment of a unified symptom monitoring period (SMP) for participating patients is of great importance, especially with regard to a multicenter setting [10,48]. Thus, an SMP based on the gathered data was generated. This SMP aligns with the WPS and WHS definitions and covers the pollen seasons of the pollen taxa with the most relevant clinical impact regarding AIT (for Southern Europe, namely Cupressaceae, Poaceae, Urticaceae) in all six centers (Figure 17, Table 7).

Table 7. *Potential symptom-monitoring period (SMP) for AIT trials for three clinical relevant pollen (Cupressaceae, Poaceae, Urticaceae) in contrast with the proportion of days with no or low pollen concentration in six Southern European cities in 2018. From Hoffmann et al. [1].*

Pollen		**monitoring period**			**extra days with low pollen*,°**					
		start	end	days	Valencia	Marseille	Rome	Messina	Istanbul	Izmir
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

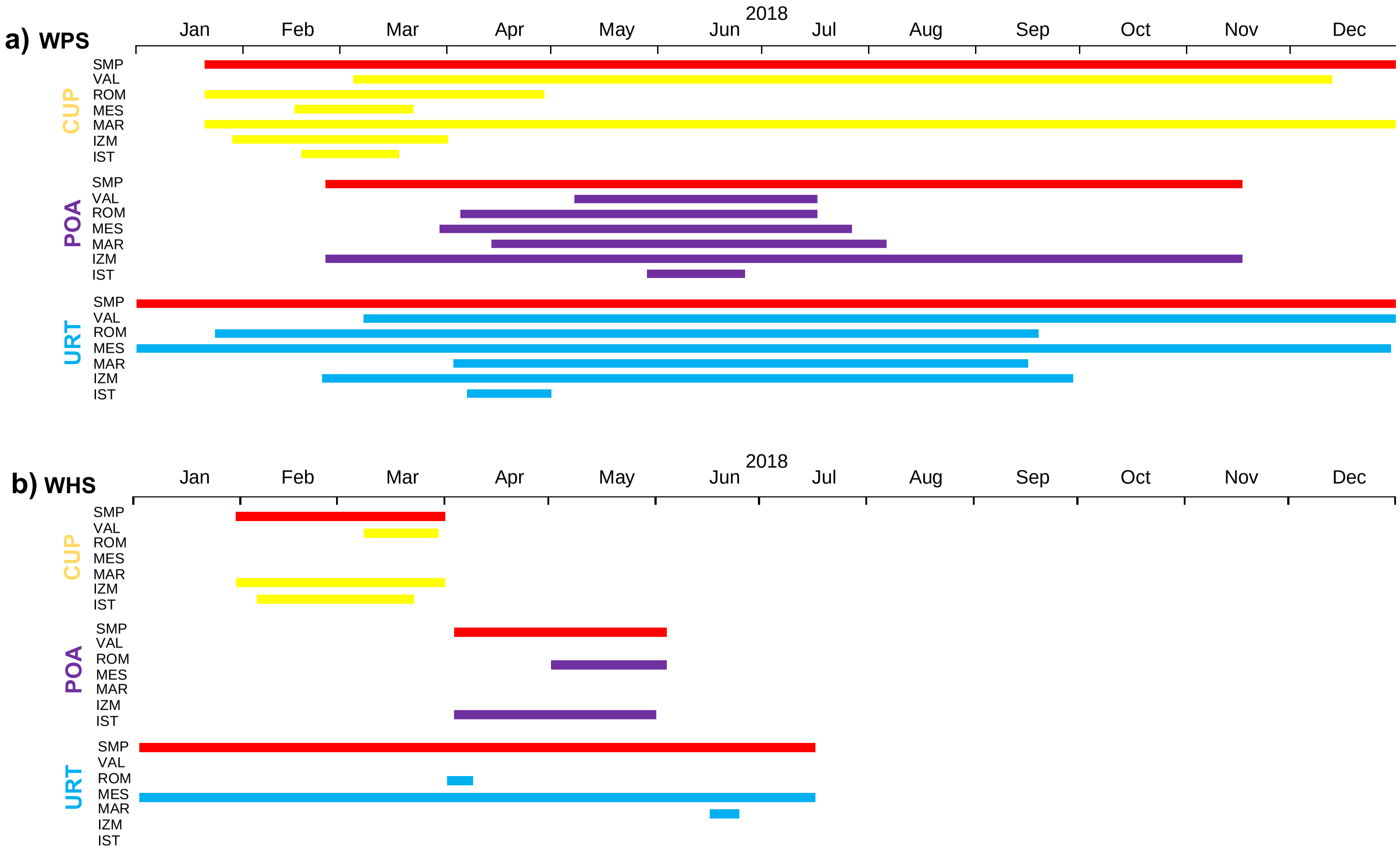

Figure 17. *Potential unified symptom-monitoring period (SMP, red) for allergen-specific immunotherapy (AIT) trial participants in Southern Europe in comparison to individual localized monitoring periods (for Valencia, VAL; Rome, ROM; Messina, MES; Marseille, MAR; Izmir, IZM; Istanbul, IST) based on* ***(A)*** *the whole pollen season (WPS) and* ***(B)*** *the whole high season (WHS) for Cupressaceae (CUP, yellow), Poaceae (POA, purple) and Urticaceae (URT, blue). From Hoffmann et al. et al [1].*

A comparison of the generated SMP with the potential monitoring period (WPS/WHS) for each center for all three pollen clarifies that the number of unnecessary symptom-monitoring days for the SMP is considerably higher (up to 341 days for WPS Urticaceae in Istanbul, Table 7) than for the local WPS/WHS periods. In fact, in 33.3% of cases for the WPS for all three pollen, the avoidable days with low pollen concentrations exceed a mark of 200 days, which is over 54.8% of the year. For the WHS, the numbers of unnecessary monitoring days are more moderate, with range from 0 days in Marseille, Messina and Izmir to 39 days in Valencia, with the two exceptions being 188 and 187 days for Urticaceae in Rome and Marseille, respectively.

3.4 The influence of rainfall on fragmented pollen season length

The meteorological rain data suggest more rainy days and higher average rainfall per day among the excluded days (intercurrent periods) than the days included in the FPSs, limited mainly to one pollen (two pollen in Rome) for the five centers (Table 8).

Table 8. *Included (incl) and excluded (excl) rainy days (>3mm per day) in/from the fragmented pollen seasons (FPS) for Cupressaceae (CUP), Fagaceae (FAG), Oleaceae (OLE), Poaceae (POA) and Urticaceae (URT) in six Mediterranean centers. Adapted from Hoffmann et al. [1].*

		WPS days		days incl in FPS				days excl from FPS				
						mm rain				mm rain		
				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
	POA	65	5	2	*3.1*	0.6	36.8	1	*20.0*	1.3	6.3	0.202
	URT	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
	POA	115	0	16	*13.9*	0.8	95.8	0	-	-	-	n/a
	URT	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
	POA	85	18	16	*18.8*	2.1	175.2	0	-	-	-	n/a
	URT	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
	POA	80	39	2	*2.5*	0.3	24.2	3	*7.7*	3.3	129.8	0.329
	URT	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
	POA	28	0	5	*17.9*	1.8	51.2	0	-	-	-	n/a
	URT	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
	POA	256	9	14	*5.5*	0.8	194.1	2	*22.2*	4.2	37.6	0.096
	URT	106	111	7	*6.6*	1.0	110.8	7	*6.3*	0.8	83.3	1.000

° rain= rainy days (>3mm per day) within the whole pollen season (WPS)

no rain= days without rain (<3 or 3mm per day) within the whole pollen season (WPS)

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

4 Discussion

4.1 Summary of the main findings

The investigation of pollen seasons for seven pollen taxa in six Southern European cities based on the EAACI pollen-exposure times definitions [45], revealed the following main findings:

i. The results demonstrated a wide variety in the lengths and patterns of the investigated pollen seasons, both between and within study centers with regard to each investigated pollen.
ii. It was observed that interrupting periods of low pollen concentrations (i.e., intercurrent periods) caused a fragmentation of pollen seasons into segments. To improve the accuracy of EAACI criteria [45], this thesis suggests an additional definition of FPS for Southern Europe.
iii. There is no realistically applicable option for a potential unified SMP for multicenter AIT trials in Mediterranean countries.
iv. The analysis demonstrates the relevance of the EAACI criteria [45] through a multicenter approach in six Southern European cities, as it was investigated for the grass and birch pollen seasons in four European countries so far [39,55–57].

4.2 Characteristics of atmospheric pollen abundancy

The length of the observed pollen seasons (WPS, FPS) always exceeded the high seasons (WHS, FHS), as expected due to the nature of the EAACI definitions [45]. Nonetheless, in a comparison of pollen and high seasons, an inconsistent pattern emerges among centers. For example, the WPS and FPS of Urticaceae were found in all six centers, whereas the WHS and FHS were observed only in Messina, Rome and Marseille. WPS and FPS of Poaceae were observed in all six centers too; although with a significant portion of their length from 28 days in Istanbul to 265 days in Izmir. According to $WHS_{Urticaceae}$, the $WHS_{Poaceae}$ was generated in only two of the six centers.

The results also demonstrated an intra-country variation in the pollen seasons, which could be seen between the Italian cities of Rome und Messina and the Turkish cities of Istanbul and Izmir. For example, the WPS and WHS for Fagales were distinguished in Rome, but not in Messina. This heterogeneity in season lengths seems to occur broader in contrast to Northern European countries; for example, a study found homogenous season lengths for *Betula* spp. for West and South Germany in two different years [77].

Nonetheless, these study sites are geographically closer – the geographical proximity may account for the similarities.

In total, the longest pollen seasons were the ones observed for Cupressaceae and Urticaceae (with the exception of Rome (Fagales) and Izmir (Poaceae)) followed by Poaceae. In Central and Northern Europe, on the other hand, Betulaceae (order Fagales) was found to be the most dominant pollen, followed by Poaceae (among the examined pollen of Betulaceae, Oleaceae, Poaceae and Asteraceae) [78]. Nonetheless, in the aforementioned study, the definition of the examined pollen seasons was based on percentages in comparison to the herein investigated thresholds [78]. This comparison reflects heterogeneity in pollen seasons across Europe and demonstrates why a local approach for pollen season definitions may be preferable for Southern Europe.

In the context of the aerobiological heterogeneity in the findings, it should be mentioned that all seven studied pollen taxa could be identified on at least one day in each of the six cities, apart from *Ambrosia* spp. in Valencia and Izmir. The levels of *Ambrosia* spp. and *Artemisia* spp. pollen concentration in these two centers were neither high enough nor continuous enough to generate WPS/WPS based on the EAACI criteria [45]. Poaceae, belonging also to the herbaceous plant order, however generated long seasons in most centers. An adaption to the same thresholds within the group of herbaceous plants was recommended in another paper [46]. Conversely, in the case of *Ambrosia* spp., another study has demonstrated variation in the distribution across Southern Europe, with decreasing levels towards the Mediterranean coasts [79].

With respect to Urticaceae, genera such as *Parietaria judaica* and *Cymbalaria muralis* favor a damp climate with mild winter periods and are well adapted to the wall constructions of Southern Italy, explaining their perennial abundance in the region [80] as reflected in the results for Messina. In contrast, only low levels of Urticaceae pollen were found in Izmir, reflecting aerobiological differences among the investigated centers. These results for Izmir are consistent with former findings from previous years [28].
For Cupressaceae, the representative genus *Cupressus sempervirens*, is particularly prevalent in France and Italy [81,82]. A review published in 2005 on cypress allergy in France concluded that measures such as avoidance of cultivating new cypress trees should be encouraged [83]. In accordance with the findings from the latter review, seasonality findings for Marseille revealed rather long WPS and WHS in comparison to

the other centers. It remains to be seen how the current @IT.2020 data on the prevalence of cypress allergy in Marseille will contribute to that matter.

4.3 Comparison to tested pollen season criteria in other studies

The produced FPS segments were generated by applying multiple start- and stop-signals to the WPS and WHS (days with the features required to start or end a season). The fragmentation of WPS using the EAACI criteria was not reported in other studies performed in mainly Northern and Central European countries [39,46,55–57]. An overview of recently performed studies can be found in Table 1 (see Objectives).

This study is the first one to consider exclusively Southern European study sites and to include seven pollen taxa in the analysis at once. Of these taxa, Urticaceae pollen seasons based on EAACI criteria [45] were first identified in this study; most other studies were performed for grass and birch only [46,55–57]. The fact that, in contrast to the other studies [39,46,55–57], not all WPS and WHS for each study center were determined, highlights the vast variety of the investigated cities and strengthens the conclusion that an individual approach, or the herein suggested fragmented method for Mediterranean cities, sheds light on possible options for overcoming this challenge in Southern Europe. However, this study's findings can confirm that the WPS and WHS were identified for Marseille, as stated in another study including study sites of the Rhônes-Alpes region [39]. Interestingly, two of those studies tested the correlation of patients' symptoms as documented in the hay fever diary (PHD) [36] and the generated birch and grass pollen seasons for Germany, Austria, Finland and France [39,57]. Significant correlations were found [39,57], except for grass in Finland/France for 2016 and for birch in Austria/Finland for 2015 [39]. However, PHD users included in the studies were not necessarily sensitized to birch and grass only. The authors expand further that birch flowered relatively little in Finland in 2015 [39]. The causes for the unsignificant correlation of pollen and symptoms in Finland remain unclear; it could be hypothesized that long periods of low pollen concentrations within the defined pollen seasons and the resulting lack of symptoms could be to blame. The aforementioned findings rise the question whether a fragmentation of the season would have substantially changed the results in this specific case.

4.4 Clinical relevance for allergen immunotherapy trials

The analysis of pollen seasons in Southern European cities was conducted as part of the clinical @IT.2020 multicenter study on the combined effect of CRD and mHealth on the diagnosis of seasonal allergic rhinitis, in the context of doctors' decisions concerning AIT prescription. Therefore, this section outlines and elaborates the relevance of the findings for AIT trials.

One of the diagnostic steps integrated in the @IT.2020 concept was the matching of electronically recorded patient symptoms with pollen data of the individually relevant aeroallergens during a fixed symptom monitoring period (see Methods). For 2018, based on WPS data, the distinguished potential SMP for Poaceae sensitized patients in the six investigated cities lasted for a total of 9.5 months in 2018, from the end of February to mid-November.

Similarities regarding the Cupressaceae SMP can be noted. It is worth noting that the SMP matches the WHS data more precisely. Nevertheless, fewer WHS periods were generated for the three pollen (Cupressaceae, Poaceae, Urticaceae) tested in those six centers. In conclusion, the more heterogeneous seasons must be unified in one generalized SMP for a trial, the more challenging it is to reliably conduct the trial. Patients' adherence results to the prescribed symptom-monitoring in the @IT.2020 pilot study indicated that from the seventh week of monitoring onward, the adherence decreased from the initial 90% in the first week to 70% [24]. Altogether, this scenario impacts a standardized design of a potential AIT multicenter trial for pollen allergies in Southern European cities.

It is plausible, that in some cases, the use of the fragmentation method proposed here could be even more meaningful: for example, when including several species of the same flowering plant family in a clinical trial, affecting the same area, having different flowering times and sharing cross-reacting major allergen molecules (e.g. PR-10 protein family of Fagales in Rome, Ole e 1 like family of Oleaceae in Marseille) [84]. This example may help setting up potential SMPs, for example, for patients sensitized to birch (Fagales) [13,66] in AIT trials [85].

Furthermore, a pollen calendar, which does not strictly refer to a calendar year (January to December), would arguably provide results of similar accuracy to those of the fragmentation method. Exemplary cases would be the pollen seasons of the Cupressaceae and Urticaceae in Valencia and of the Cupressaceae in Marseille. The

FPS, unlike the WPS, indicates that the “true (coherent) season” is likely shifted (e.g. from October/November to March/April for Cupressaceae in Valencia). Indeed, this is the case for Cupressaceae; findings from Southern Spain show that season start and end dates are near the beginning and end of the winter season [86]. Intriguingly, in one of the studies the thresholds for Cupressaceae were raised to over 30 pollen grains/m^3. The rationale was that Cupressaceae pollen are abundant over a long period of the year, and therefore the authors aimed to avoid intermediate periods of low pollen concentrations [86]. The question therefore arises as to whether the fragmentation method might comprise an adequate alternative to a modified pollen calendar that considers different start and end dates for each individual pollen, and is independet from the fixed boundaries of the months January to December. The importance of the clinical use of pollen calendars for allergic patients has been demonstrated previously [5,44].

The results show the significance of tailoring the symptom monitoring periods of multicenter studies in Southern Europe to the local aerobiological conditions of each Mediterranean region rather than following a standardized strategy. This conclusion implies that in some cases, the decision of which definition to use would greatly alter the outcome, both diagnostically and therapeutically [10]. Currently studied approaches to assess individual pollen exposure (e.g. via electronic tools) may help to overcome this burden by adding a new variable – the patient’s standpoint [57,87]. Feasible solutions such as the utilization of symptom scores documented in e-Diaries, may improve season definitions [87].

4.5 Limitations

Primarily, a very small percentage of days with missing pollen counts in each center was noted (see Methods, Table 2). Additionally, the analysis shows a vast range of flowering characteristics (within the Mediterranean basin) among the six centers which were included in this study. Therefore, conclusions drawn from the results may not be appliable for other regions. Moreover, the analyzed pollen data is based on 2018 data exclusively. The results should hence be further investigated further and confirmed using data from other years, as well as symptom data from e-Diaries to confirm the fragmentation method for clinical purposes [39]. Furthermore, the position of the pollen traps on rooftops may have led to an underestimation of herbaceous pollen [88]. In addition, the season fragmentation may be the product of multiple factors. For example, meteorological factors (e.g. periods of rainy days) may have interrupted long pollen seasons by lowering the amount of abundant pollen in the air [7]. Data from a study on spring rains shows that, in fact, pollen grains decrease during rainfall; on the contrary, certain peaks of certain pollen fragments were observed during heavy rainfalls and persisted up to 11 hours [7]. Other findings (in this case explicitly for group 5 grass allergens) suggest that a higher pollen concentration is not associated with pollen augmentation at higher humidity levels. It was hypothesized that more and smaller allergen-containing particles are released under these humid conditions [89]. Whether and how this conclusion might be clinically relevant for patients with allergic rhinitis remains to be seen and investigated in future clinical trials. Finally, the inclusion of multiple Fagales species (see Methods) hinders the investigation of each genus. As previously mentioned, however, researchers have found cross-reactivity caused by PR-10 molecules within the group of birch-homologous species [63].

4.6 Conclusions

Concerning their time of occurrence, distributions and lengths, the flowering periods of major allergenic plants are quite diverse in Southern European countries. A uniform traditional pollen calendar would therefore be desirable in a standardized methodology for study centers included in AIT studies. It would, however, be challenging to create such as calendar, which would reflect the aforementioned geographic variation.

The results of this present study demonstrate that applying the EAACI pollen season criteria to Southern European regions, a method validated primarily for Central and Northern European countries, generates rather long seasons including days or weeks with no or low pollen concentrations. Potential SMPs for AIT studies based on the EAACI criteria could thus include intermittent periods of low pollen concentrations. This inclusion is problematic as the effectiveness assessment of AIT is based on patient symptoms during these defined pollen-exposure times. The introduced FPS method based on the EAACI criteria offers a greater accuracy than the same EAACI methodology. In conclusion, the proposed fragmented method created shorter, and more specific, albeit fragmented, pollen seasons in Southern European countries. Whether this approach's results are similar to those of other geographic regions in Southern Europe should be further investigated; and it remains to be seen whether this approach is useful in clinical trials in Southern Europe.

5 Bibliography

1. Hoffmann TM, Acar Şahin A, Aggelidis X, Arasi S, Barbalace A, Bourgoin A, Bregu B, Brighetti MA, Caeiro E, Caglayan Sozmen S, Caminiti L, Charpin D, Couto M, Delgado L, Di Rienzo Businco A, Dimier C, Dimou MV, Fonseca JA, Goksel O, Guvensen A, Hernandez D, Jang DT, Kalpaklioglu F, Lame B, Llusar R, Makris MP, Mazon A, Mesonjesi E, Nieto A, Öztürk A, Pahus L, Pajno GB, Panasiti I, Panetta V, Papadopoulos NG, Pellegrini E, Pelosi S, Pereira AM, Pereira M, Pinar M, Pfaar O, Potapova E, Priftanji A, Psarros F, Sackesen C, Sfika I, Suarez J, Thibaudon M, Travaglini A, Tripodi S, Verdier V, Villella V, Xepapadaki P, Yazici D, Matricardi PM, Dramburg S. “Whole” vs. “fragmented” approach to EAACI pollen season definitions: A multicenter study in six Southern European cities. Allergy. 2020;75:1659–71.
2. Pawankar R, Canonica GW, Holgate ST, Lockey RF, Blaiss M. The WAO White Book on Allergy (Update 2013): [edition unknown]. Milwaukee: World Allergy Organization (WAO); 2013.
3. Vandenplas O, Vinnikov D, Blanc PD, Agache I, Bachert C, Bewick M, Cardell L-O, Cullinan P, Demoly P, Descatha A, Fonseca J, Haahtela T, Hellings PW, Jamart J, Jantunen J, Kalayci Ö, Price D, Samolinski B, Sastre J, Tian L, Valero AL, Zhang X, Bousquet J. Impact of Rhinitis on Work Productivity: A Systematic Review. The Journal of Allergy and Clinical Immunology: In Practice. 2018;6:1274–1286.e9. doi:10.1016/j.jaip.2017.09.002.
4. Liu J, Zhang X, Zhao Y, Wang Y. The association between allergic rhinitis and sleep: A systematic review and meta-analysis of observational studies. PLoS One. 2020;15:e0228533. doi: 10.1016/j.dld.2007.09.006.
5. Skoner DP. Allergic rhinitis: Definition, epidemiology, pathophysiology, detection, and diagnosis. Journal of Allergy and Clinical Immunology. 2001; 108:S2–8
6. Grote M, Vrtala S, Niederberger V, Wiermann R, Valenta R, Reichelt R. Release of allergen-bearing cytoplasm from hydrated pollen: A mechanism common to a variety of grass (Poaceae) species revealed by electron microscopy. Journal of Allergy and Clinical Immunology. 2001;108:109–15.
7. Hughes DD, Mampage CBA, Jones LM, Liu Z, Stone EA. Characterization of Atmospheric Pollen Fragments during Springtime Thunderstorms. Environ Sci Technol Lett. 2020;7:409–14.

8. Bousquet J, Anto JM, Bachert C, Baiardini I, Bosnic-Anticevich S, Walter Canonica G, Melén E, Palomares O, Scadding GK, Togias A, Toppila-Salmi S. Allergic rhinitis. Nat Rev Dis Primers. 2020;6:95.
9. Senna G, Caminati M, Lockey RF. Allergen Immunotherapy Adherence in the Real World: How Bad Is It and How Can It Be Improved? Curr Treat Options Allergy. 2015;2:39–53.
10. Pfaar O, Alvaro M, Cardona V, Hamelmann E, Mösges R, Kleine-Tebbe J. Clinical trials in allergen immunotherapy: current concepts and future needs. Allergy. 2018;73:1775–83.
11. Bousquet P-J, Chinn S, Janson C, Kogevinas M, Burney P, Jarvis D. Geographical variation in the prevalence of positive skin tests to environmental aeroallergens in the European Community Respiratory Health Survey I: Prevalence of positive skin tests to environmental aeroallergens. Allergy. 2007;62:301–9.
12. Ballero M, Maxia A. Pollen spectrum variations in the atmosphere of Cagliari, Italy. Aerobiologia. 2003;19:251–9.
13. D'Amato G, Cecchi L, Bonini S, Nunes C, Annesi-Maesano I, Behrendt H, Liccardi G, Popov T, Cauwenberge PV. Allergenic pollen and pollen allergy in Europe. Allergy. 2007;62:976–90.
14. Pfaar O, Bonini S, Cardona V, Demoly P, Jakob T, Jutel M, Kleine-Tebbe J, Klimek L, Klysner S, Kopp MV, Kuna P, Larché M, Muraro A, Schmidt-Weber CB, Shamji MH, Simonsen K, Somoza C, Valovirta E, Zieglmayer P, Zuberbier T, Wahn U. Perspectives in allergen immunotherapy: 2017 and beyond. Allergy. 2018; 73(Suppl 104):5–23.
15. Agache I, Akdis C, Jutel M, Virchow JC. Untangling asthma phenotypes and endotypes. Allergy. 2012;67:835–46.
16. Matricardi PM, Dramburg S, Alvarez-Perea A, Antolín-Amérigo D, Apfelbacher C, Atanaskovic-Markovic M, Berger U, Blaiss MS, Blank S, Boni E, Bonini M, Bousquet J, Brockow K, Buters J, Cardona V, Caubet J-C, Cavkaytar Ö, Elliott T, Esteban-Gorgojo I, Fonseca JA, Gardner J, Gevaert P, Ghiordanescu I, Hellings P, Hoffmann-Sommergruber K, Kalpaklioglu AF, Marmouz F, Calderón ÁM, Mösges R, Nakonechna A, Ollert M, Oteros J, Pajno G, Panaitescu C, Perez-Formigo D, Pfaar O, Pitsios C, Rudenko M, Ryan D, Sánchez-García S, Shih J, Tripodi S, Poel L-AV der, Os-Medendorp H van, Varricchi G, Wittmann J, Worm M, Agache I. The role of mobile

health technologies in allergy care: An EAACI position paper. Allergy. 2020;75:259–72.

17. Matricardi PM, Dramburg S, Potapova E, Skevaki C, Renz H. Molecular diagnosis for allergen immunotherapy. Journal of Allergy and Clinical Immunology. 2019;143:831–43.
18. Pablos I, Wildner S, Asam C, Wallner M, Gadermaier G. Pollen Allergens for Molecular Diagnosis. Curr Allergy Asthma Rep. 2016;16:31.
19. Arasi S, Castelli S, Di Fraia M, Villalta D, Tripodi S, Perna S, Dramburg S, Brighetti MA, Conte M, Martelli P, Sfika I, Travaglini A, Verardo PL, Villella V, Matricardi PM. @IT2020: An innovative algorithm for allergen immunotherapy prescription in seasonal allergic rhinitis. Clin Exp Allergy. 2021. doi:10.1111/cea.13867. [Epub ahead of print].
20. Douladiris N, Savvatianos S, Roumpedaki I, Skevaki C, Mitsias D, Papadopoulos NG. A Molecular Diagnostic Algorithm to Guide Pollen Immunotherapy in Southern Europe: Towards Component-Resolved Management of Allergic Diseases. IAA. 2013;162: 163–72.
21. Barber D, de la Torre F, Lombardero M, Antépara I, Colas C, Dávila I, Tabar AI, Vidal C, Villalba M, Salcedo G, Rodríguez R. Component-resolved diagnosis of pollen allergy based on skin testing with profilin, polcalcin and lipid transfer protein pan-allergens. Clinical & Experimental Allergy. 2009;39:1764–73.
22. Tripodi S, Giannone A, Sfika I, Pelosi S, Dramburg S, Bianchi A, Pizzulli A, Florack J, Villella V, Potapova E, Matricardi PM. Digital technologies for an improved management of respiratory allergic diseases: 10 years of clinical studies using an online platform for patients and physicians. Ital J Pediatr. 2020;46:105.
23. Matricardi PM, Potapova E, Forchert L, Dramburg S, Tripodi S. Digital allergology: Towards a clinical decision support system for allergen immunotherapy. Pediatr Allergy Immunol. 2020;31:61–4.
24. Di Fraia M, Tripodi S, Arasi S, Dramburg S, Castelli S, Villalta D, Buzzulini F, Sfika I, Villella V, Potapova E, Perna S, Brighetti MA, Travaglini A, Verardo P, Pelosi S, Zicari AM, Matricardi PM. Adherence to Prescribed E-Diary Recording by Patients With Seasonal Allergic Rhinitis: Observational Study. J Med Internet Res. 2020;22:e16642. doi: 10.2196/16642.
25. Lipp T, Acar Şahin A, Aggelidis X, Arasi S, Barbalace A, Bourgoin A, Bregu B, Brighetti MA, Caeiro E, Caglayan Sozmen S, Caminiti L, Charpin D, Couto M, Delgado L, Di Rienzo Businco A, Dimier C, Dimou MV, Fonseca JA, Goksel O, Guvensen A,

Hernandez D, Hoffmann TM, Jang DT, Kalpaklioglu F, Lame B, Llusar R, Makris M, Mazon A, Mesonjesi E, Nieto A, Öztürk A, Pahus L, Pajno G, Panasiti I, Papadopoulos NG, Pellegrini E, Pelosi S, Pereira AM, Pereira M, Pinar NM, Potapova E, Priftanji A, Psarros F, Sackesen C, Sfika I, Suarez J, Thibaudon M, Travaglini A, Tripodi S, Verdier V, Villella V, Xepapadaki P, Yazici D, Matricardi PM, Dramburg S. Heterogeneity of Pollen Food Allergy Syndrome in Seven Southern European Countries: the @IT.2020 Multicenter Study. Allergy. 2021. doi:10.1111/all.14742. [Epub ahead of print].

26. Hoffmann TM, Travaglini A, Brighetti MA, Acar Şahin A, Arasi S, Bregu B, Caeiro E, Caglayan Sozmen S, Charpin D, Delgado L, Dimou M, Fiorilli M, Fonseca JA, Goksel O, Kalpaklioglu F, Lame B, Mazon A, Mesonjesi E, Nieto A, Öztürk A, Pajno G, Papadopoulos NG, Pellegrini E, Pereira AM, Pereira M, Pinar NM, Pinter E, Priftanji A, Sackesen C, Sfika I, Suarez J, Thibaudon M, Tripodi S, Ugus U, Villella V, Matricardi PM, Dramburg S. Cumulative Pollen Concentration Curves for Pollen Allergy Diagnosis. J Investig Allergol Clin Immunol. 2020. doi:10.18176/jiaci.0646. [Epub ahead of print].
27. Werchan M, Werchan B, Bergmann K-C. German pollen calendar 4.0 – update based on 2011–2016 pollen data. Allergo J Int. 2018;27:69–71.
28. Guvensen A, Ozturk M. Airborne pollen calendar of Izmir - Turkey. Ann Agric Environ Med. 2003;10:37–44.
29. Katotomichelakis M, Nikolaidis C, Makris M, Zhang N, Aggelides X, Constantinidis TC, Bachert C, Danielides V. The clinical significance of the pollen calendar of the Western Thrace/northeast Greece region in allergic rhinitis. International Forum of Allergy & Rhinology. 2015;5:1156–63.
30. Geller-Bernstein C, Portnoy JM. The Clinical Utility of Pollen Counts. Clin Rev Allergy Immunol. 2019;57:340–349.
31. Wayne P, Foster S, Connolly J, Bazzaz F, Epstein P. Production of allergenic pollen by ragweed (Ambrosia artemisiifolia L.) is increased in CO2-enriched atmospheres. Ann Allergy Asthma Immunol. 2002;88:279–82.
32. Lake IR, Jones NR, Agnew M, Goodess CM, Giorgi F, Hamaoui-Laguel L, Semenov MA, Solomon F, Storkey J, Vautard R, Epstein MM. Climate Change and Future Pollen Allergy in Europe. Environmental Health Perspectives. 2017;125:385–91.
33. Barnes CS. Impact of Climate Change on Pollen and Respiratory Disease. Curr Allergy Asthma Rep. 2018;18:59.

34. Levetin E, Van de Water PK. Pollen count forecasting. Immunology and Allergy Clinics of North America. 2003;23:423–42.
35. Rodríguez-Rajo FJ, Valencia-Barrera RM, Vega-Maray AM, Suárez FJ, Fernández-González D, Jato V. Prediction of airborne Alnus pollen concentration by using ARIMA models. Ann Agric Environ Med. 2017;13:25–32.
36. Berger UE, Jäger S, Bergmann KC. PHD, the electronic patient's hayfever diary. European Respiratory Journal. 2011;38:3213.
37. Oteros J, Pusch G, Weichenmeier I, Heimann U, Möller R, Röseler S, Traidl-Hoffmann C, Schmidt-Weber C, Buters JTM. Automatic and Online Pollen Monitoring. International Archives of Allergy and Immunology. 2015;167:158–66.
38. Bastl K, Kmenta M, Berger M, Berger U. The connection of pollen concentrations and crowd-sourced symptom data: new insights from daily and seasonal symptom load index data from 2013 to 2017 in Vienna. World Allergy Organization Journal. 2018;16;11:24. doi: 10.1186/s40413-018-0203-6.
39. Pfaar O, Karatzas K, Bastl K, Berger U, Buters J, Darsow U, Demoly P, Durham SR, Galán C, Gehrig R, Wijk RG van, Jacobsen L, Katsifarakis N, Klimek L, Saarto A, Sofiev M, Thibaudon M, Werchan B, Bergmann K-C. Pollen season is reflected on symptom load for grass and birch pollen-induced allergic rhinitis in different geographic areas—An EAACI Task Force Report. Allergy. 2020;75:1099–106.
40. Peeters AG. Cumulative temperatures for prediction of the beginning of ash (Fraxinus excelsior L.) pollen season. Aerobiologia. 1998;14:375.
41. Davies JM, Berman D, Beggs PJ, Ramón GD, Peter J, Katelaris CH, Ziska LH. Global Climate Change and Pollen Aeroallergens. Immunology and Allergy Clinics of North America. 2021;41:1–16.
42. Beck I, Jochner S, Gilles S, McIntyre M, Buters JTM, Schmidt-Weber C, Behrendt H, Ring J, Menzel A, Traidl-Hoffmann C. High Environmental Ozone Levels Lead to Enhanced Allergenicity of Birch Pollen. Hogan SP, editor. PLoS ONE. 2013;8:e80147. doi: 10.1371/journal.pone.0080147.
43. Jacobs RL, Harper N, He W, Andrews CP, Rather CG, Ramirez DA, Ahuja SK. Effect of confounding cofactors on responses to pollens during natural season versus pollen challenge chamber exposure. Journal of Allergy and Clinical Immunology. 2014;133:1340–1346.e7. doi: 10.1016/j.jaci.2013.09.051.
44. De Weger L, Dahl Å, Bergmann K., Belmonte J, Rantio-Lehtimaaki A, Thibaudon M, Dechamp C, Brandao R, Selig A, Gehrig R, Buters J, Emberlin J, Ceccchi, L,

Besancenot JP, Galán C, Waisel Y. Chapter 6. Impact of pollen. In: Sofiev M, Bergmann KC, editors. Allergenic pollen. A review of the production, release, distribution and health impacts. Dordrecht: Springer; 2012. p. 161–203.

45. Pfaar O, Bastl K, Berger U, Buters J, Calderon MA, Clot B, Darsow U, Demoly P, Durham SR, Galán C, Gehrig R, Wijk RG van, Jacobsen L, Klimek L, Sofiev M, Thibaudon M, Bergmann KC. Defining pollen exposure times for clinical trials of allergen immunotherapy for pollen-induced rhinoconjunctivitis – an EAACI position paper. Allergy. 2017;72:713–22.
46. Bastl K, Kmenta M, Berger UE. Defining Pollen Seasons: Background and Recommendations. Curr Allergy Asthma Rep. 2018;18:73.
47. Jato V, Rodríguez-Rajo FJ, Alcázar P, De Nuntiis P, Galán C, Mandrioli P. May the definition of pollen season influence aerobiological results? Aerobiologia. 2006;22:13–25.
48. Canonica GW, Baena-Cagnani CE, Bousquet J, Bousquet PJ, Lockey RF, Malling H-J, Passalacqua G, Potter P, Valovirta E. Recommendations for standardization of clinical trials with Allergen Specific Immunotherapy for respiratory allergy. A statement of a World Allergy Organization (WAO) taskforce. Allergy. 2007;62:317–24.
49. Creticos PS, Maloney J, Bernstein DI, Casale T, Kaur A, Fisher R et al.Randomized controlled trial of a ragweed allergy immunotherapy tablet in North American and European adults. J Allergy Clin Immunol. 2013;131:1342–9.
50. Durham SR, Nelson HS, Nolte H, Bernstein DI, Creticos PS, Li Z, Andersen JS. Magnitude of efficacy measurements in grass allergy immunotherapy trials is highly dependent on pollen exposure. Allergy. 2014;69:617–23.
51. Voukantsis D, Karatzas K, Jaeger S, Berger U, Smith M. Analysis and forecasting of airborne pollen–induced symptoms with the aid of computational intelligence methods. Aerobiologia. 2013;29:175–85.
52. Mösges R, Bachert C, Panzner P, Calderon MA, Haazen L, Pirotton S, Wathelet N, Durham SR, Bonny M-A, Legon T, Frenckell R von, Pfaar O, Shamji MH. Short course of grass allergen peptides immunotherapy over 3 weeks reduces seasonal symptoms in allergic rhinoconjunctivitis with/without asthma: A randomized, multicenter, double-blind, placebo-controlled trial. Allergy. 2018;73:1842–50.
53. Gorczynski RM. Pollen aero allergens and the climate in mediterranean region and allergen sensitivity in allergic rhinoconjunctivitis and allergic asthma patients. Med Sci Monit. 2013;19:102–10.

54. Nikolaidis C, Katotomichelakis M, Nena E, Makris M, Tsakas M, Michopoulos I, Constantinidis TC, Danielides V. Seasonal variations of allergenic pollen in a Mediterranean region – Alexandroupolis, north-east Greece. Ann Agric Environ Med. 2015;22:685–9.
55. Bergmann KC, Buters J, Karatzas K, Tasioulis T, Werchan B, Werchan M, Pfaar O. The development of birch pollen seasons over 30 years in Munich, Germany—An EAACI Task Force report*. Allergy. 2020;75:3024–6.
56. Karatzas K, Riga M, Berger U, Werchan M, Pfaar O, Bergmann KC. Computational validation of the recently proposed pollen season definition criteria. Allergy. 2018;73:5–7.
57. Karatzas K, Katsifarakis N, Riga M, Werchan B, Werchan M, Berger U, Pfaar O, Bergmann K-C. New European Academy of Allergy and Clinical Immunology definition on pollen season mirrors symptom load for grass and birch pollen-induced allergic rhinitis. Allergy. 2018;73:1851–9.
58. Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, Zuberbier T, Baena-Cagnani CE, Canonica GW, Weel CV, Agache I, Aït-Khaled N, Bachert C, Blaiss MS, Bonini S, Boulet L-P, Bousquet P-J, Camargos P, Carlsen K-H, Chen Y, Custovic A, Dahl R, Demoly P, Douagui H, Durham SR, Wijk RGV, Kalayci O, Kaliner MA, Kim Y-Y, Kowalski ML, Kuna P, Le LTT, Lemiere C, Li J, Lockey RF, Mavale-Manuel S, Meltzer EO, Mohammad Y, Mullol J, Naclerio R, O'Hehir RE, Ohta K, Ouedraogo S, Palkonen S, Papadopoulos N, Passalacqua G, Pawankar R, Popov TA, Rabe KF, Rosado-Pinto J, Scadding GK, Simons FER, Toskala E, Valovirta E, Cauwenberge PV, Wang D-Y, Wickman M, Yawn BP, Yorgancioglu A, Yusuf OM, Zar H, Annesi-Maesano I, Bateman ED, Kheder AB, Boakye DA, Bouchard J, Burney P, Busse WW, Chan-Yeung M, Chavannes NH, Chuchalin A, Dolen WK, Emuzyte R, Grouse L, Humbert M, Jackson C, Johnston SL, Keith PK, Kemp JP, Klossek J-M, Larenas-Linnemann D, Lipworth B, Malo J-L, Marshall GD, Naspitz C, Nekam K, Niggemann B, Nizankowska-Mogilnicka E, Okamoto Y, Orru MP, Potter P, Price D, Stoloff SW, Vandenplas O, Viegi G, Williams D. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008*. Allergy. 2008;63(s86):8–160.
59. GINA-Main-Report-2021-V2-WMS.pdf [Internet]. [cited 2021 Jun 15]. Available from: https://ginasthma.org/wp-content/uploads/2021/05/GINA-Main-Report-2021-V2-WMS.pdf

60. Florack J, Brighetti MA, Perna S, Pizzulli A, Pizzulli A, Tripodi S, Costa C, Travaglini A, Pelosi S, Bianchi A, Tsilochristou O, Gabrielli F, Matricardi PM. Comparison of six disease severity scores for allergic rhinitis against pollen counts a prospective analysis at population and individual level. Pediatric Allergy and Immunology. 2016;27:382–90.
61. Euro Immun. Details Product No. 3211-1 [Internet]. 2021 [cited 2021 May 12]. Available from: https://www.euroimmun.com/products/allergy-diagnostics/pd/dpa-dx/3211-1/3/86726/.
62. Lionello P, Malanotte-Rizzoli P, Boscolo R, Alpert P, Artale V, Li L, Luterbacher J, May W, Trigo R, Tsimplis M, Ulbrich U, Xoplaki E. The Mediterranean climate: An overview of the main characteristics and issues. In: Lionello P, Malanotte-Rizzoli P, Boscolo R, editors. Developments in Earth and Environmental Sciences: [edition unknown]. Amsterdam: Elsevier; 2006. p. 1–26.
63. Hauser M, Asam C, Himly M, Palazzo P, Voltolini S, Montanari C, Briza P, Bernardi ML, Mari A, Ferreira F, Wallner M. Bet v 1-like pollen allergens of multiple Fagales species can sensitize atopic individuals. Clinical & Experimental Allergy. 2011;41:1804–14.
64. Ribeiro H, Oliveira M, Ribeiro N, Cruz A, Ferreira A, Machado H, Reis A, Abreu I. Pollen allergenic potential nature of some trees species: A multidisciplinary approach using aerobiological, immunochemical and hospital admissions data. Environmental Research. 2009;109:328–33.
65. EMA Europa. Allergen Products Production [Internet]. 2008 [cited 2019 May 13]. Available from: https://www.ema.europa.eu/en/allergen-products-production-quality-issues.
66. Lorenz AR, Lüttkopf D, May S, Scheurer S, Vieths S. The Principle of Homologous Groups in Regulatory Affairs of Allergen Products – A Proposal. IAA. 2009;148:1–17.
67. WHO/IUIS Allergen Nomenclature Sub-Comittee. Allergen Search Results [Internet]. 2003 [cited 2021 April 24]. Available from: http://www.allergen.org/search.php?allergenname=&allergensource=&TaxSource=Plantae+Magnoliopsida&TaxOrder=Fagales&foodallerg=all&bioname=.
68. United States Department of Agriculture/Natural Resources Conservation Service. Classification | USDA PLANTS [Internet]. No date [cited 2021 April 24]. Available from: https://plants.usda.gov/classification.html.

69. Asturias JA, Ibarrola I, Eraso E, Arilla MC, Martinez A. The major Platanus acerifolia pollen allergen Pla a 1 has sequence homology to invertase inhibitors. Clin Exp Allergy 2003; 33:978–985.
70. Asam C, Hofer H, Wolf M, Aglas L, Wallner M. Tree pollen allergens—an update from a molecular perspective. Allergy. 2015;70:1201–11.
71. EAS QC Working Group, Galán C, Smith M, Thibaudon M, Frenguelli G, Oteros J, Gehrig R, Berger U, Clot B, Brandao R. Pollen monitoring: minimum requirements and reproducibility of analysis. Aerobiologia. 2014;30:385–95.
72. DIN. DIN EN 16868 Ambient air – Sampling and analysis of airborne pollen grains and fungal spores for networks related to allergy – Volumetric Hirst method [Internet]. 2019 [cited 2021 May 06]. Available from: https://www.din.de/en/getting-involved/standards-committees/krdl/publications/wdc-beuth:din21:300169880.
73. Núñez A, Amo de Paz G, Rastrojo A, García AM, Alcamí A, Montserrat Gutiérrez-Bustillo A, Moreno DA. Monitoring of airborne biological particles in outdoor atmosphere. Part 2: Metagenomics applied to urban environments. International Microbiology. 2016;19:69–80.
74. Galán C, Ariatti A, Bonini M, Clot B, Crouzy B, Dahl A, Fernandez-González D, Frenguelli G, Gehrig R, Isard S, Levetin E, Li DW, Mandrioli P, Rogers CA, Thibaudon M, Sauliene I, Skjoth C, Smith M, Sofiev M. Recommended terminology for aerobiological studies. Aerobiologia. 2017;33:293–5.
75. Mandrioli P. Basic aerobiology. Aerobiologia. 1998;14:89.
76. Rojo J, Picornell A, Oteros J. AeRobiology: The computational tool for biological data in the air. Methods Ecol Evol. 2019;10:1371–1376.
77. Buters JTM, Kasche A, Weichenmeier I, Schober W, Klaus S, Traidl-Hoffmann C, Menzel A, Huss-Marp J, Krämer U, Behrendt H. Year-to-Year Variation in Release of Bet v 1 Allergen from Birch Pollen: Evidence for Geographical Differences between West and South Germany. International Archives of Allergy and Immunology. 2008;145:122–30.
78. Smith M, Jäger S, Berger U, Šikoparija B, Hallsdottir M, Sauliene I, Bergmann K-C, Pashley CH, Weger L de, Majkowska-Wojciechowska B, Rybníček O, Thibaudon M, Gehrig R, Bonini M, Yankova R, Damialis A, Vokou D, Bustillo AMG, Hoffmann-Sommergruber K, Ree R van. Geographic and temporal variations in pollen exposure across Europe. Allergy. 2014;69:913–23.

79. Sikoparija B, Skjøth CA, Celenk S, Testoni C, Abramidze T, Alm Kübler K, Belmonte J, Berger U, Bonini M, Charalampopoulos A, Damialis A, Clot B, Dahl Å, de Weger LA, Gehrig R, Hendrickx M, Hoebeke L, Ianovici N, Kofol Seliger A, Magyar D, Mányoki G, Milkovska S, Myszkowska D, Páldy A, Pashley CH, Rasmussen K, Ritenberga O, Rodinkova V, Rybníček O, Shalaboda V, Šaulienė I, Ščevková J, Stjepanović B, Thibaudon M, Verstraeten C, Vokou D, Yankova R, Smith M. Spatial and temporal variations in airborne Ambrosia pollen in Europe. Aerobiologia. 2017;33:181–9.
80. Brandes D. Flora und Vegetation von Stadtmauern. Tuexenia. 1992;12:315–39.
81. Caudullo G, de Rigo D. Cupressus sempervirens in Europe; distribution, habitat, usage and threats. Luxembourg: Publication Office of the European Union; 2016.
82. Bagnoli F, Vendramin GG, Buonamici A, Doulis AG, González-Martínez SC, Porta NL, Magri D, Raddi P, Sebastiani F, Fineschi S. Is Cupressus sempervirens native in Italy? An answer from genetic and palaeobotanical data. Molecular Ecology. 2009;18:2276–86.
83. Charpin D, Calleja M, Lahoz C, Pichot C, Waisel Y. Allergy to cypress pollen. Allergy. 2005;60:293–301.
84. Matricardi PM, Kleine-Tebbe J, Hoffmann HJ, Valenta R, Hilger C, Hofmaier S, Aalberse RC, Agache I, Asero R, Ballmer-Weber B, Barber D, Beyer K, Biedermann T, Bilò MB, Blank S, Bohle B, Bosshard PP, Breiteneder H, Brough HA, Caraballo L, Caubet JC, Crameri R, Davies JM, Douladiris N, Ebisawa M, EIgenmann PA, Fernandez-Rivas M, Ferreira F, Gadermaier G, Glatz M, Hamilton RG, Hawranek T, Hellings P, Hoffmann-Sommergruber K, Jakob T, Jappe U, Jutel M, Kamath SD, Knol EF, Korosec P, Kuehn A, Lack G, Lopata AL, Mäkelä M, Morisset M, Niederberger V, Nowak-Węgrzyn AH, Papadopoulos NG, Pastorello EA, Pauli G, Platts-Mills T, Posa D, Poulsen LK, Raulf M, Sastre J, Scala E, Schmid JM, Schmid-Grendelmeier P, Hage M van, Ree R van, Vieths S, Weber R, Wickman M, Muraro A, Ollert M. EAACI Molecular Allergology User's Guide. Pediatric Allergy and Immunology. 27:1–250.
85. Kleine-Tebbe J, Zuberbier T, Werfel T, Krüll M, Wagenmann M, Johansen N, Würtzen PA, Wolf H, Mücke V, Wüstenberg E, Biedermann T. Is allergy immunotherapy with birch sufficient to treat patients allergic to pollen of tree species of the birch homologous group? Allergy. 2020;75:1327–36.
86. Velasco-Jiménez MJ, Alcázar P, Díaz de la Guardia C, Trigo M del M, de Linares C, Recio M, Galán C. Pollen season trends in winter flowering trees in South Spain. Aerobiologia. 2020;36:213–24.

87. Bastl K, Kmenta M, Geller-Bernstein C, Berger U, Jäger S. Can we improve pollen season definitions by using the symptom load index in addition to pollen counts? Environmental Pollution. 2015;204:109–16.

88. Rojo J, Oteros J, Pérez-Badia R, Cervigón P, Ferencova Z, Gutiérrez-Bustillo AM, Bergmann K-C, Oliver G, Thibaudon M, Albertini R, Rodríguez-De la Cruz D, Sánchez-Reyes E, Sánchez-Sánchez J, Pessi A-M, Reiniharju J, Saarto A, Calderón MC, Guerrero C, Berra D, Bonini M, Chiodini E, Fernández-González D, García J, Trigo MM, Myszkowska D, Fernández-Rodríguez S, Tormo-Molina R, Damialis A, Kolek F, Traidl-Hoffmann C, Severova E, Caeiro E, Ribeiro H, Magyar D, Makra L, Udvardy O, Alcázar P, Galán C, Borycka K, Kasprzyk I, Newbigin E, Adams-Groom B, Apangu GP, Frisk CA, Skjøth CA, Radišić P, Šikoparija B, Celenk S, Schmidt-Weber CB, Buters J. Near-ground effect of height on pollen exposure. Environmental Research. 2019;174:160–9.

89. Buters J, Prank M, Sofiev M, Pusch G, Albertini R, Annesi-Maesano I et al. Variation of the group 5 grass pollen allergen content of airborne pollen in relation to geographic location and time in season. J Allergy Clin Immunol. 2015;136:87–95.

6 Appendix

6.1 Workshop material: questionnaires on the graphical presentation of cumulative pollen concentrations

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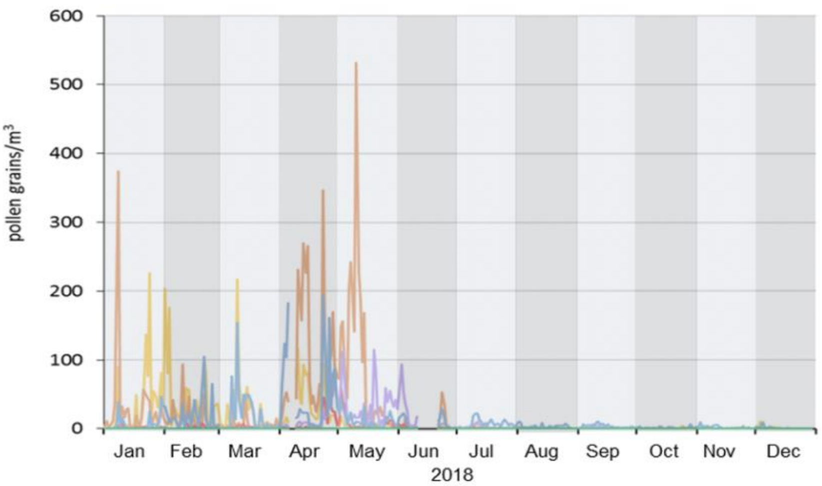
Representation of Pollen Concentrations – Validation

ID: WS-_ _ _

In the following, two representations of pollen concentration trajectories are shown to you. Please answer the questions below, multiple answers are possible.

a) Daily Pollen Concentration Trajectory

The following figure provides information on the **daily pollen concentration** of Cupressaceae (CUP), Fagales (FAG), Oleaceae (OLE), Graminaceae (GRA), *Parietaria (PAR)*, *Ambrosia (AMB)* and *Artemisia (ART)* in Rome, for 2018.



(Please complete the sentence by making a cross (X) for the ones you consider):

1. I can distinguish the trajectories of ...

☐ CUP ☐ FAG ☐ OLE ☐ GRA ☐ PAR ☐ AMB ☐ ART ☐ None

2. I can see the highest pollen concentration peaks of...

☐ CUP ☐ FAG ☐ OLE ☐ GRA ☐ PAR ☐ AMB ☐ ART ☐ None

3. I can see that May is the month with highest concentration of...

☐ CUP ☐ FAG ☐ OLE ☐ GRA ☐ PAR ☐ AMB ☐ ART ☐ None

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Representation of Pollen Concentrations – Validation

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In the following, two representations of pollen concentration trajectories are shown to you. Please answer the questions below, multiple answers are possible.

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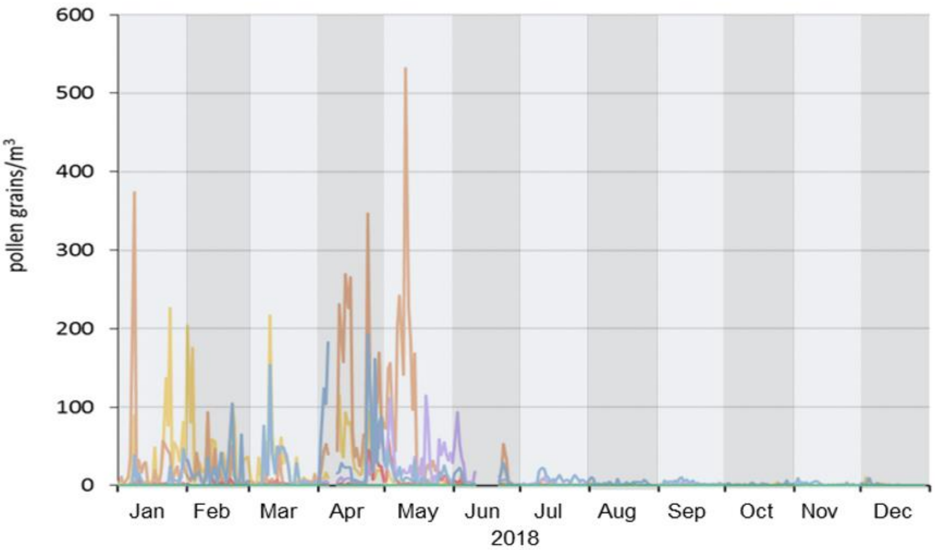

(Please complete the sentence by making a cross (X) for the ones you consider):

1. I can distinguish the trajectories of ...

☐ CUP ☐ FAG ☐ OLE ☐ GRA ☐ PAR ☐ AMB ☐ ART ☐ None

2. I can see the highest pollen concentration peaks of...

☐ CUP ☐ FAG ☐ OLE ☐ GRA ☐ PAR ☐ AMB ☐ ART ☐ None

3. I can see that May is the month with highest concentration of...

☐ CUP ☐ FAG ☐ OLE ☐ GRA ☐ PAR ☐ AMB ☐ ART ☐ None

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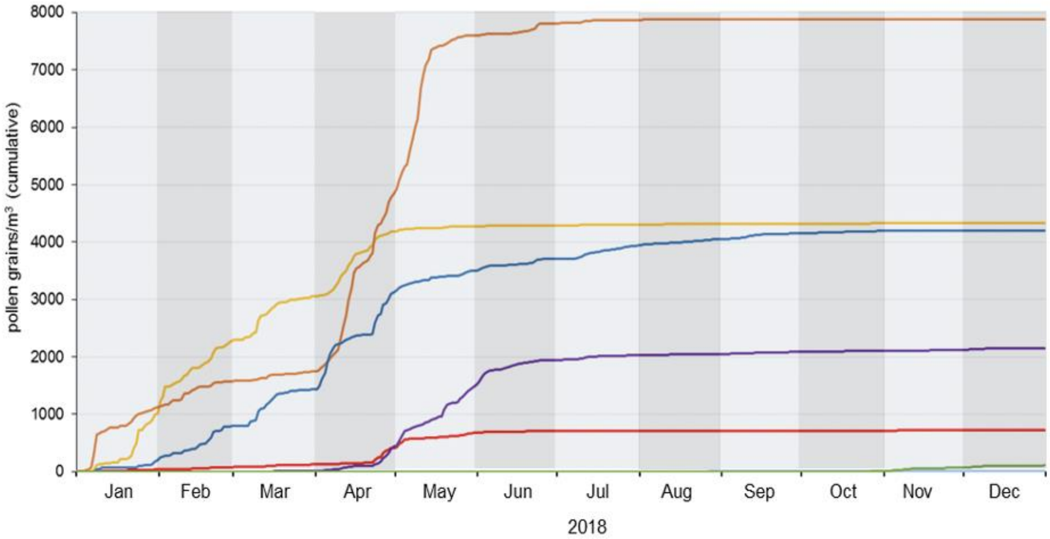
b) Cumulative Pollen Concentration Trajectory

The following figure provides information on the **cumulative pollen concentration** of Cupressaceae (CUP), Fagales (FAG), Oleaceae (OLE), Graminaceae (GRA), *Parietaria (PAR), Ambrosia (AMB)* and *Artemisia (ART)* in Rome, for 2018.



(Please complete the sentence by making a cross (X) for the ones you consider):

4. I can distinguish the trajectories of ...

☐ CUP ☐ FAG ☐ OLE ☐ GRA ☐ PAR ☐ AMB ☐ ART ☐ None

5. I can see the highest pollen concentration peaks of...

☐ CUP ☐ FAG ☐ OLE ☐ GRA ☐ PAR ☐ AMB ☐ ART ☐ None

6. I can see that May is the month with highest concentration of...

☐ CUP ☐ FAG ☐ OLE ☐ GRA ☐ PAR ☐ AMB ☐ ART ☐ None

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c) Daily and Cumulative Pollen Concentration Trajectory

The following figure provides information on the **daily pollen concentration trajectories (Figure 1)** and the **cumulative pollen concentration trajectories (Figure 2)** of Cupressaceae (CUP), Fagales (FAG), Oleaceae (OLE), Graminaceae (GRA), *Parietaria (PAR), Ambrosia (AMB)* and *Artemisia (ART)* in Rome, for 2018.

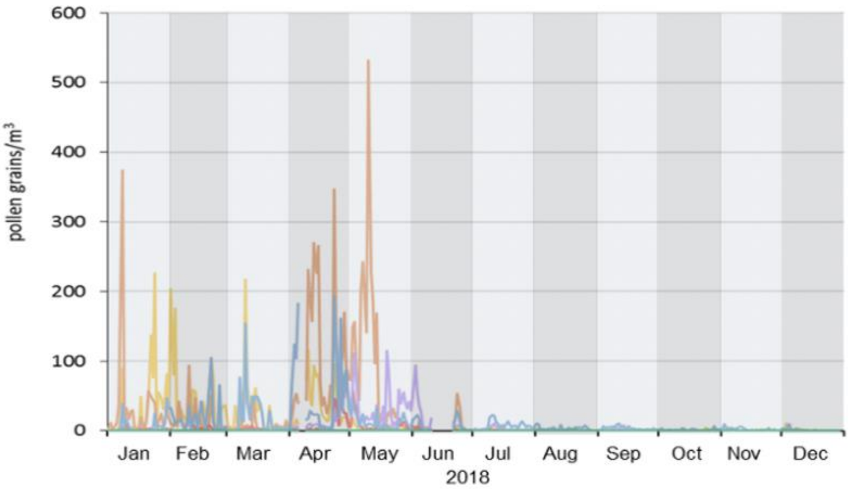

Figure 1

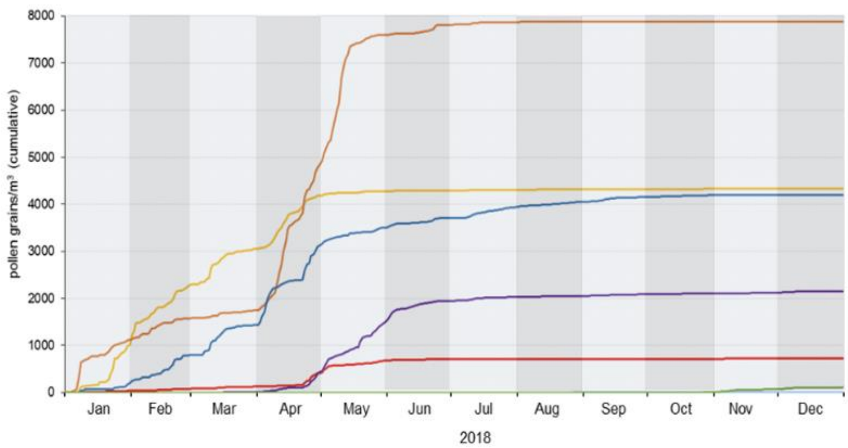

Figure 2

7a) In which month do you see the highest peak in the pollen concentration (pollen grains/m³) for...?

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
CUP	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
FAG	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
OLE	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
GRA	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
PAR	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
AMB	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
ART	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

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b) From which trajectory did you take the information to answer question 7a?

☐ daily pollen concentration trajectory (figure 1) ☐ cumulative pollen concentration trajectory (figure 2)

8a) What is the final cumulative pollen concentration (pollen grains/m³) for ...?

CUP	☐ 2000-3000	☐ 3000-4000	☐ 4000-5000
FAG	☐ 5000-6000	☐ 6000-7000	☐ 7000-8000
OLE	☐ 0-1000	☐ 2000-3000	☐ 3000-4000
GRA	☐ 0-1000	☐ 2000-3000	☐ 3000-4000
PAR	☐ 4000-5000	☐ 5000-6000	☐ 6000-7000
AMB	☐ 0-1000	☐ 2000-3000	☐ 3000-4000
ART	☐ 0-1000	☐ 2000-3000	☐ 3000-4000

b) From which trajectory did you take the information to answer question 8a?

☐ daily pollen concentration trajectory (figure 1) ☐ cumulative pollen concentration trajectory (figure 2)

9a) During which month(s) do you consider the pollen concentration(s) of the given sources clinically relevant?

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
CUP	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
FAG	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
OLE	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
GRA	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
PAR	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
AMB	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
ART	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

b) From which trajectory did you take the information to answer question 9a?

☐ daily pollen concentration trajectory (figure 1) ☐ cumulative pollen concentration trajectory (figure 2)

d) Conclusion

Which of the presented trajectories (daily pollen concentration trajectory/ cumulative pollen concentration trajectory) do you think could be clinically relevant?

☐ Both ☐ Only daily pollen concentration trajectories ☐ Only cumulative pollen concentration trajectories

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Im Rahmen des Projekts @IT.2020 entstand meine für den Manteltext ausgewählte Publikation. Im Oktober 2017 bis Mai 2019 unterstützte ich meine Betreuer*innen Dr. med. Stephanie Dramburg und Privatdozent Paolo Maria Matricardi nach sorgfältiger Einarbeitung mit der Organisation und Durchführung der Datenerhebung des Projekts. Ich übernahm die Mitverantwortung für das Erreichen der Studienteilnehmer*innen-Rekrutierungsziele in den neun Zentren und blieb im stetigen und regen Kontakt zu den 37 Studienärzt*innen vor Ort. Dies beinhaltete unter anderem das Klären von Rückfragen zu technischen Hürden, betreffend der mHealth Technologie, die wir für das Projekt nutzten. Ich erfasste in regelmäßigen Reports den Rekrutierungsstand und besprach den Zeitplan mit den Studienärzt*innen. Hierfür kontrollierte ich auch regelmäßig die erhobenen elektronischen Fragebögen auf Eingabefehler und Vollständigkeit, um dies gegebenenfalls zurückzumelden. Anfang 2018 schlossen wir die Rekrutierung von 815 Studienteilnehmer*innen ab. Die erhobenen Daten fügte ich zu einer Primärdatenbank zusammen und erstellte hierfür eine ausführliche Legende. Diese diente unter anderem auch meiner Kollegin Theresa Lipp zur Erstellung ihrer Doktorarbeit und dem Paper (nicht im Manteltext enthalten):

Lipp T, Acar Şahin A, Aggelidis X, Arasi S, Barbalace A, Bourgoin A, Bregu B, Brighetti MA, Caeiro E, Caglayan Sozmen S, Caminiti L, Charpin D, Couto M, Delgado L, Di Rienzo Businco A, Dimier C, Dimou MV, Fonseca JA, Goksel O, Guvensen A, Hernandez D, **Hoffmann TM**, Jang DT, Kalpaklioglu F, Lame B, Llusar R, Makris M, Mazon A, Mesonjesi E, Nieto A, Öztürk A, Pahus L, Pajno G, Panasiti I, Papadopoulos NG, Pellegrini E, Pelosi S, Pereira AM, Pereira M, Pinar NM, Potapova E, Priftanji A, Psarros F, Sackesen C, Sfika I, Suarez J, Thibaudon M, Travaglini A, Tripodi S, Verdier V, Villella V, Xepapadaki P, Yazici D, Matricardi PM, Dramburg S. Heterogeneity of Pollen Food Allergy Syndrome in Seven Southern European Countries: the @IT.2020 Multicenter Study. Allergy. 2021. doi: 10.1111/all.14742. [Epub ahead of print].

Im Jahr 2018 erfolgte dann die Beobachtungsphase der Studienteilnehmer*innen, welche entsprechend unseres zugewiesenen Zeitraums tägliche ihre Symptome registrieren

sollten. Um die Studienärzt*innen zu unterstützen, erstellte ich über das Jahr 2018 hinweg wöchentliche Reports zum Registrierungsverhalten aller 815 Studienteilnehmer*innen. So konnten entsprechend unserer Standard Operating Procedures die Studienärzt*innen rechtzeitig Rücksprache mit dem/der Studienteilnehmer*in halten.
In gemeinsamer Absprache mit den zehn Aerobiolog*innen des @IT.2020 Projekts und Herrn Matricardi legten wir die aerobiologischen Pollenaufzeichnungsziele und relevanten elektronischen Aufzeichnungsräume für die Studienteilnehmer*innen fest. Über das gesamte Jahr 2018 sendeten mir die Aerobiolog*innen wöchentlich die Pollenmessungen zu, welche ich in einer Primärdatenbank für alle Studienzentren zusammenfügte und auf fehlende Messungen und Stimmigkeit kontrollierte. Auf Anfrage sendeten mir die Aerobiolog*innen auch meteorologische Daten zu, sodass ich diese ebenso in einer Datenbank erfassen konnte.
Nach Ende des Beobachtungszeitraums der Studienteilnehmer*innen, unterstützte ich Frau Dramburg bei der Erstellung der Fragebögen und weiteren Materialien für die Workshops des @IT.2020 Projekts zur Erfassung der Daten zu dem Clinical Decision Support System im März bis Juni 2019. Ich erstellte auch meinen eigenen Fragebogen mit vorausgehendem Einführungsteil für die Studienteilnehmer*innen, der als Grundlage der folgenden Publikation (nicht im Manteltext enthalten) diente:
Hoffmann TM, Travaglini A, Brighetti MA, Acar Şahin A, Arasi S, Bregu B, Caeiro E, Caglayan Sozmen S, Charpin D, Delgado L, Dimou M, Fiorilli M, Fonseca JA, Goksel O, Kalpaklioglu F, Lame B, Mazon A, Mesonjesi E, Nieto A, Öztürk A, Pajno G, Papadopoulos NG, Pellegrini E, Pereira AM, Pereira M, Pinar NM, Pinter E, Priftanji A, Sackesen C, Sfika I, Suarez J, Thibaudon M, Tripodi S, Ugus U, Villella V, Matricardi PM, Dramburg S; @IT.2020 study team. Cumulative Pollen Concentration Curves for Pollen Allergy Diagnosis. J Investig Allergol Clin Immunol. 2020. doi: 10.18176/jiaci.0646. [Epub ahead of print].

Im Zuge der Workshops erstellte ich eine weitere elektronische Primärdatenbank aus den erhobenen Daten in Papierform und erfasste systematisch die Variablen, welche die Grundlage noch derzeit laufender Datenauswertungen bilden.

Datenauswertung/-veröffentlichung und Präsentation

Als Erstautorin des vorausgehend genannten Papers (Cumulative Pollen Concentration Curves for Pollen Allergy Diagnosis) war ich unter ständiger Supervision meiner Betreuer*innen zuständig für die Auswertung der Daten, als auch das Schreiben des Manuskripts und das Erstellen aller Tabellen und Abbildungen (Tables e1, e2, e3, e4; Figures 1, e2) hierfür. Außerdem stellte ich diese Ergebnisse im Rahmen einer Postersession des 14. Deutschen Allergologiekongress in Hannover vor. Des Weiteren erhielt ich die Möglichkeit auf einem Studienmeeting in Porto, Portugal, sowie auf einem weiteren Meeting während des EAACI Jahreskongresses in Lissabon, Portugal, Zwischenergebnisse vorzustellen. Weitere Daten durfte ich unserem Kooperationspartner EUROIMMUN AG in Lübeck präsentieren.

Beitrag im Einzelnen zu der erfolgten Publikation dieses Manteltextes:

Publikation: Hoffmann TM, Acar Şahin A, Aggelidis X, Arasi S, Barbalace A, Bourgoin A, Bregu B, Brighetti MA, Caeiro E, Caglayan Sozmen S, Caminiti L, Charpin D, Couto M, Delgado L, Di Rienzo Businco A, Dimier C, Dimou MV, Fonseca JA, Goksel O, Guvensen A, Hernandez D, Jang DT, Kalpaklioglu F, Lame B, Llusar R, Makris MP, Mazon A, Mesonjesi E, Nieto A, Öztürk A, Pahus L, Pajno GB, Panasiti I, Panetta V, Papadopoulos NG, Pellegrini E, Pelosi S, Pereira AM, Pereira M, Pinar M, Pfaar O, Potapova E, Priftanji A, Psarros F, Sackesen C, Sfika I, Suarez J, Thibaudon M, Travaglini A, Tripodi S, Verdier V, Villella V, Xepapadaki P, Yazici D, Matricardi PM, Dramburg S. "Whole" vs. "fragmented" approach to EAACI pollen season definitions: A multicenter study in six Southern European cities. Allergy. 2020;75(7):1659–71.

Vorbereitung

Im Laufe der Datenerfassung legten meine Betreuer*innen und ich mein Promotionsthema fest. Ich führte eine Literaturrecherche durch und fand wichtige vorausgegangene Arbeiten, um die Datenanalyse der Pollendaten durchzuführen. Hierfür stand ich auch im engen Kontakt mit den Aerobiolog*innen unserer Studie.

Erstellung der Datenbank und Datenauswertung

Aus den wie oben beschrieben erhobenen Daten, erstellte ich nach Absprache mit meinen Betreuer*innen aus der Primärdatenbank die Sekundärdatenbank(en) inklusive

der in der Methodik beschriebenen Interpolation der Datenlücken mit Excel, die den weiteren Datenanalysen dieser Publikation diente.
Frau Panetta erstellte die statistische Auswertung und Übertragung von R in Excel der Pollensaisondefinitionen für Januar bis September 2019 in rudimentärer Form (Angaben in 0,1). Ich führte die Vervollständigung der konkreten Pollensaisondaten für das gesamte Jahr 2018 aus, sowie alle weiteren darauf basierenden Berechnungen aller in der Publikation enthaltenen Tabellen (Tables 1, 2, 3, 4, e1, e2, e3 - ausschließlich der Berechnungen der Spalte p Values in Table e3) und Abbildungen (graphical abstract, Figures 1, 2, 3, e1, e2, e3). Im Zuge dessen überlegte ich mir eine sinnvolle Reihenfolge meiner Ergebnisse in Tabellen und Abbildungen. Diese habe ich im ständigen Austausch mit meinen Betreuer*innen verbessert und diskutiert.

Veröffentlichung der Ergebnisse und Präsentation
Meine noch nicht veröffentlichten Ergebnisse stellte ich im Rahmen des wöchentlichen Journal Clubs der Klinik für Pädiatrie mit Schwerpunkt Pneumologie, Immunologie und Intensivmedizin vor.
Anhand der mit Herrn Matricardi festgelegten Reihenfolge der Tabellen und Abbildungen entwickelte ich unter weiterer Literaturrecherche und Kontrolle der Formalitäten des Journals, das Manuskript. Ich setzte inhaltliche Verbesserungsvorschläge und sprachliche Korrekturen um. Für die Point-by-Point Reply ergaben sich weitere Literaturrecherchen und Korrekturen des Papers, die ich gemeinsam mit Frau Dramburg Durchführte. Die Co-Autoren, mit Ausnahme von Herr Matricardi, Frau Dramburg und Frau Panetta, waren ausschließlich an der Erhebung der Primärdaten, sowie der Korrektur des Manuskripts beteiligt. Der Artikel wurde am 11. Mai 2020 online veröffentlicht. Die Ergebnisse dieser Publikation stellte ich in Form eines Posters im Juni 2020 auf dem EAACI Jahreskongresses (digital) vor.

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Unterschrift, Datum und Stempel des/der erstbetreuenden Hochschullehrers/in

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Unterschrift der Doktorandin

Auszug aus der Journal Summary List

Journal Data Filtered By: **Selected JCR Year: 2017** Selected Editions: SCIE,SSCI
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Gesamtanzahl: 18 Journale

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY	49,229	13.258	0.083800
2	Journal of Allergy and Clinical Immunology-In Practice	2,802	6.966	0.009670
3	CLINICAL REVIEWS IN ALLERGY & IMMUNOLOGY	2,741	6.442	0.005880
4	ALLERGY	16,476	6.048	0.025790

Publikation dieses Manteltextes

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

Im Folgenden erfolgt der Sonderdruck der Publikation des Manteltextes, erschienen als open access Artikel bei Wiley online:

Hoffmann TM, Acar Şahin A, Aggelidis X, Arasi S, Barbalace A, Bourgoin A, Bregu B, Brighetti MA, Caeiro E, Caglayan Sozmen S, Caminiti L, Charpin D, Couto M, Delgado L, Di Rienzo Businco A, Dimier C, Dimou MV, Fonseca JA, Goksel O, Guvensen A, Hernandez D, Jang DT, Kalpaklioglu F, Lame B, Llusar R, Makris MP, Mazon A, Mesonjesi E, Nieto A, Öztürk A, Pahus L, Pajno GB, Panasiti I, Panetta V, Papadopoulos NG, Pellegrini E, Pelosi S, Pereira AM, Pereira M, Pinar M, Pfaar O, Potapova E, Priftanji A, Psarros F, Sackesen C, Sfika I, Suarez J, Thibaudon M, Travaglini A, Tripodi S, Verdier V, Villella V, Xepapadaki P, Yazici D, Matricardi PM, Dramburg S. "Whole" vs. "fragmented" approach to EAACI pollen season definitions: A multicenter study in six Southern European cities. Allergy. 2020;75(7):1659-1671.

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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.

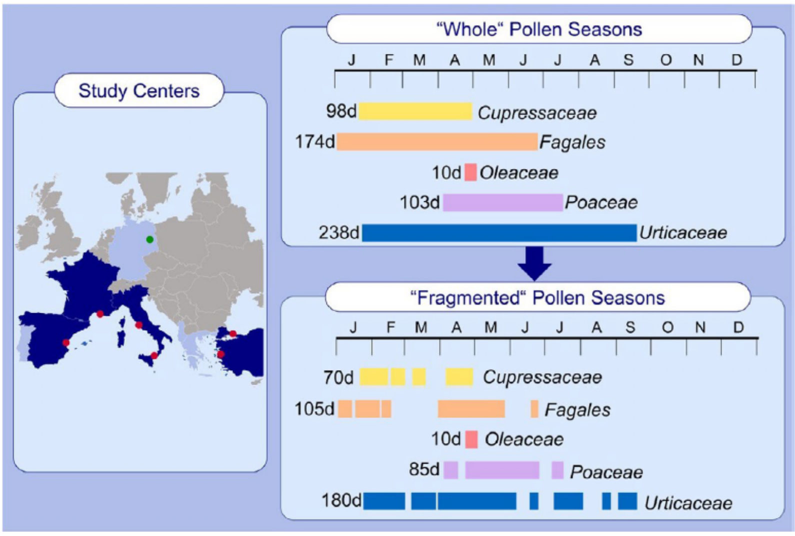
Objective: To test the pollen season definitions proposed by EAACI in six Mediterranean cities for seven pollen *taxa*.

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,

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/m³.

[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/m³ (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

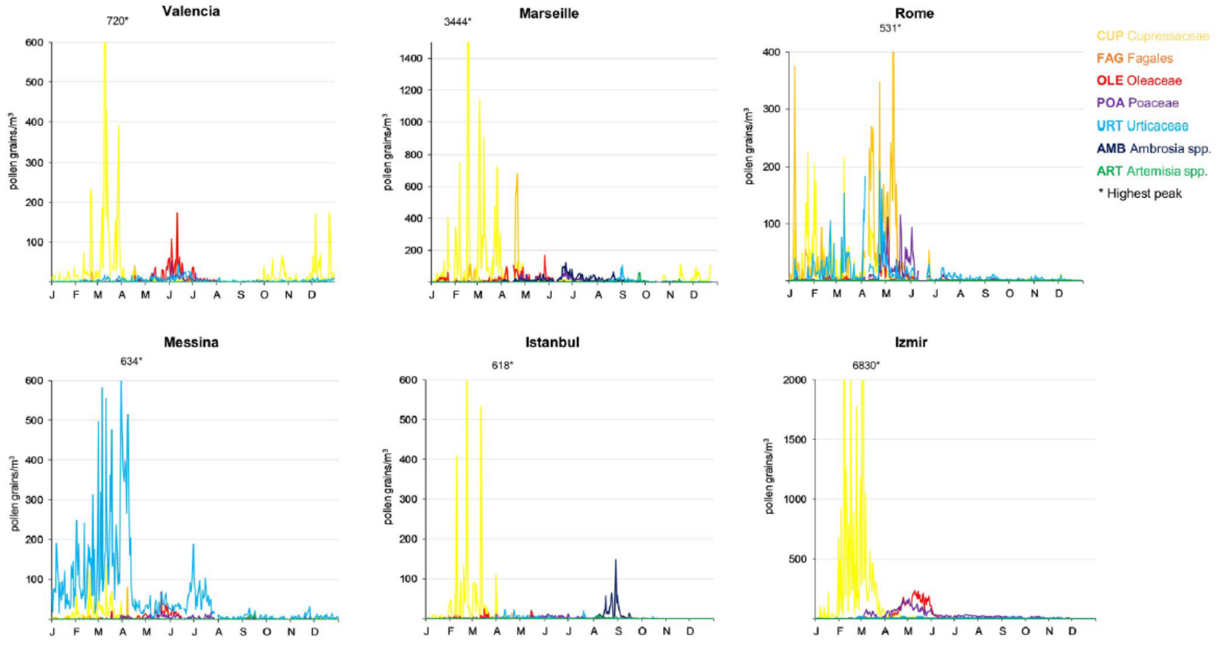


FIGURE 1 Daily pollen concentration (pollen grains/m³) of Cupressaceae, Fagales, Oleaceae, Poaceae, Urticaceae, *Ambrosia* spp., and *Artemisia* spp. in six Southern European/Mediterranean cities in 2018

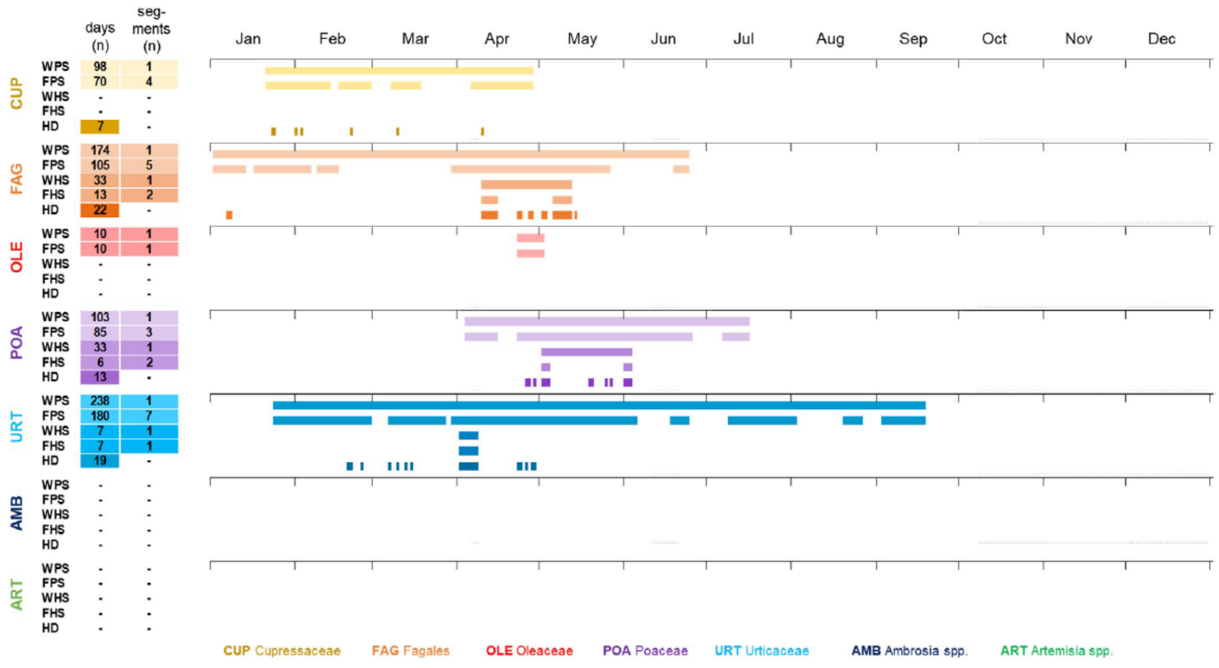


FIGURE 2 Whole pollen season (WPS), fragmented pollen season (FPS), whole high season (WHS), fragmented high season (FHS), and high days (HD) for the year 2018 in Rome as defined on the basis of the EAACI criteria[24] with further adaptation (see methods). On the left side, the length of each season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

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

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.

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).

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

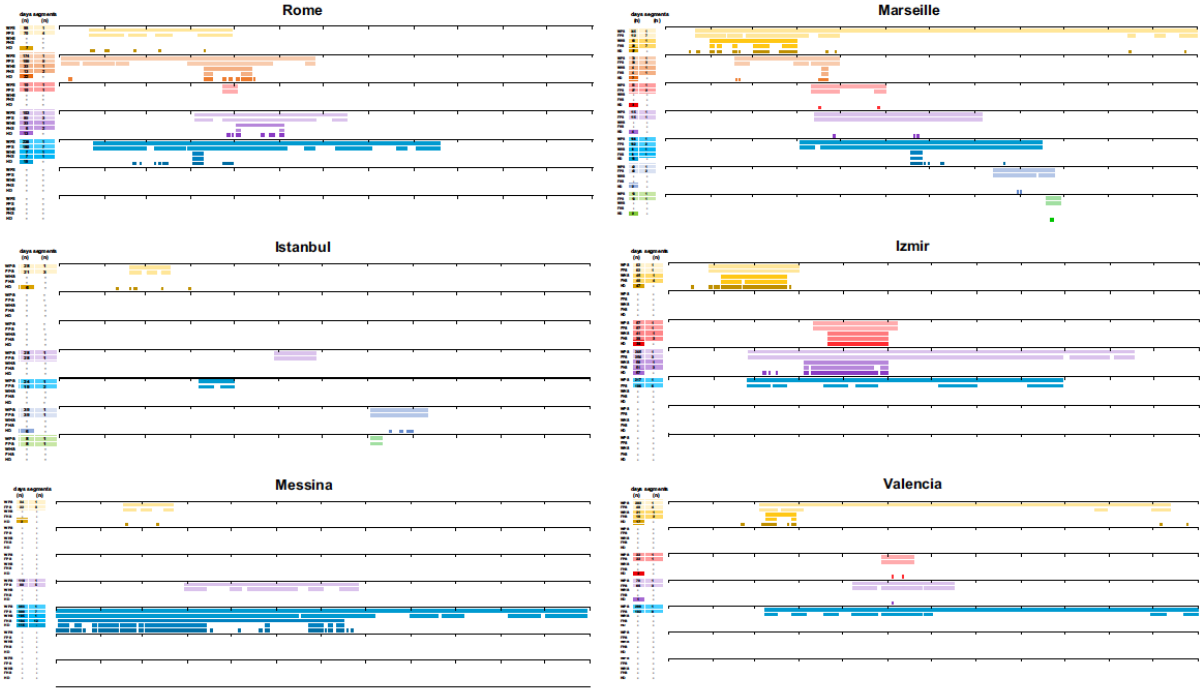


FIGURE 3 Pollen seasons of Cupressaceae, Fagales, Oleaceae, Urticaceae, Poaceae, *Ambrosia* spp., and *Artemisia* spp. in six Southern European/Mediterranean cities in 2018. Graphical representation of the days included in the whole pollen season (WPS), fragmented pollen season (FPS), whole high season (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

AM, Pereira M, Potapova E, Priftanji A, Psarros F, Sackensen C, Sfika I, Tripodi S, Verdier V, Villella V, Xepapadaki P, Yazici D, Matricardi PM, Dramburg S were involved in the coordination of the study as well as the patient recruitment and data collection.

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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REFERENCES

1. Pawankar R, Canonica GW, Holgate ST, Lockey RF, Blaiss M. *The WAO White Book on Allergy*. (Update. 2013). Milwaukee, WI: World Allergy Organization; 2013.
2. D'Amato G, Cecchi L, Bonini S, et al. Allergenic pollen and pollen allergy in Europe. *Allergy*. 2007;62:976-990.
3. Skoner DP. Allergic rhinitis: definition, epidemiology, pathophysiology, detection, and diagnosis. *J Allergy Clin Immunol*. 2001;108:S2-S8.
4. Camacho IC. Airborne pollen in Funchal city, (Madeira Island, Portugal) – first pollinic calendar and allergic risk assessment. *Ann Agric Environ Med*. 2015;22:608-613.
5. Katotomichelakis M, Nikolaidis C, Makris M, et al. The clinical significance of the pollen calendar of the Western Thrace/northeast Greece region in allergic rhinitis. *Int Forum Allergy Rhinol*. 2015;5:1156-1163.
6. Yalcin AD, Basaran S, Bisgin A, Polat HH, Gorczyzynski M. Pollen aero allergens and the climate in Mediterranean region and allergen sensitivity in allergic rhinoconjunctivitis and allergic asthma patients. *MedSci Monit*. 2013;19:102-110.
7. Guvensen A, Ozturk M. Airborne pollen calendar of Izmir - Turkey. *Ann Agric Environ Med AAEM*. 2003;10:37-44.
8. Geller-Bernstein C, Portnoy JM. The clinical utility of pollen counts. *Clin Rev Allergy Immunol*. 2019;57(3):340-349.
9. Barber D, de la Torre F, Lombardero M, et al. Component-resolved diagnosis of pollen allergy based on skin testing with profilin, polcalcin and lipid transfer protein pan-allergens. *Clin Exp Allergy*. 2009;39:1764-1773.
10. Barnes CS. Impact of climate change on pollen and respiratory disease. *Curr Allergy Asthma Rep*. 2018;18:59.
11. Ziello C, Sparks TH, Estrella N, et al. Changes to airborne pollen counts across Europe. *PLoS One*. 2012;7:e34076.
12. Levetin E, Van de Water PK. Pollen count forecasting. *Immunol Allergy Clin North Am*. 2003;23:423-442.
13. Rodríguez-Rajo FJ, Valencia-Barrera RM, Vega-Maray AM, Suárez FJ, Fernández-González D, Jato V. Prediction of airborne Alnus pollen concentration by using ARIMA models. *Ann Agric Environ Med*. 2017;13:25-32.
14. Jato V, Rodríguez-Rajo FJ, Alcázar P, De Nuntiis P, Galán C, Mandrioli P. May the definition of pollen season influence aerobiological results? *Aerobiologia*. 2006;22:13-25.
15. Nilsson S, Persson S. Tree pollen spectra in the stockholm region (Sweden), 1973–1980. *Grana*. 1981;20:179-182.
16. Andersen TB. A model to predict the beginning of the pollen season. *Grana*. 1991;30:269-275.
17. Sánchez Mesa JA, Smith M, Emberlin J, Allitt U, Caulton E, Galan C. Characteristics of grass pollen seasons in areas of southern Spain and the United Kingdom. *Aerobiologia*. 2003;19:243-250.
18. Driessen MNBM, van Herpen RMA, Moelands RPM, Spieksma FTM. Prediction of the start of the grass pollen season for the western part of the Netherlands. *Grana*. 1989;28:37-44.
19. Bastl K, Kmenta M, Geller-Bernstein C, Berger U, Jäger S. Can we improve pollen season definitions by using the symptom load index in addition to pollen counts? *Environ Pollut*. 2015;204:109-116.
20. Galán C, Emberlin J, Domínguez E, Bryant RH, Villamandos F. A comparative analysis of daily variations in the gramineae pollen counts at Córdoba, Spain and London, UK. *Grana*. 1995;34:189-198.
21. Creticos PS, Maloney J, Bernstein DI, et al. Randomized controlled trial of a ragweed allergy immunotherapy tablet in North American and European adults. *J Allergy Clin Immunol*. 2013;131:1342-1349.
22. Canonica GW, Baena-Cagnani CE, Bousquet J, et al. Recommendations for standardization of clinical trials with allergen specific immunotherapy for respiratory allergy. A statement of a world allergy organization (WAO) taskforce. *Allergy*. 2007;62:317-324.
23. Durham SR, Nelson HS, Nolte H, et al. Magnitude of efficacy measurements in grass allergy immunotherapy trials is highly dependent on pollen exposure. *Allergy*. 2014;69:617-623.

24. Pfaar O, Bastl K, Berger U, et al. Defining pollen exposure times for clinical trials of allergen immunotherapy for pollen-induced rhinoconjunctivitis – an EAACI position paper. *Allergy*. 2017;72:713-722.
25. Cecchi L. Introduction. In: Sofiev M, Bergmann K-C, eds. *Allergenic pollen. A review of the production, release, distribution and health impacts*. Dordrecht: Springer; 2013: 1-7.
26. Nikolaidis C, Katotomichelakis M, Nena E, et al. Seasonal variations of allergenic pollen in a Mediterranean region – Alexandroupolis, north-east Greece. *Ann Agric Environ Med*. 2015;22(4):685-689.
27. Ballero M, Maxia A. Pollen spectrum variations in the atmosphere of Cagliari, Italy. *Aerobiologia*. 2003;19:251-259.
28. Karatzas K, Riga M, Berger U, Werchan M, Pfaar O, Bergmann KC. Computational validation of the recently proposed pollen season definition criteria. *Allergy*. 2018;73:5-7.
29. Bastl K, Kmenta M, Berger UE. Defining pollen seasons: background and recommendations. *Curr Allergy Asthma Rep*. 2018;18:73.
30. DIN EN 16868 Ambient air - Sampling and analysis of airborne pollen grains and fungal spores for networks related to allergy - Volumetric Hirst method; German and English version prEN 16868:2017 [Internet]. https://www.din.de/en/getting-involved/standards-committees/krdl/drafts/wdc-beuth:din21:277928625. Accessed June 24, 2019.
31. Galán C, Ariatti A, Bonini M, et al. Recommended terminology for aerobiological studies. *Aerobiologia*. 2017;33:293-295.
32. Mandrioli P. Basic aerobiology. *Aerobiologia*. 1998;14:89.
33. EAS QC Working Group, Galán C, Smith M, et al. Pollen monitoring: minimum requirements and reproducibility of analysis. *Aerobiologia*. 2014;30(4):385-395.
34. Rojo J, Picornell A, Oteros J. AeRobiology: the computational tool for biological data in the air. *Methods Ecol Evol*. 2019;10:1371-1376.
35. Sikoparija B, Skjøth CA, Celenk S, et al. Spatial and temporal variations in airborne *Ambrosia*pollen in Europe. *Aerobiologia*. 2017;33:181-189.
36. Matricardi PM, Kleine-Tebbe J, Hoffmann HJ, et al. EAACI molecular allergology user's guide. *Pediatr Allergy Immunol*. 2016;27:1-250.
37. Pfaar O, Alvaro M, Cardona V, Hamelmann E, Mösges R, Kleine-Tebbe J. Clinical trials in allergen immunotherapy: current concepts and future needs. *Allergy*. 2018;73:1775-1783.
38. Karatzas K, Katsifarakis N, Riga M, et al. New European academy of allergy and clinical immunology definition on pollen season mirrors symptom load for grass and birch pollen-induced allergic rhinitis. *Allergy*. 2018;73:1851-1859.
39. Rojo J, Oteros J, Pérez-Badia R, et al. Near-ground effect of height on pollen exposure. *Environ Res*. 2019;174:160-169.
40. Smart IJ, Tuddenham WG, Knox RB. Aerobiology of grass pollen in the city atmosphere of melbourne: effects of weather parameters and pollen sources. *Aust J Bot*. 1979;27:333-342.
41. Buters J, Prank M, Sofiev M, et al. Variation of the group 5 grass pollen allergen content of airborne pollen in relation to geographic location and time in season. *J Allergy Clin Immunol*. 2015;136:87-95.
42. Guideline on Allergen Products: Production and Quality Issues. EMEA/CHMP/BWP/304831/2007. European Medicines Agency. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-allergen-products-production-quality-issues_en.pdf. Accessed September 25, 2019.
43. Lorenz AR, Lüttkopf D, May S, Scheurer S, Vieths S. The principle of homologous groups in regulatory affairs of allergen products–a proposal. *Int Arch Allergy Immunol*. 2009;148:1-17.
44. Hauser M, Asam C, Himly M, et al. Bet v 1-like pollen allergens of multiple Fagales species can sensitize atopic individuals. *Clin Exp Allergy*. 2011;41:1804-1814.

SUPPORTING INFORMATION

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

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Appendix S1

"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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Key words: EAACI, pollen, Mediterranean, pollen allergy, pollen season, season definitions, seasonal allergic rhinitis
Word count: 632

Tables: 3

Figures: 3

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.

References

e1.Pfaar O, Bastl K, Berger U, Buters J, Calderon MA, Clot B, et al. Defining pollen exposure times for clinical trials of allergen immunotherapy for pollen-induced rhinoconjunctivitis – an EAACI position paper. Allergy. 2017;72:713–22.

e2. Emberlin J. The effects of patterns in climate and pollen abundance on allergy. Allergy. 1994;49:15–20.

e3. Buters JTM, Kasche A, Weichenmeier I, Schober W, Klaus S, Traidl-Hoffmann C, et al. Year-to-Year Variation in Release of Bet v 1 Allergen from Birch Pollen: Evidence for Geographical Differences between West and South Germany. Int Arch Allergy Immunol. 2008;145:122–30.

e4. D'Amato G, Cecchi L, Bonini S, Nunes C, Annesi-Maesano I, Behrendt H, et al. Allergenic pollen and pollen allergy in Europe. Allergy. 2007;62:976–90.

e5. Jaeger S. Exposure to grass pollen in Europe. Clin Exp Allergy Rev. 2008;8:2–6.

e6. De Weger LA, Bergmann KC, Rantio-Lehtimaaki A, Dahl Å, Buters J, Déchamp C et al. Impact of Pollen. Netherlands: SPringer; 2013.

e7. D'amato G, Liccardi G. Pollen-related allergy in the European Mediterranean area. Clin Exp Allergy. 1994;24:210–9.

e8. D'Amato G, Lobefalo G. Allergenic pollens in the southern Mediterranean area. J Allergy Clin Immunol. 1989;83:116–22.

e9. Brandes D. Flora und Vegetation von Stadtmauern. Tuexenia. 1992;12:315–39.

e10. Fotiou C, Damialis A, Krigas N, Halley JM, Vokou D. *Parietaria judaica* flowering phenology, pollen production, viability and atmospheric circulation, and expansive ability in the urban environment: impacts of environmental factors. Int J of Biometeorol. 2011;55:35-50.

e11. Guvensen A, Ozturk M. Airborne pollen calendar of Izmir - Turkey. Ann Agric Environ Med AAEM. 2003;10:37–44.

e12. Charpin D, Calleja M, Lahoz C, Pichot C, Waisel Y. Allergy to cypress pollen. Allergy. 2005;60:293–301.

e13. Caiola MG, Mazzitelli A, Capucci E, Travaglini A. Monitoring pollinosis and airborne pollen in a Rome university. Aerobiologia. 2002;18:267–75.

e14. The Gymnosperm Database-Cupressus [Internet]. [cited 2019 May 27]. Available from: https://www.conifers.org/cu/Cupressus_sempervirens.php

e15. Caudullo G, de Rigo D. Cupressus sempervirens in Europe; distribution, habitat, usage and threats. Luxembourg: Publication Office of the European Union; 2016.

Legends to figures

Figure e1 - 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.

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

Figure e3 - Comparison between a "unified" (monitoring period) vs. "localized" season approach to definition of pollen seasons of Cupressaceae, Poaceae, and Urticaceae in six Southern European/Mediterranean cities for participating patients in potential AIT trials. The overall length of the monitoring periods (red bars representing number of days during the month) is reported. (a) showing the potential Whole Pollen Season monitoring period (WPS MP) in comparison to the individual Whole Pollen Season (WPS) length in the centers and (b) comparing the Whole High 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 continuos 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/m^3) of pollen seasons, defined according to EAACI criteria in six cities throughout 2018.°

		Valencia		Marseille		Rome		Messina		Istanbul		Izmir	
	Pollen Seasons	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

Figure e1

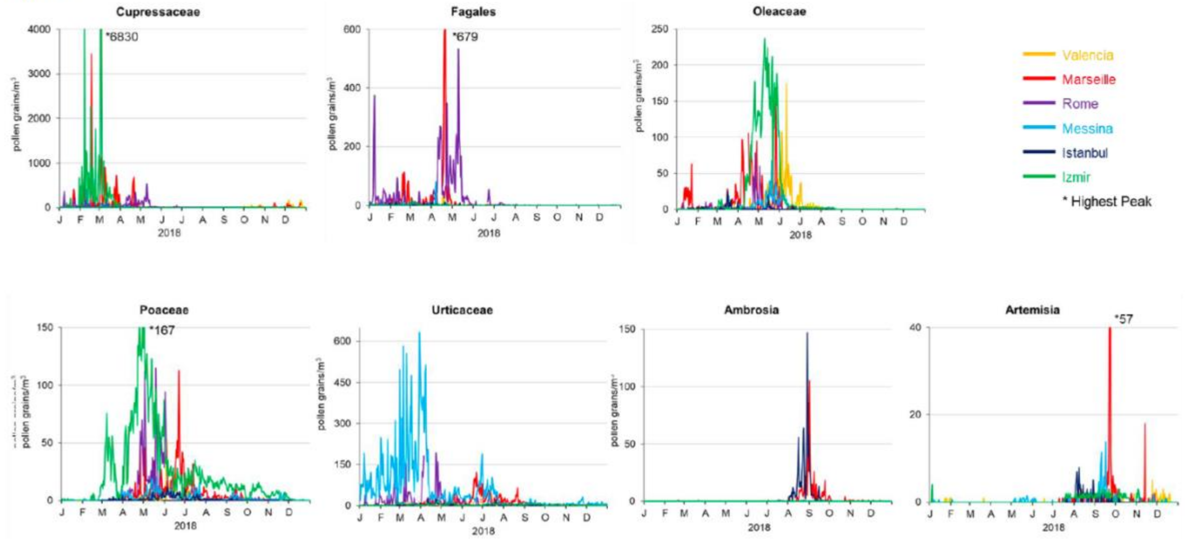

Cupressaceae
*6830
Fagales
*679
Oleaceae
Poaceae
*167
Urticaceae
Ambrosia
Artemisia
*57
pollen grains/m3
2018
Valencia
Marseille
Rome
Messina
Istanbul
Izmir
* Highest Peak

Figure e2

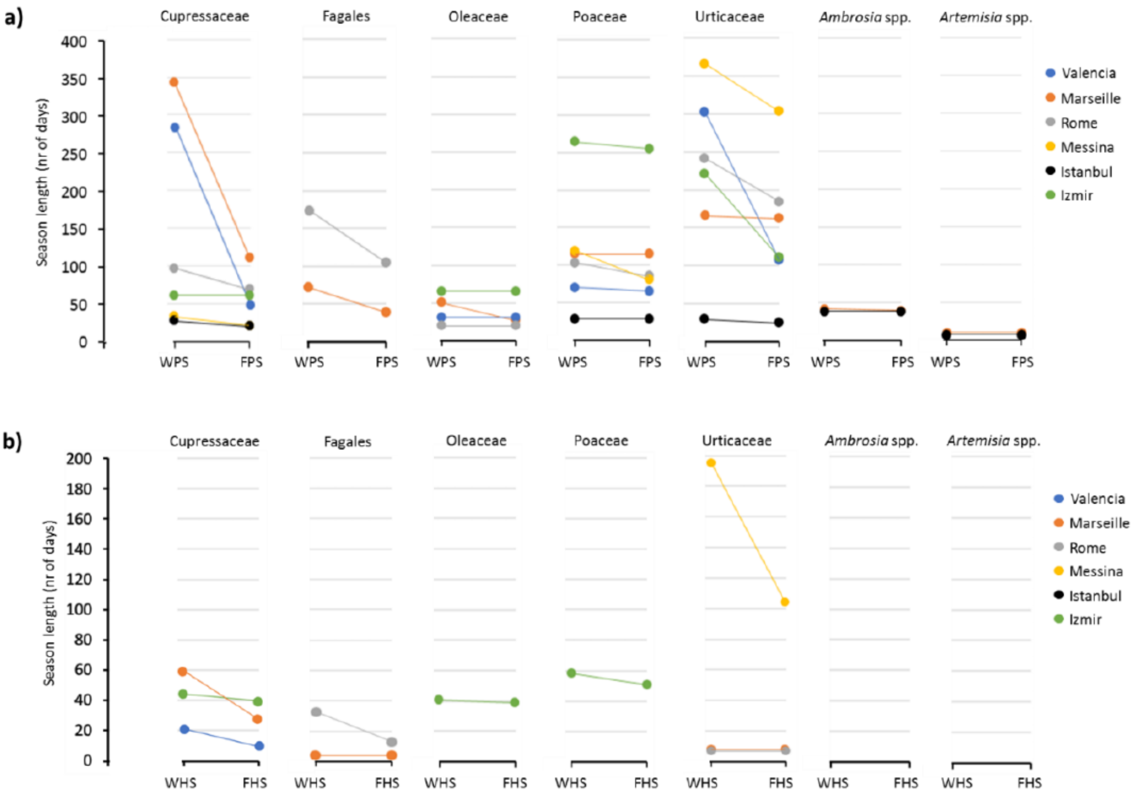
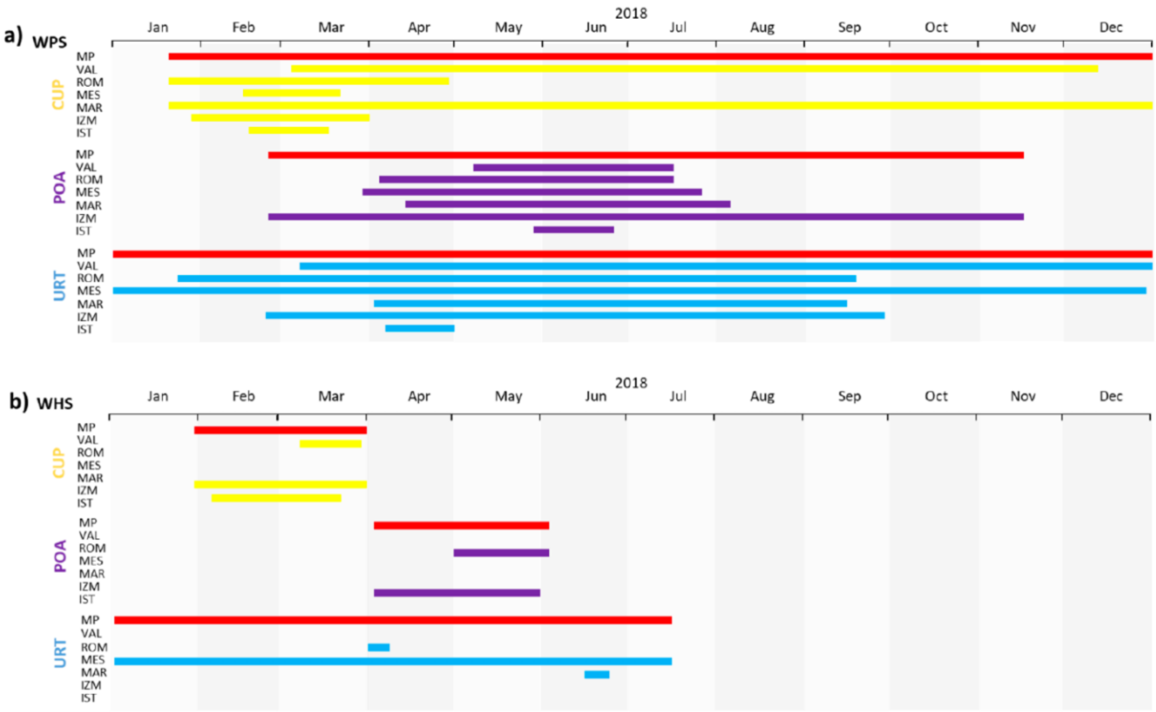

a)
Cupressaceae
Fagales
Oleaceae
Poaceae
Urticaceae
Ambrosia spp.
Artemisia spp.
Season length (nr of days)
400
350
300
250
200
150
100
50
0
WPS
FPS
Valencia
Marseille
Rome
Messina
Istanbul
Izmir
b)
Cupressaceae
Fagales
Oleaceae
Poaceae
Urticaceae
Ambrosia spp.
Artemisia spp.
Season length (nr of days)
200
180
160
140
120
100
80
60
40
20
0
WHS
FHS
Valencia
Marseille
Rome
Messina
Istanbul
Izmir

Figure e3


a) WPS
b) WHS
2018
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
CUP
POA
URT
MP
VAL
ROM
MES
MAR
IZM
IST

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

Publikationsliste

1. **Hoffmann TM**, Acar Şahin A, Aggelidis X, Arasi S, Barbalace A, Bourgoin A, Bregu B, Brighetti MA, Caeiro E, Caglayan Sozmen S, Caminiti L, Charpin D, Couto M, Delgado L, Di Rienzo Businco A, Dimier C, Dimou MV, Fonseca JA, Goksel O, Guvensen A, Hernandez D, Jang DT, Kalpaklioglu F, Lame B, Llusar R, Makris MP, Mazon A, Mesonjesi E, Nieto A, Öztürk A, Pahus L, Pajno GB, Panasiti I, Panetta V, Papadopoulos NG, Pellegrini E, Pelosi S, Pereira AM, Pereira M, Pinar M, Pfaar O, Potapova E, Priftanji A, Psarros F, Sackesen C, Sfika I, Suarez J, Thibaudon M, Travaglini A, Tripodi S, Verdier V, Villella V, Xepapadaki P, Yazici D, Matricardi PM, Dramburg S. "Whole" vs. "fragmented" approach to EAACI pollen season definitions: A multicenter study in six Southern European cities. Allergy. 2020;75:1659-1671.

2. **Hoffmann TM**, Travaglini A, Brighetti MA, Acar Şahin A, Arasi S, Bregu B, Caeiro E, Caglayan Sozmen S, Charpin D, Delgado L, Dimou M, Fiorilli M, Fonseca JA, Goksel O, Kalpaklioglu F, Lame B, Mazon A, Mesonjesi E, Nieto A, Öztürk A, Pajno G, Papadopoulos NG, Pellegrini E, Pereira AM, Pereira M, Pinar NM, Pinter E, Priftanji A, Sackesen C, Sfika I, Suarez J, Thibaudon M, Tripodi S, Ugus U, Villella V, Matricardi PM, Dramburg S. Cumulative Pollen Concentration Curves for Pollen Allergy Diagnosis. J Investig Allergol Clin Immunol. 2020. doi: 10.18176/jiaci.0646. [Epub ahead of print]. [Impact Factor: 8,07, Stand 2019]

3. Lipp T, Acar Şahin A, Aggelidis X, Arasi S, Barbalace A, Bourgoin A, Bregu B, Brighetti MA, Caeiro E, Caglayan Sozmen S, Caminiti L, Charpin D, Couto M, Delgado L, Di Rienzo Businco A, Dimier C, Dimou MV, Fonseca JA, Goksel O, Guvensen A, Hernandez D, **Hoffmann TM**, Jang DT, Kalpaklioglu F, Lame B, Llusar R, Makris M, Mazon A, Mesonjesi E, Nieto A, Öztürk A, Pahus L, Pajno G, Panasiti I, Papadopoulos NG, Pellegrini E, Pelosi S, Pereira AM, Pereira M, Pinar NM, Potapova E, Priftanji A, Psarros F, Sackesen C, Sfika I, Suarez J, Thibaudon M, Travaglini A, Tripodi S, Verdier V, Villella V, Xepapadaki P, Yazici D, Matricardi PM, Dramburg S. Heterogeneity of Pollen Food Allergy Syndrome in Seven Southern European Countries: the @IT.2020 Multicenter Study. Allergy. 2021. doi: 10.1111/all.14742. [Epub ahead of print].

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