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Título de la tesis:

Estudio de sensibilización de alergenos de polen de Estambul

Istanbul Pollen Allergen Sensitization Study

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Estudio de sensibilización de alergenos de polen de Estambul

Istanbul Pollen Allergen Sensitization Study

A dissertation submitted to

Universidad de Córdoba

for the degree of

Doctor of Sciences

presented by

FRANZISKA ZEMMER

Mag. ner. nat, MSc

accepted on the recommendation of

examiner: Carmen Galán, PhD University of Cordoba

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Instituto de Estudios de Postgrado

TÍTULO DE LA TESIS: Estudio de sensibilización de alergenos de polen de Estambul.

DOCTORANDO/A: Franziska Zemmer

INFORME RAZONADO DEL/DE LOS DIRECTOR/ES DE LA TESIS

(se hará mención a la evolución y desarrollo de la tesis, así como a trabajos y publicaciones derivados de la misma).

La doctoranda ha realizado un estudio sobre sensibilización a alérgenos del polen en Estambul, abordando múltiples aspectos relacionados con el monitoreo aerobiológico estandarizado de polen, con un muestreador volumétrico tipo Hirst, en Estambul. Un primer estudio se ha enfocado en la polinosis, tratando de presentar una recomendación para el Prueba de Punción Cutánea (SPT) para el área geográfica de Estambul. Un segundo estudio ha sido dirigido a la definición de la estación polínica y sobre la intensidad de la floración en Estambul, presentando el papel que juegan los parámetros meteorológicos en estas variables gracias al uso de Modelos Aditivos Generalizados (GAMs). El tercer estudio se ha enfocado en una evaluación de la relación dosis-respuesta, con polen de Poaceae, Betulaceae/Fagales, Ambrosia, Total de Polen y PM10, para los índices de síntomas y medicamentos. Para este estudio se han añadido resultados de otro estudio realizado en Yuvojvod, Serbia. Los umbrales del polen estudiado se han definido mediante una interpretación visual de curvas no lineales, habiendo podido evaluar el Riesgo Relativo (RR) asociado con la morbilidad a partir de estimaciones con GAM. En el RR, ya sea para desarrollar síntomas o para el uso de medicación, o para ambos, se ha observado una tendencia creciente de los umbrales. La doctoranda ha publicado 2 trabajos científicos relacionados con el tema de la tesis en revistas indexadas en el Journal Citation Report (JCR). La doctoranda ha realizado las actividades del Plan de Formación recomendadas para nuestro programa.

Por todo ello, se autoriza la presentación de la tesis doctoral

Córdoba, 14 de marzo de 2022

Firma de los directores

GALAN SOLDEVILLA CARMEN - 30449394Q

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Dr. Åslög Dahl

Prof. Carmen Galán

TITLE OF THE THESIS: Istanbul Pollen Allergen Sensitization Study

DOCTORAL STUDENT: Franziska Zemmer

REASONED REPORT OF THE DIRECTORS OF THE THESIS

(Mention will be made of the evolution and development of the thesis, as well as works and publications derived from it).

The PhD student has carried out a study on sensitization to pollen allergens in Istanbul, addressing multiple aspects related to standardized aerobiological pollen monitoring, with a Hirst-type volumetric sampler in Istanbul. A first study has focused on pollinosis, trying to present a recommendation for the Skin Prick Test (SPT) for the geographical area of Istanbul. A second study has focused to the definition of the pollen season and the intensity of flowering in Istanbul, presenting the role that the meteorological parameters play in these variables thanks to the use of Generalized Additive Models (GAMs). The third study has focused to assess the dose response relationship with pollen from Poaceae, Betulaceae/Fagales, Ambrosia, Total Pollen and PM10, for symptom and drug indices. For this study we have added results from another study conducted in Yuvojvod, Serbia. The thresholds for studied pollen have been defined by means of a visual interpretation of non-linear curves, trying to evaluate the Relative Risk (RR) associated with morbidity with GAM stimations. In the RR, either to develop symptoms or to use medication, or both, an increasing trend in thresholds has been observed. The doctoral student has published 2 scientific papers related to the topic of the thesis in journals indexed in the Journal Citation Report (JCR). The doctoral student has carried out the activities of the Training Plan recommended for our program.

For all these reasons, the presentation of the doctoral thesis is authorized.

Córdoba, 14 march, 2022

Directors Signature

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SUMMARY

The Istanbul Pollen Allergen Sensitization Study (IPASS) addresses multiple aspects linked to standardized aerobiological pollen monitoring with a volumetric Hist type sampler in western Istanbul from 2013 - June 2016.

One aspect concerns the management of pollinosis in collaboration between aerobiologists and health practitioners. In a clinical panel study (Chapter 1) sensitizations rates of 60 patients to allergens relevant for the area were delineated using the European Standard Panel (ESP) for Skin Prick Testing (SPT) and additional single allergens corresponding to pollen taxa identified in the pollen monitoring. The output was a recommendation on a SPT panel designed for the geographical area of Istanbul. The study proved that the neophyte *Ambrosia* has started sensitizing allergy sufferers in Istanbul. The ESP was found to be suitable for Istanbul when grasses from the Pooideae subfamily (*Phelum pratense*) and Panicoideae subfamily (*Sorghum halepense*) were included. Besides grasses, Fagales, Oleaceae (*Fraxinus* and *Olea*) were important allergens. *Morus* and *Rumex* sensitizations often appeared in polysensitized patients, which might involve pan-allergen sensitization. A key message for the implementation of future clinical SPT studies is to refrain from including cereal allergens, as they may distort results.

The next issue addressed is the pollen season in Istanbul – this time from the environmental perspective (Chapter 2). Research software designed for aerobiological analysis, the AeReobiology package in R, facilitated this comprehensive study on pollen seasons of individual taxa, and the intensity of the flowering. Poaceae, Cupressaceae, Pinaceae, Fagaceae and *Ambrosia* were the five pollen taxa with the greatest intensity in western Istanbul. The time series of the monitoring was disturbed by a change in the trap height due to a construction project in 2013. The location of the pollen trap at higher altitude mainly affected pollen concentrations of taxa growing in the immediate surrounding, especially of Poaceae. The definition of the pollen season, referring to the period when 95 % of the Annual Pollen integral (APIn) is dispersed, seemed adequate due to low concentrations of airborne pollen. Also 5 non zero daily records were used

to define the pollen season start. For Pinaceae, *Platanus*, *Fraxinus*, *Olea*, as well as for Poaceae and *Plantago*, the method of consecutive 5 non zero daily records could be an alternative to the percent-method. Over the study period there was great variability in the seasons, especially in woody taxa. An outstanding high tree pollen season was observed in 2016. Pollen data were analysed regarding the influence of meteorological factors. Abundant water availability a year ahead was linked to this high tree pollen season in taxa of the Betulaceae family, but not only. Therefrom the hypothesis of a masting phenomenon across taxa and populations arose. Indeed, research from East Europe on high birch pollen levels in 2016 endorses this assumption. The effect of meteorological factors was studied with Generalized Additive Models (GAMs). The effect on the phenology of trees flowering in spring was the opposite on weeds flowering mainly in summer. GAMs showed short term effects during the peak flowering period. Temperature had a negative and relative humidity a positive effect in spring flowering trees. Drops in temperature slowed anther dehiscence, while sunny spells after rainfall favoured it. Mean relative humidity changes over the day, so that rather decreasing humidity levels during the day caused dehiscence when (due to rain) humidity was actually elevated. Summer flowering weeds dispersed their pollen when temperatures increased as the season progressed. The effect was, thus, positive. GAMs can be used to study the oscillating effect of meteorological variables.The city stretches over 80 km along the coast from west to east, so that the pollen spectrum on the less urbanized western fringes of the city were different compared to published pollen data from Central Istanbul. Differences in the importance of pollen contributing taxa between the city centre and the suburbs suggest that one trap in the city centre and one each at the western and eastern outskirts of the city would assure representable pollen information for Istanbul.

In Turkey, unlike in many western European countries, public pollen warning is developing. Threshold levels of pollen concentrations leading to symptoms have not been defined. For a sound definition of low, moderate or high exposure risks, threshold levels need to address the local pollen situation. In the third study (Chapter 3) GAMs were used again. This time, to assess the dose response relationship of Poaceae, Betulaceae/Fagales, *Ambrosia*, Total pollen and PM10 on symptom and medicine scores from crowd sourced data. Hereby the Patient's Hay-fever Diary (PHD), for allergy patients recording their symptoms, provided data on morbidity. For the scope of a research paper on Southeast Europe, data from Yuvojvod, Serbia, was included in the study. Thresholds of Poaceae, Betulaceae/Fagales, *Ambrosia* and Total pollen were determined by visual interpretation of non-linear effect curves. A saturation in the effect curves of Poaceae after 20 p/m^3 in Istanbul, and of *Ambrosia* after 15 p/m^3 in Yuvojvod, was the threshold for severe symptoms. At the beginning of the season patients suffered more than later in the season and morbidity did not further increase at higher pollen concentrations. From the effect estimates of the GAM outputs the Relative Risk (RR) associated with morbidity can be assessed. The RR, either to develop symptoms or to use medication or both, was increased in all independent variables. The RR for Poaceae, Betulaceae and *Ambrosia* was pronounced (110-120%), while Total pollen and PM10 had an increased RR of 1-3%. Total pollen can provide a small additive effect to the risk of symptom development, but cannot be an indicator for symptoms caused by taxa with varying allergenicity and seasonality.

To conclude, IPASS provides allergists with information that will aid them in the selection of allergens to use in testing and treatment. It facilitated patient allergy self-management by propagating the electronic PHD. Knowledge on pollen seasons in Istanbul is important to understand future environmental effects caused by climate change in this vulnerable area. The challenge of issuing threshold levels on symptoms and medicine use has been addressed by using crowd sourced data from the PHD of Istanbul, Turkey and Yuvojvod, Serbia. We proposed a way to delineate regional threshold levels by means of GAMs that could be applied all over Europe within the European Aeroallergen Network (EAN). This study adds to aerobiological knowledge in Turkey in the light of developing public pollen information.

RESUMEN

Este estudio, sobre sensibilización a alérgenos del polen en Estambul (IPASS, siglas en inglés), aborda múltiples aspectos relacionados con el monitoreo aerobiológico estandarizado de polen, con un muestreador volumétrico tipo Hirst, en Estambul desde 2013 hasta junio de 2016.

En primer lugar, se presenta el estudio sobre polinosis como resultado de una colaboración entre aerobiológos y profesionales de la salud. Sobre el panel clínico para el área de estudio (Capítulo 1), se presentaron las tasas de sensibilización a alérgenos relevantes en 60 pacientes en base al Panel Estándar Europeo (ESP, siglas en inglés) para pruebas cutáneas (SPT, siglas en inglés), añadiendo alérgenos individuales adicionales correspondientes a taxones de polen identificados en el monitoreo de polen. El resultado ha sido presentar una recomendación para el SPT diseñado para el área geográfica de Estambul. Este estudio ha demostrado que *Ambrosia artemisiifolia*, especie recientemente invasora en esta región, ha comenzado a sensibilizar a los pacientes de alergia en Estambul. El ESP ha sido presentado para Estambul, una vez incluida la subfamilia Pooideae (con *Phleum pratense*) y la subfamilia Panicoideae (con *Sorghum halepense*). Además de las gramíneas, el orden Fagales y la familia Oleaceae (*Fraxinus* y *Olea*) han sido considerados como alérgenos importantes. Por otro lado, la sensibilización al polen de *Morus* y *Rumex* se ha presentado en pacientes polisensibilizados, lo que podría considerarse como una sensibilización panalergénica. Un mensaje clave, para la puesta en marcha de futuros estudios clínicos de SPT, es evitar incluir alérgenos de cereales, ya que estos podrían distorsionar los resultados.

Un segundo paso se ha dirigido a la definición de la estación polínica de Estambul, en esta ocasión desde una perspectiva ambiental (Capitulo 2). El software AeReobiology package in R, diseñado para realizar análisis aerobiológicos, ha facilitado realizar un estudio exhaustivo sobre la estación polínica para diferentes taxones, y sobre la intensidad de la floración. Poaceae, Cupressaceae, Pinaceae, Fagaceae y *Ambrosia* fueron los cinco taxones polínicos mejor representados en el oeste de Estambul. Durante el periodo de muestreo, hubo un cambio en altura del captador, debido a un

proyecto de construcción en 2013. Esta altura más elevada repercutió, principalmente, en la detección de polen de taxones que crecen en el entorno inmediato, especialmente de Poaceae. La definición de la estación polínica, considerando el 95 % de la Integral de Polen Anual (APIn, siglas en inglés), pareció adecuada debido a las bajas concentraciones de polen en el aire. Para definir el inicio de estación polínica se consideraron, además, cinco días consecutivos con polen. Para Pinaceae, *Platanus*, *Fraxinus*, *Olea*, así como para Poaceae y *Plantago*, el método de cinco días consecutivos con polen podría ser una alternativa al método porcentual.

Durante el período de estudio hubo una gran variabilidad en las estaciones polínicas, especialmente en taxones leñosos. En 2016 se observó alta concentración de polen de especies leñosas. Se ha realizado un análisis sobre la influencia de factores meteorológicos sobre el contenido de polen en el aire. La disponibilidad hídrica del año previo está relacionada con elevadas concentraciones polen en taxones de la familia Betulaceae, además de otros. De ahí surgió la hipótesis de partida sobre el fenómeno de vecería entre taxones y poblaciones. De hecho, el estudio sobre los altos niveles de polen de abedul, en Europa del Este durante 2016, corrobora esta suposición. El papel de los parámetros meteorológicos se ha estudiado con Modelos Aditivos Generalizados (GAMs, siglas en inglés), observándose una relación con la fenología de los árboles que florecen en primavera, contraria al de las plantas herbáceas que florecen principalmente en verano. Los GAMs mostraron un impacto a corto plazo durante el período de máxima floración. La temperatura tuvo una relación negativa y la humedad positiva en los árboles de floración primaveral. Los descensos de temperatura retrasaron la dehiscencia de las anteras, mientras que los períodos soleados, posteriores a las lluvias, la favorecieron. La humedad relativa cambia a lo largo del día, observándose que descensos de humedad, tras eventos de lluvia, favorecen la dehiscencia. Las especies herbáceas que florecen en verano liberan su polen cuando aumentan las temperaturas a medida que avanzaba la temporada. El papel de la precipitación fue negativo. Los GAMs pueden ser una alternativa para estudiar el impacto de variables meteorológicas dependiendo de su rango de variación. La ciudad se extiende a lo largo de 80 km de la costa de oeste a este, por lo que el espectro de polen en las franjas occidentales menos urbanizadas de la ciudad fue diferente en comparación con los datos de polen en el centro de Estambul. Las diferencias en la importancia de los taxones que contribuyen al polen entre el centro de la ciudad y la periferia sugieren la necesidad de contar con un captador de polen en el centro de la ciudad y otro en los alrededores del este y oeste de la ciudad, de esta forma se asegura una información representativa sobre el polen de Estambul.

En Turquía, a diferencia de muchos países de Europa occidental, está surgiendo la necesidad de un sistema público de alerta. De momento no se han definido los niveles de umbral de las concentraciones de polen que conducen a síntomas. Para definir bien los riesgos de exposición bajos, moderados o altos, se deben presentar los niveles de umbral del polen local. En el tercer estudio (Capítulo 3) se han vuelto a utilizar modelos GAMs. En esta ocasión se ha tratado de evaluar la relación dosis-respuesta con Poaceae, Betulaceae/Fagales, *Ambrosia*, Total de Polen y PM10 en los índices de síntomas y medicamentos a partir de fuentes múltiples. La App pollen diary, utilizada por pacientes de alergia para registrar sus síntomas (PHD, siglas en inglés), proporcionó datos sobre la morbilidad. Tratando de ampliar el estudio al sudeste de Europa, se ha contado con datos de Yuvojvod, Serbia. Los umbrales de Poaceae, Betulaceae/Fagales, *Ambrosia* y Polen Total se han definido mediante una interpretación visual de curvas no lineales. Para Estambul 20 p/m3 de Poaceae se ha considerado como umbral de síntoma grave, en Yuvojvod se han considerado para este síntoma 15 p/m3 de *Ambrosia*. Los pacientes sufrieron más síntomas al inicio de la estación polínica que al final y la morbilidad no aumentó con el aumento de las concentraciones de polen. A partir de las estimaciones del efecto de los resultados del GAM, se ha podido evaluar el Riesgo Relativo (RR) asociado con la morbilidad. El RR, ya sea para desarrollar síntomas o para el uso de medicación, o para ambos, se observó una tendencia creciente de los umbrales. El RR para Poaceae, Betulaceae y *Ambrosia* fue pronunciado (110-120%), mientras que para el Total de Polen y partículas PM10 se obtuvo un RR que aumentó de 1-3%. El Polen Total proporciona un pequeño efecto aditivo al riesgo de desarrollo de síntomas, pero no puede ser un indicador de los síntomas causados por taxones que cuentan con una alergenicidad y estacionalidad variable.

Para concluir, IPASS ofrece a los alergólogos una información que ayuda a la selección de alérgenos para las pruebas de alergia y para su tratamiento. La participación en el PHD ha facilitado el autocontrol de la alergia del paciente. El conocimiento sobre las estaciones polínicas en Estambul permitirá comprender mejor futuros efectos ambientales causados por el cambio climático en esta área vulnerable. El desafío de presentar niveles de umbral sobre los síntomas y el uso de medicamentos se ha conseguido con el uso de datos de fuentes múltiples del PHD de Estambul, Turquía, y Yuvojvod, Serbia. Propusimos una forma de determinar niveles de umbral regionales por medio de GAM que podrían aplicarse en toda Europa dentro de la Red Europea de Aeroalérgenos (EAN). Este estudio se suma al conocimiento aerobiológico en Turquía a la luz del desarrollo de información pública sobre el polen.

ABBREVIATIONS

APIn	Annual Pollen Integral
EAN	European Aeroallergen Network
EAS	European Aerobiology Society
ESP	European Standard Panel
GAM	Generalized Additive Model
IPASS	Istanbul Pollen Allergen Sensitization Study
MPS	Main Pollen Season
MSS	Main Spore Season
PHD	Patient’s Hay-fever Diary
RR	Relative Risk
SPIn	Seasonal Pollen Integral
SPT	Skin Prick Testing

General Introduction

Background

In the first decade of the new millennium, patients with autumn hay-fever started to visit an allergy clinic in Istanbul. Clinicians did not understand its origin, as this phenomenon was new and Skin Prick Testing (SPT) did not provide an answer. The need to identify an "unknown pollen" that might be the origin for allergy exacerbations out of the usual pollen season, led to the beginning of the pollen monitoring with a volumetric Hirst type trap in Büyükçekmece on today's campus of the Medical Faculty of Istanbul University. It turned out that highly allergenic *Ambrosia* pollen was present in the atmosphere of Istanbul, when the distribution of this alien weed in the region was still not known.

In 2012 the Istanbul pollen monitoring station adhered to the European Aeroallergen Network (EAN) for standardized monitoring and to the European Aerobiology Society (EAS) for knowledge transfer. On this ground, the Istanbul Pollen Allergen Sensitization Study (IPASS) was conceived to address research needs in allergology in Istanbul and Turkey.

In Turkey, public pollen information is at its beginnings. To present, no regular public pollen information is available at national level. However, channelled through the EAN, weekly pollen information can be transferred to the public by means of the portal pollendiary.com (Berger et al., 2011), coordinated by the Medical University of Vienna. Members of the EAN upload standardized pollen data (EN 16868, 2019) to the system on a weekly basis. Users of pollendiary.com (PHD) can learn which pollen were airborne at the time they recorded their symptoms (Berger et al., 2013). The station TRISFU in Büyükçekmece, Istanbul, which was run by the author of this dissertation from 2012-2016, supplied data to the EAN. The use of the PHD in Istanbul was promoted in the frame of IPASS. Recently, Ege University has resumed the endeavour to supply pollen information to the public in a pilot project with the State Meteorological Service (Göksel & Güvensen, 2021).

The starting hypothesis of this work is based on the assumption that the population of Istanbul is sensitized to pollen from both Mediterranean and Middle European species. *Ambrosia* (ragweed) pollen has been observed for the first time by the author in Istanbul in 2007 (Zemmer, 2012). Till then *Ambrosia* had not been mentioned in pollen studies related to Istanbul (Celenk et al., 2010). Not only sensitization to ragweed but allergic symptom development was expected to be due to fast increasing airborne pollen concentrations of ragweed (Zemmer, 2014). *Ambrosia* is believed to be a novel allergen in the atmosphere of Istanbul.

Although there are sensitization studies related to the study area available from the Anatolian side of Istanbul (Aydin et al., 2009), and Edirne, western Thrace (Yazicioglu et al., 2004) the allergen selection for SPT required an update on relevant allergens based on aerobiological data. Updated local aerobiological information ought to be considered for the clinical assessment of pollen allergy (Ansotegui et al., 2020; Bastl et al., 2019). This call for interdisciplinarity has rarely been explicitly addressed by means of a clinical SPT panel based on aeroallergens. A recommendation on a test panel for the metropolis Istanbul has the potential to be used for the Marmara Region.

A comprehensive analysis of the allergenic pollen season in relation to meteorological parameters monitored at the western fringes of Istanbul has been overdue. The last analysis on the pollen spectra of the city concerns one year data from spring 2005 – spring 2006 and is related to central Istanbul (Celenk et al., 2010). Hoffmann et al. (2020) have contributed one year data from Istanbul for the assessment of clinical seasons in South Europe. In times of climate change, knowledge on the environmental aspects of pollen helps to make inferences on possible shifts in the vegetation (Bogdziewicz et al., 2017; Ziello et al., 2012; García-Mozo et al., 2010). Hereby effects on human health and the environment can be derived (Damialis et al., 2019; Katelaris & Beggs, 2018).

The identification of pollen threshold levels that induce symptoms of allergy can improve public pollen warnings (Šukienė et al., 2021). It is problematic to issue universal thresholds since there are regional peculiarities in pollen exposure (Becker et al., 2021; Kitinoja et al., 2020). Hay fever symptom severity may change during the course of the pollen season (de Weger et al., 2011). There have been a few studies reporting non-linear dose-response relationships between pollen exposure and pollen intensities (Caillaud et al., 2012; Caillaud et al., 2014). These have implications for the delineations of thresholds themselves (Caillaud et al., 2012; Caillaud, et al., 2014), which impact the implementation of allergy therapies and clinical trials (Ansotegui et al., 2020; Pfaar et al., 2017). Threshold levels have not been previously assessed in Turkey. Istanbul at the southwestern end of the Balkan Peninsula can be a reference for Southeast Europe.

Objectives and Research Questions

Given the population density of Istanbul and limited information in pollen allergy epidemiology, the purpose of this study is i) to issue a recommendation on a SPT panel for adult hay fever patients, based on a multidisciplinary approach designed for the biogeographical area of Istanbul; ii) to delineate the pollen season for western Istanbul in relation to meteorological factors; iii) to identify threshold levels and the relative risk (RR) of allergens leading to symptom development and medicine use among the population in western Istanbul by means crowd sourced data. To obtain further insight on thresholds in Southeast Europe, Serbia (region Yuvojvod) was included in this study.

Study Concept

The Istanbul Pollen Allergen Sensitization Study (IPASS) (Fig. 1) emerges from routine airborne pollen monitoring. From the airborne pollen taxa, potential allergenic taxa were identified and used to issue an Istanbul Test Panel (Chapter 1). To do so, allergen extracts of identified taxa were applied in a prospective clinical panel study on adult participants with a history of allergy. From there, sensitization rates were identified. Participants of the clinical study were instructed to keep an electronic hay fever diary (pollendiary.com) and to register their symptoms. This way, patients were able to see which pollen was airborne, when symptoms appeared and to have their personal pollen information.

The intensity and the duration of the pollen seasons in western Istanbul was assessed on the base of the data form the routine monitoring (Chapter 2).

Crowd sourced symptom data gathered from pollendiary.com were eventually used to identify threshold levels for symptom development and medicine consumption (Chapter 3).

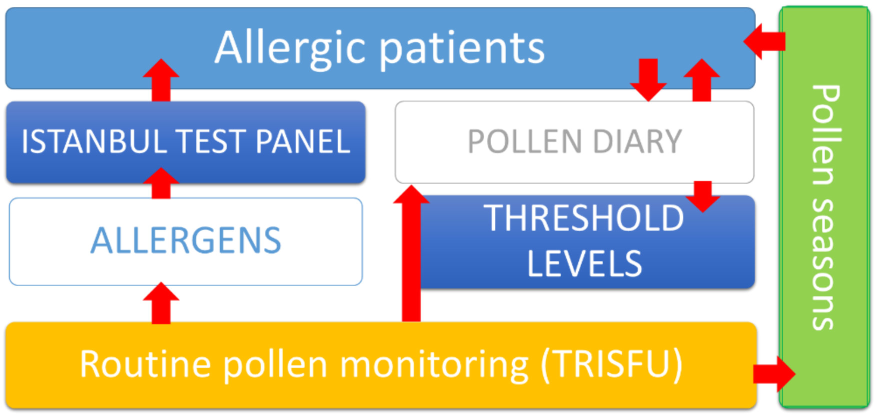

Fig. 1 The concept of the Istanbul Pollen Allergen Sensitization Study. Red arrows show how the modules of the study are connected.

STRUCTURE OF THE THESIS

This thesis is structured into three chapters based on the goals of IPASS. Each chapter is a concluded study conceived as a research paper. The complete bibliography is included at the end of the dissertation.

Chapter 1 The Istanbul Test Panel

The purpose of this study was to avoid significant absences or redundancies in the diagnostic panels for Skin Prick Tests (SPT) used by specialists operating in this densely populated area of Turkey. The results were generally similar to those found in European Community countries. Novel is the proven sensitization to *Ambrosia* in Istanbul. The study refers to a territorial area that had not yet been studied in detail and takes the experience of European countries as a reference.

Open Access Research Paper

This study was published as the first article in the January 2022 edition of the *European Annals of Allergy and Immunology*, Q3 in Journal Citation Reports (SCI). An extended version of the published article is presented in this chapter.

"A Multidisciplinary Approach of Outdoor Aeroallergen Selection for Skin Prick Testing in the Geographical Area of Greater Istanbul", DOI: 10.23822/EurAnnACI.1764-1489.188

A MULTIDISCIPLINARY APPROACH OF OUTDOOR AEROALLERGEN SELECTION FOR SKIN PRICK TESTING IN THE GEOGRAPHICAL AREA OF GREATER ISTANBUL

ABSTRACT

Background: Allergen selection for Skin Prick Testing (SPT) and the interpretation of results require to be in line with the occurrence of allergenic sources of the specific geographic area.

Objective: In this study we aim to 1) identify an allergen test panel designed for a specific geographic area 2) shed light on sensitization to new environmental allergens 3) interpret results according to the state of the art.

Methods: The European Standard Panel (ESP) for SPT has been adapted for use in a Csa climate region according to the Köppen-Geiger classification based on aerobiological observations, which reflect the occurrence of allergenic sources of the area and considering cross reactivity patterns of selected allergens. Atopic adult patients (n = 60) filled in a questionnaire and were skin prick tested with 36 allergens. Aerobiological monitoring was performed with a volumetric spore trap abiding to European Aeroallergen Network (EAN) standards. SPT results were interpreted in the light of aerobiological parameters and correlations of multiple sensitizations.

Results: 65 % of patients reacted to a panel of 34 inhalant allergens. The total grass sensitization was 30 % of which *Phleum pratense* (25.8 %), *Sorghum halepense* (22.6 %), *Anthoxanthum odoratum* (21.0 %), *Cynodon dactylon* (19.4 %), and *Poa pratensis* 14.5 %. The total woody plants sensitization was 15 %, of which *Fraxinus excelsior* accounted for 8.1 %, *Corylus avellana*, *Olea europaea* and *Morus alba* each for 6.5 %, *Juniperus ashei* for 4.8 %, *Quercus rubra*, *Betula alba*, *Acer negundo* each for 3.2 %, *Alnus glutinosa, Celtis australis* for 1.6 %, and *Platanus orientalis* and *Salix alba* for 0 %. The total weed sensitization was 15 %, of which *Rumex crispus* accounted for 12.9 %, *Ambrosia artemisiifolia* and *Artemisia vulgaris* each for 4.8 %, *Amaranthus retroflexus* for 3.2 %, *Xanthium strumarium* for 3.2 %, and *Parietaria Judaica* for 0 %. Of non-pollen allergens, *Alternaria alternata* accounted for 3.2 %, *Dermatophagoides farinae* for 16.1%, *Dermatophagoides pteronyssinus* for 12.9%, *Blatella germanica* for 1.6 %, *Felis silvestris* for 1.6 % and *Canis familiaris* for 0 *%.*

Conclusion: The ESP is suitable for Istanbul; however, *Sorghum halepense* should be included when testing for grass pollinosis. *Morus* sensitization was connected with polysensitization.

Key words: Pollen allergy; Allergens, Skin Tests; Environmental Monitoring; Symptoms

1.1 Introduction

Anyone who works in allergology knows that development of allergy (asthma, rhinitis, conjunctivitis, atopic dermatitis, etc.) is connected with multiple factors and often considered a disorder rather than a well-defined disease with clear causes and treatments (Jenerowicz et al., 2012). The epidemiology depends on the genetic makeup of the individual (Ferreira et al., 2014; Abbas et al., 2012). Allergy can be further triggered by environmental conditions such as exposure to chemical agents in indoor and outdoor air as well as to non-biogenic and biogenic particulate matter (Baldacci et al., 2015). Income status (Sipahi et al., 2017), alimentation, and early life antigen exposure (Geller-Bernstein et al., 2002) play a role, too. Moreover, people who live in environments with an elevated degree of biodiversity are less likely to suffer from allergies (Hanski et al., 2012).

Allergy, also called atopy, is a reaction of the immune system to specific environmental antigens. Such environmental antigens are known as allergens. From a cellular and molecular perspective, TH_2 cells, immunoglobulin E (IgE), mast cells, basophils and eosinophils are involved. An allergic immune response is defined by the production of allergen-specific IgE antibodies. The induction of IgE production upon contact with an allergen is called sensitization. When an allergen enters the circulation, and is presented to receptors expressed on T-cells, sensitization may occur. Instead of inducing the production of IgG-antibodies, which is the “normal” answer, the T-cells will stimulate the priming of IgE-producing plasma-cells, derived from so called B-cells. At subsequent exposure to allergen, T-cells and B-cells will again be activated to produce specific IgE-antibodies, which will bind to the allergen and then to mast cells which release mediators of immediate hypersensitivity (Abbas et al., 2012).

Type I allergic disorders are detected either in vivo by means of Skin Prick Testing (SPT) or in vitro by IgE in the serum (Bignardi et al., 2019; Heinzerling et al., 2013). The SPT methodology is based on an immediate reaction upon an intradermal administration of antigen to a previously sensitized individual. At the site of the challenge a wheal (softly swelled tissue due to plasma infiltration) develops. Subsequently, blood vessels on the margin of the wheal dilate and red blood cells accumulate to form a red rim – the flare. In the test, the size of the wheal functions as indicator of allergy severity. The wheal and the flare develop within 10 minutes after antigen administration and lasts no longer than an hour (Heinzerling, et al., 2013; Abbas, et al., 2012).

SPT is a generally safe procedure widely used in sensitization studies (Şahiner et al., 2012; Aydin et al., 2009; Bousquet et al., 2009; Dursun et al., 2008; Sin et al., 2001; Yazicioglu et al., 2004). The procedure has great specificity and sensitivity for inhalant allergy and is cheap (Ansotegui et al., 2020).

The inhalation of aeroallergens from pollen and fungal spores may lead to respiratory allergy in susceptible individuals. It is known that aeroallergens often correlate with symptom development (Kiotseridis et al., 2013) and symptom inducing pollen concentrations vary from place to place (Bastl et al., 2018). Atmospheric pollen concentrations differ greatly in time and space because they are dependent on local vegetation and weather conditions (Dahl et al., 2013). Moreover, biotic and abiotic particles from remote sources due to air mass movements add to the health burden through atmospheric transport (Grewling et al., 2019; Cecchi et al., 2006).

Epidemiological studies on IgE mediated sensitization patterns in atopic individuals reveal which allergens are relevant in a particular population since sensitized individuals often develop symptoms of allergic disease (Ansotegui et al., 2020). In this regard, a standard test panel for Europe has been proposed by Heinzerling et al. (2013) including species from the Eurosiberian and Mediterranean plant regions, and the neophyte

Ambrosia artemisiifolia. Throughout Europe, Poaceae allergens play a significant role in allergic disease (García-Mozo, 2017; Kmenta et al., 2017; Kiotseridis et al., 2013). In addition, *Betula* is relevant in Northern Europe as well as in Middle Europe (Ritenberga et al., 2016; Pfaar et al., 2017; D'Amato et al., 2007). In Middle Europe additional tree species from the order Fagales such as *Corylus* (Grewling et al., 2014) add to the burden of hay fever sufferers. In terms of weed pollen, *Artemisia* and *Ambrosia* are most relevant (D'Amato et al., 2007). Common allergens in Southern Europe are *Olea*, *Cupressus* and *Parietaria* (Cebrino et al., 2017; Galán et al., 2013; Jato et al., 2010; D'Amato et al., 2007). *Ambrosia* has become a feared allergen in many parts of Europe with hot spots in the Rhone valley in France, Lombardy in Italy, the Pannonian Plain, and the Ukraine (Smith et al., 2013; Skjøth et al., 2010) conquering aggressively new areas such as Turkish Thrace and the megacity Istanbul. Airborne pollen concentrations in Istanbul have now reached values comparable to those in infested regions in Europe (Zemmer, 2014). Several plant populations of *Ambrosia artemisiifolia* in Thrace, including the metropolitan area of Istanbul (Ozaslan et al., 2016), pose an alarming threat to the atopic population of the region.

Allergy incidence in Turkey has increased rapidly over the last decade. While in 2012 less than 10 % of the population aged over 14 suffered from atopic allergy including allergic rhinitis, food allergy, dermatitis, and asthma, in 2019 21.2 % of the population were affected. In short, at a population of over 83 million (Turkish Statistical Institute), 2020) 17.7 million people suffer from asthma or a form of allergy in the country, which makes 3 million in the 15 million metropolis of Istanbul alone.

The vegetation in the surroundings of Istanbul pertains to the Eurosiberian (Euxine province) and the Mediterranean floral regions (Kavgaci et al., 2015). Considering this vegetation mixed with typical urban and ruderal floristic elements, the floristic diversity consists of 2048 taxa in the province of Istanbul (Bakis et al., 2020).

Species detected in aerobiological monitoring mirror the regional anemophilous flora and can aid allergy practitioners in diagnosis. In fact, clinical experience in Istanbul and data from aerobiological monitoring indicated the need for an allergen test panel for clinical purposes designed for this region. Efforts have been made to issue a recommendation on a minimal SPT-panel to use in Turkey in order to reduce costs (Şahiner et al., 2012). However, in a follow up study it was indicated that minimal batteries bare the risk to miss out allergens relevant in a specific area (Cavkaytar et al., 2015). The selection of the right allergens for a battery can enhance diagnosis and aid targeted treatment, thus reducing its cost. In the selection of allergens for clinical studies using SPT methodology, it is fundamental to consider cross reactivity patterns between species for a sound interpretation of the results (Ansotegui et al., 2020). When using standardized extract mixtures, though, the de facto sensitizing allergen remains unknown. In contrast to previous epidemiological studies on allergen sensitization prevalence in Istanbul (Aydin et al., 2009), and Turkish Thrace (Yazicioglu et al., 2004), the present investigation included single allergens rather than mixed extracts. This allows, for example, discussing possible cross-reactivity patterns (Matricardi et al., 2016; Asam et al., 2015; Weber, 2007) at multiple sensitizations.

For this reason, the aim of this observational study was 1) to identify an allergen test panel designed to use in the region of Istanbul based on aerobiological observations and taking into account cross reactivity patterns in the selection of allergens; 2) to shed light on trends in ragweed sensitization rates in Istanbul and 3) to report on clinical symptoms connected with sensitization.

1.2 METHODOLOGY

STUDY AREA

The study area includes most of European Istanbul, defined by a theoretical 30 km range of the monitoring site at 41°05'24.6"N 28°37'16.7"E on the western fringes of the metropolis. The degree of urbanisation increases city inwards towards east and south-east. To the south, the area is delineated by the Marmara Sea, to the north by the Black Sea. Following agricultural lands, grasslands and smaller dwellings, deciduous forests are found from the northwest to the northeast at a distance of 10-15 km. The agricultural land use consists in the growth of annual crops (sunflower, canola, cereals). The landscape is undulating, not exceeding an elevation of 300 m above sea level. The climate is between temperate and Mediterranean corresponding to the Köppen-Geiger Csa-type (Peel et al., 2007). The average annual temperature is 14.1 °C, and the average annual rainfall is 747 mm (Climate-Data.org, 2020). The main wind direction is north-easterly.

POLLEN AND FUNGAL SPORE MONITORING

Regional pollen monitoring was performed with a volumetric Hirst-Type pollen sampler (Hirst 1952), located in Büyükçekmece, at 10 m height on a rooftop. We followed the minimum requirements from the European Aerobiology Society (EAS) (Galán et al., 2014) and current standards (EN 16868, 2019). The daily average pollen and spore concentrations were expressed as per cubic meter of air after applying a conversion factor. Data were sent to the European Allergen Network (EAN). The Main Pollen Season (MPS) and Main Spore Season (MSS) were defined according to the 95 % method suitable for the local sampling circumstances (Bastl et al., 2018) and which was already applied in Turkey (Tosunoglu et al., 2015).

ALLERGEN SELECTION

For the clinical trial 36 allergens from airborne particles were selected on the base of 1) the European Standard Panel (ESP) (Ansotegui et al., 2020; Heinzerling et al., 2013); 2) taking into consideration cross reactivity patterns; and 3) own additions in line with the allergenic sources of the specific geographic area according to atmospheric pollen occurrence as well as observations on the local allergenic flora in the range of the trap location. Standardized extracts were used to obtain comparable results.

STUDY PARTICIPANTS

After approval by an ethic commission (2014/11/03 Bakirkoy Dr. Sadik Konuk Egitim ve Aristirma Hastanesi) a recruitment campaign was launched in 2015. Patients admitted to testing were ≥ 18 years old, had symptoms of respiratory allergy, had lived in Istanbul for three years, were not immunocompromised, and lived, worked or studied in the range of 30 km from the trap location (Kiotseridis, 2013). Patients were asked to stop histamine medication three days before SPT as well as long-lasting histamines and immunosuppressants ten days prior to testing. All participants compiled a questionnaire and their informed consent was obtained.

THE QUESTIONNAIRE

Before undergoing the skin test, patients had to fill in a questionnaire (Annex I). The questionnaire comprised two parts (Tab. 1). Part A included question items adapted from the Allergic Rhinitis and its Impact on Asthma (ARIA) (Ansotegui et al., 2020; Heinzerling et al., 2013) to assess the degree of morbidity.

Part B was intended to investigate in more depth the severity (proposed as problems) and type of symptoms of the eyes, nose, and lungs. Participants rated problems related to organs from none to mild, moderate and severe and were respectively recorded as 1, 2, 3 or 4. Items concerning symptoms were nominal and multiple answers were possible. Those nominal items were recorded as absent (0) or present (1). For example, a participant with moderate eye problems (logged as 3) indicated that the problems were caused by itching (logged as 1) and foreign body sensation (2). Redness and watering were left empty (each logged as 0). The same applied to the type of medication used. All items corresponded to the ones in the online Patient's Hay-fever Diary (PHD), pollendiary.com (Berger et al., 2011). Qualitative data from section B were summed up to obtain a nose, eye, lung score, a total symptom score and a total medical score.

Tab. 1 Content of the patient questionnaire

Section A	
I.	Personal data
II. and III.	Allergy history; Family history of allergy
IV. and V.	Organs affected by symptoms; Frequency and general severity of allergy
VI.	Consent to use the pollendiary.com
VII.	Consent to skin testing
VIII.	Name, date and signature
Section B	
	"Static" symptom scores as per PHD, pollendiary.com

Skin prick testing (SPT)

The SPT procedure was performed according to current standards (Ansotegui et al., 2020; Heinzerling et al., 2013). A test was positive if the largest diameter of the wheal was ≥ 3 mm. Results were reported in five categories: grade 0 (no reaction); 1+ (erythema ≤ 15 mm, no oedema); 2+ (erythema > 15 mm, oedema < 3 mm); 3+ (oedema 3-6 mm); 4+ (oedema > 6 mm) to obtain the degree of sensitization. Tests were performed by a physician in a clinic located within the range of 30 km of the trap location.

Statistics

Descriptive statistics for quantitative data included frequency, standard deviations, and percentages, for qualitative data frequencies and percentages. We discerned SPT positive and negative groups as independent variables. For quantitative data we used a Mann Whitney U as hypothesis test. Moreover, we performed non parametric correlation analysis (Spearman's rho) to identify relationships between airborne particle concentrations, symptom scores, medical scores and sensitization rates. We used a significance level of at least 95 % ($\alpha = 0.05$). All analysis was performed with IBM SPSS Statistics 2.0.

1.3 Results

Allergens used

Refer to Tab. 2 for the comprehensive list of allergens.

Grasses: Single grass allergens, rather than grass mixtures were chosen. In order to evaluate the importance of allergens of Poaceae-subfamilies in our study area we included Pooideae, Panicoideae and Chloroideae subfamilies, which have unique allergens (Ansotegui et al., 2020; Gangl et al., 2013; Weber, 2007). At Poaceae family level there is strong cross-allergenicity between species due to homology of major allergen groups 1, 2/3, and 5. From the subfamily Pooideae we included *Phleum pratense*, *Poa pratensis* as proposed in the ESP and *Anthoxanthum odoratum* due to possible unique allergens (Weber, 2007). *Sorghum halepense* (Panicoideae) and *Cynodon dactylon* (Choloroideae) were own additions. *Sorghum halepense* is a late summer blooming grass common in the Mediterranean and bears the potential to extend the grass pollen season into autumn. The similar applies to *Cynodon dactylon* (Ghitarrini et al., 2017). *Cynodon dactylon* and *Sorghum halepense* have a widespread distribution in both agricultural and non-agricultural areas in the Thrace region (Özer et al., 1999). By testing representative species of different subfamilies sensitization patterns to each subfamily will become evident.

Trees: Cupressaceae were represented by *Juniperus ashei* extract, which is possibly more effective in specific IgE detection than *Cupressus sempervirens* extract (Bousquet et al., 2009). There is strong cross-reactivity between Cupressaceae family members because of shared homologous major allergens (Weber, 2007). Also, within the order Fagales (e.g. Betulaceae, Fagaceae) homologous allergens can lead to cross-reactivity (Ansotegui et al., 2020; Matricardi et al., 2016, D'Amato et al., 2007). Besides the allergens proposed in the ESP, we included *Quercus* ssp. based on aerobiological observations. With the prospective to observe cross-sensitization between allergen members of the Oleaceae family (Ansotegui et al., 2020; Matricardi et al., 2016; Weber, 2007), both *Olea europea* and *Fraxinus excelsior* were included. The remainder of the tree-allergen extracts selected for this study was added due to known allergenicity or aerobiological records.

Weeds: The allergenicity of different species of the Amaranthaceae family may vary (Weber, 2008). Therefore, a selection of allergens (*Chenopodium album, Atriplex canescens, Salsola kali, Amaranthus retroflexus)* was included to identify the most relevant species for Istanbul.

With regard to the Asteraceae family, we selected *Ambrosia artemisiifolia*, *Artemisia vulgaris* from the ESP as well as *Xanthium strumarium* as own addition due to the occurrence in the study area and indications in the literature (Weber, 2007). To represent Urticaceae allergens we used *Parietaria judaica* since *Urtica*-species are generally not allergenic (Vega-Maray et al., 2006). However, in aerobiological monitoring this pollen type is recorded at Urticaceae family level, because the pollen of species belonging to this family (except *U. membranacea*) is morphologically similar and difficult to distinguish under the optical microscope. Finally, we included *Rumex crispus* and *Plantago lanceaolata* (Ansotegui et al., 2020).

Fungi: From the fungal allergens proposed by Heinzerling et al. (2013) we tested *Alternaria alternata* extract.

Animal allergens: According to the ESP cat, dog, house dust mite and cockroach were included.

Tab. 2 List of allergens

Group	Family	Subfamily/Tribe	Latin name	Common name	Source
Grasses					
	Poaceae	Pooideae	*Phleum pratense*	Timothy grass	1, 2
			Anthoxanthum odoratum	Sweet vernal grass	2
			Poa pratensis	Meadow grass	1
		Chloroideae	*Cynodon dactylon*	Bermuda grass	2
		Panicoideae	*Sorghum halepense*	Johnson grass	2
Trees					
	Cupressaceae		*Juniperus ashei*	Juniper	2, 1 (*C. sempervirens*)
	Betulaceae		*Corylus avellana*	Hazelnut	1
			Betula alba	Birch	1
			Alnus glutinosa	Alder	1 (*A. incana*)
	Fagaceae		*Quercus rubra*	Oak	2,3
	Oleaceae		*Fraxinus excelsior*	Ash	2
			Olea europaea	Olive	1
	Moraceae		*Morus alba*	Mulberry	2,3
	Aceraceae		*Acer negundo*	Box elder	2
	Salicaceae		*Populus alba*	Poplar	2,3
			Salix alba	Willow	2,3
	Cannabaceae		*Celtis occidentalis*	Hackberry	2,3
	Platanaceae		*Platanus occidentalis*	Sycamore	1 (*P. vulgaris*)
Weeds					
	Polygonaceae		*Rumex crispus*	Dock	2,3
	Amaranthaceae		*Chenopodium album*	Goosefoot	2
			Atriplex canescens	Fourwing saltbush	2
			Salsola kali	Russian thistle	2
			Amaranthus retroflexus	Redroot amaranth	2
	Asteraceae	Asteroideae	*Artemisia vulgaris*	Mugwort	1
			Ambrosia artemisiifolia	Ragweed	1
			Xanthium strumarium	Cockelbur	2
	Urticaceae		*Parietaria judaica*	Wall pellitory	1,2
	Plantaginaceae		*Plantago lanceolata*	Plantain	2,3
Funghi					
	Pleosporaceae		*Alternaria alternata*		1
Animal allergens					
	Pyroglyphidae		*Dermatophagoides farinae*	American house dust mite	1
	Pyroglyphidae		*D. pteronysinus*	European house dust mite	1
	Blattellidae		*Blatella germanica*	Cockroach	1
	Felidae		*Felis silvestris catus*	Cat fur	1
	Canidae		*Canis lupus familiaris*	Dog fur	1
Control	Negative		Salin		
	Positive		Histamin		

Source: 1) Heinzerlig et al. (2013); 2) Weber (2007); 3) own addition

Patient demographics and clinical aspects

All participants (n = 66) received a questionnaire for completion prior to testing. Six participants were removed from the evaluation because they did not comply with the eligibility criteria. The following demographic data (Tab. 3) were obtained from the part A of the questionnaire.

Tab. 3 Patient demographics versus SPT results

		Positive SPT	Negative SPT
Female	n	19	12
Male	n	19	9
Total	n	39 (65%)	21 (35%)
Allergic parent	n	15 (r = 0.265; p = 0.04)	3
Age group			
18-29	n	17	9
30-39	n	9	5
40-49	n	7	2
50+	n	1	1
Years allergy	n	32	13
	Mean	8,3	4,2
	Std. Dev.	8,1	5,2
Age	n	34	17
	Mean	30,9	31,1
	Std. Dev.	8,6	7,7
Weight	n	33	17
	Mean	72,7	67,4
	Std. Dev.	15,8	18,8

Personal data. 31 of the respondents were female and 28 male, 6 did not respond to this question item. The mean age was 31 years (SD 8) within a range of 18-52 years.

Personal history of allergy. Respondents (n = 45) had suffered from allergy on average for 7.1 years (Sd 7.5) ranging from 1-32 years previous to the testing day. Two years marked the first quartile. Five years the second and 10 years the third quartile of positive answers.

Family history of allergy. 70 % of respondents (n = 42) did not have an allergic parent, while 25 % (n = 15) had one and 5 % (n = 3) had both parents suffering from an allergic condition. We found a significant relationship between family history of allergy and SPT positive participants (Spearman's rho $r = 0.261$; $p < 0.05$).

Symptom scores and Medication

In order to obtain scale type data for statistical analysis, qualitative answers from the section B of the questionnaire were summed to a total symptom score and a total medical score. The distribution of total symptom scores was the same ($p > 0.05$) among positive and negative tested participants (Mann Whitney U Test). However, there was a significant difference in the use of medication ($p < 0.05$) (Fig. 1).

The total eye symptoms (mean = 3.47) were significantly different ($p < 0.05$) between positively and negatively tested participants. The total nose (mean = 4.7) and total lung symptoms (mean = 2.03) were not significantly different (Fig. 2).

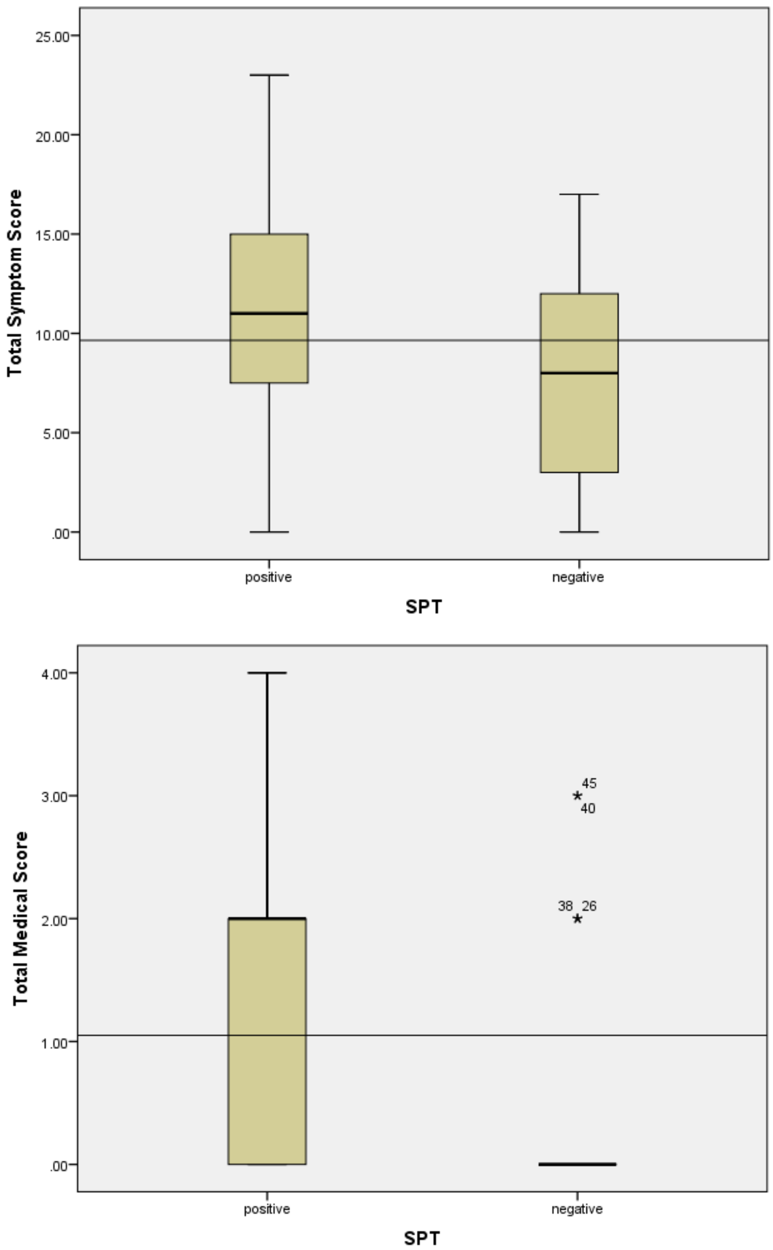

Fig. 1 Mann-Whitney U significance test on total symptom scores (top) and total medical scores (bottom) between positively and negatively tested participants. The crossing line is the common mean (1.05; 9.65).

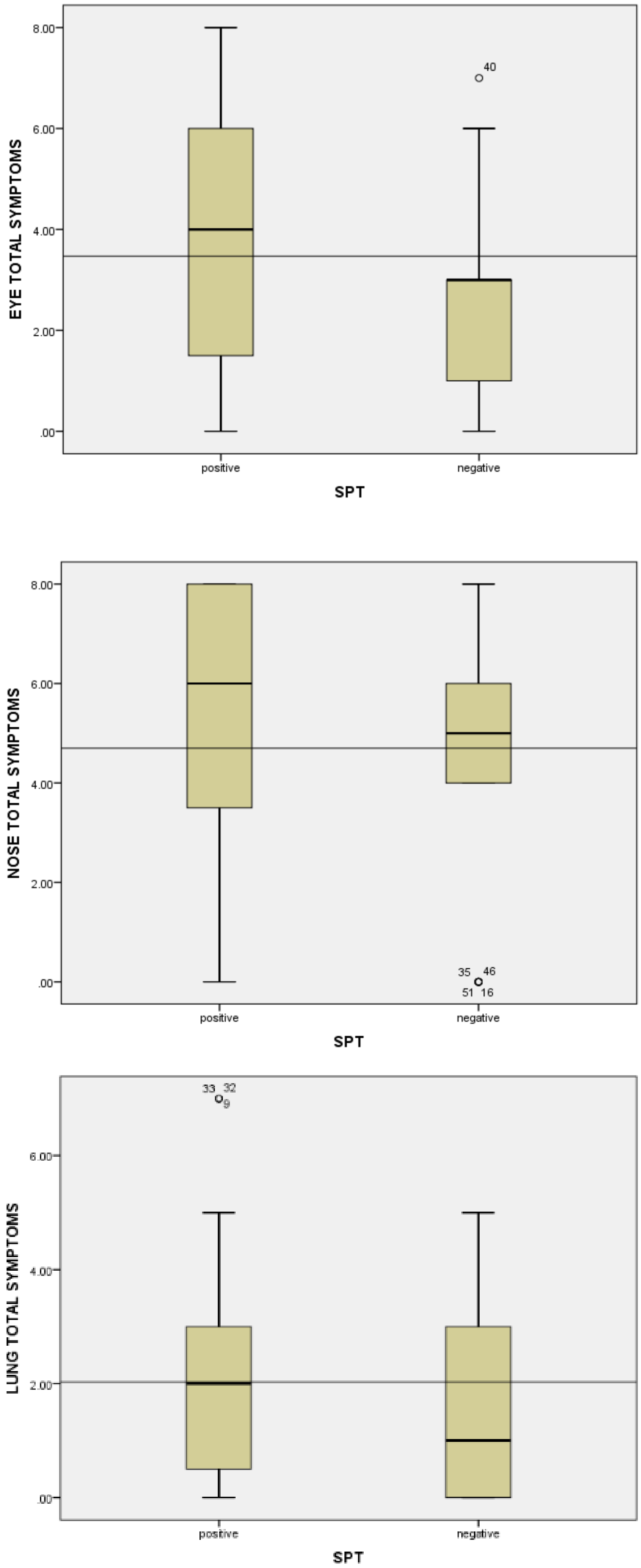

Fig. 2 Mann-Whitney U significance test on symptom scores of eyes (top), nose (middle), and lungs (bottom) between positively and negatively tested participants. The crossing Lines is the common mean.

AIRBORNE POLLEN AND FUNGAL SPORE CONCENTRATIONS

To understand the relationship between prevalence of sensitization and aerobiological features, relevant airborne particles monitored during 2015, Pollen Season Start (PSS) and Pollen Season End (PSE), MPS and MSS, maximal daily concentrations, Annual Pollen Integrals (APIn) and the number of sensitized individuals to the corresponding allergen of the airborne particle is reported (Tab. 4). The APIn of all pollen types monitored in Büyükçekmece that year was 7959. With reference to allergens used in SPT, Poaceae, Amaranthaceae and Urticaceae, pollen was identified to botanical family level. The list includes additional pollen types, which were not included as single allergens i.e., *Carpinus* (Betulaceae) and *Castanea* (Fagaceae), both of them pertaining to the order Fagales. Figures related to Oleaceae pollen, i.e., other species than *Olea europaea* belonging to this family, were summarized and included in the table for the same reason.

Tab. 4 Aeroallergens, start and end date, Main Pollen Season (MPS), Main Spore Season (MSS), the ratio of the Annual Pollen Integral (APIn) to the total pollen, maximum daily pollen concentrations and sensitizations in 2015.

	Airborne particles in Büyükcekmece	Start date:	End date:	MPS/MSS [days]	APIn and ASIn	APIn/total pollen [%]	Maximum daily concentration [p/m3]	SPT positive [n]	Sensitization [%]
Grasses	Poaceae	26.04.	23.09.	151	901.00	11.32	66.00	18	30.00
Trees	Corylus	02.02.	24.03.	51	33.00	0.41	5.00	4	6.67
	Alnus	24.02.	23.03.	28	62.00	0.78	7.00	1	1.67
	Carpinus	24.03.	02.06.	71	241.26	3.03	65.72	-	
	Betula	09.04.	06.05.	28	19.00	0.24	4.00	2	3.33
	*Betulaceae**	*02.02.*	*02.06.*	*121*	*355.26*	*4.46*		*7*	*11.67*
	Fagus	17.04.	16.06.	61	37.82	0.47	31.62	-	
	Quercus	21.04.	29.05.	39	657.52	8.26	62.00	2	3.33
	Castanea	09.06.	24.07.	46	20.06	0.25	3.10	-	
	*Fagales**	*02.02.*	*24.07.*	*173*	*1425.92*	*17.91*	*65.72*	9	15.00
	Fraxinus	09.02.	11.05.	92	91.14	1.14	6.82	5	8.33
	Olea	15.05.	13.06.	30	235.76	2.96	66.34	4	6.67
	*Oleaceae**	*09.02.*	*13.06.*	*122*	*326,9*	*4.11*	*66,34*	*9*	*15.00*
	Morus	15.04.	21.05.	36	169.26	2.13	16.74	4	6.67
	Acer	06.03.	26.04.	51	31.62	0.39	4.34	2	3.33
	Populus	14.04.	23.03.	23	34.72	0.44	19.22	1	1.67
	Salix	31.03.	10.05.	41	95.48	1.19	11.16	0	0
	Platanus	14.04.	28.04.	15	349.06	4.38	79.98	0	0
	Celtis	21.02.	21.02.	1	1.86	0.02		1	1.67
	Cupressaceae	22.02.	21.05.	89	1808.05	22.71	422.22	2	3.33
Weeds	Polygonaceae	17.04.	26.06.	71	46.66	0.59	7.44	8	13.33
	Amaranthaceae	25.05.	24.09.	123	257.47	3.23	31.00	4	6.67
	Urticaceae	05.04.	22.08.	140	291.33	3.66	17.98	0	0
	Plantaginaceae	23.04.	08.11.	200	112.36	1.41	8.06	2	3.33
	Artemisia	14.07.	05.10.	84	80.24	1.01	7.44	3	5.00
	Ambrosia	06.08.	01.10.	57	628.17	7.89	137.64	3	5.00
	Xanthium	09.08.	14.09.	37	29.37	0.37	4.34	2	3.33
	*Asteroideae**	*14.07.*	*05.10.*	*84*	*737,8*	*9.27*		*8*	*13.33*
Fungal spores	Alternaria	23.05.	10.11.	172	3881.1	48.76	145.86	2	3.33

*summarized for interpretation

In this paragraph main results regarding the aerobiological parameters (Tab. 4) are emphasized. The Poaceae pollen season in Istanbul lasted for five months, stretching from spring into autumn. In relation to other pollen types, airborne grass pollen concentrations were comparably high. Fagales pollen was airborne between February and July. Among Betulaceae, *Carpinus* flowered with greatest intensity and had the longest season. Among Fagaceae, *Quercus* flowering was most intense, but sensitizations to Betulaceae were more frequent. From Oleaceae, *Fraxinus* had a longer season than *Olea*, but its flowering was less intense. Slightly more patients reacted to ash than to *Olea*. Despite the short pollen season of *Morus*, close to 7 % had a skin reaction. The season of *Platanus* was short but intense. None of the participants reacted to this taxon. *Juniperus ashei* as representative allergen for Cupressaceae induced a skin reaction in 3.3 % of our participants, in spite of the highest pollen concentrations. The highest sensitization rate in weed allergen extracts was found with dock (*Rumex crispus*) (13.3 %), although its APIn was low. The highest pollen concentrations among weeds, however, were observed for *Ambrosia*. The pollen season of *Artemisia* was longer and less intense than of *Ambrosia* but sensitized the same number of patients. Despite an Urticaceae season of over four months and a comparably high pollen load, we found no patient reactions to *Parietaria*.

Alternaria spores were airborne in high concentrations mainly during summer. Two people out of 60 reacted to this fungal allergen.

Prevalence of sensitization and symptom development

Out of 66 patients 60 were eligible for SPT evaluation. Six were removed since they had a Histamine reaction below grade 3. None of the patients reacted to saline solution, the negative control. 65 % of participants had a positive SPT test reaction. We found 15 % of participants each sensitized to one or two allergens; 3.3 % were sensitized to 3 allergens; 13.3 % to 4; 1.7% to 5; 8.3 % to 6; 6.7 % to 7 and 1.7 % to 10 allergens each. Sensitization rates correlated significantly ($p < 0.05$) with the length of the MPS ($r = 0.471$). Consult Tab. 4 for an overview of sensitization rates to aeroallergens.

Grass allergen sensitization. The distribution of grass-subfamily sensitization among grass sensitized patients is visualized in Fig. 3.

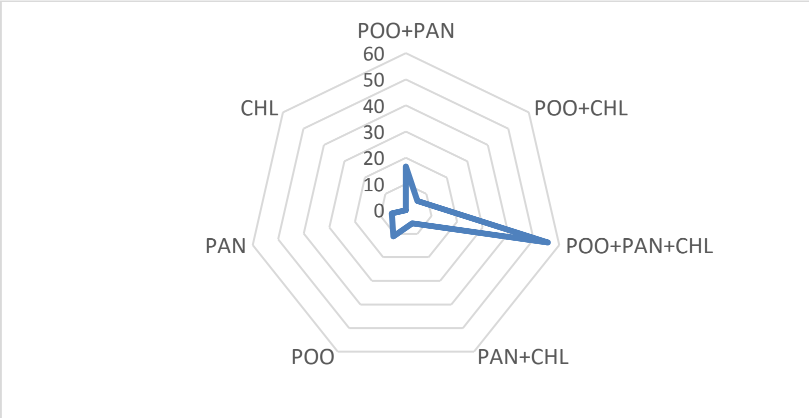

Fig. 3 Multiple grass subfamily sensitization rates [%] of Pooideae (POO), Panicoideae (PAN) and Chloroideae (CHL)

Of 39 positive tested patients, 18 (56 %) were sensitized to all three grass subfamilies. 17 % were sensitized to both Pooideae and Panicoideae, while 11% were monosensitized to Pooideae and 6 % to Panicoideae. 5 % were sensitized to both Pooideae and Choroideae subfamilies. No one was monosensitized to Chloroideae. The correlations between sensitizations to all Poaceae species were significant at 99 % (Tab. 5).

Tab. 5 Correlations of sensitizations to Poaceae allergens

		Cynodon	Sorghum	Poa	Anthoxanthum	Phleum
Cynodon	r	1,000	.781**	.523**	.579**	.761**
Sorghum	r	.781**	1,000	.594**	.797**	.835**
Poa	r	.523**	.594**	1,000	.742**	.731**
Anthoxanthum	r	.579**	.797**	.742**	1,000	.875**
Phleum	r	.761**	.835**	.731**	.875**	1,000

**. Correlation is significant at the 0.01 level (2-tailed).

There was a highly significant relationship ($p < 0.01$) between the total grass allergen sensitization score with the total medical score ($r = 0.372$) and a significant ($p < 0.05$) relationship with the total symptom score ($r = 0.304$). In detail, out of tested grass allergens we found *Poa pratensis* to correlate with the total symptom score at a significance level of 99 %, but the correlation with *Phleum pratense* and *Anthoxanthum odoratum* was significant at 95 %. On the other hand, sensitization to *Cynodon dactylon* and *Sorghum halepense* did not significantly correlate with the total symptom score. Likewise, the total medical score correlated significantly with all tested Pooideae allergens ($p < 0.01$) and Panicoideae ($p < 0.05$) but not with Chloroideae. All grass allergens, except *Cynodon dactylon*, correlated significantly with total eye symptoms ($p < 0.01$) and total nose symptoms ($p < 0.05$). *Poa pratensis* displayed the strongest correlation with nose symptoms ($r = 0.304$; $p = 0.018$). None of the Poaceae allergens affected the total lung symptom score.

The size of the wheal and flare reaction is an indicator for allergy severity. In Poaceae sensitizations grade 4+ prevailed (Fig. 4). Note that *Poa pratensis* had the highest relative rate of grade 4 reactions with respect to the sensitization rate.

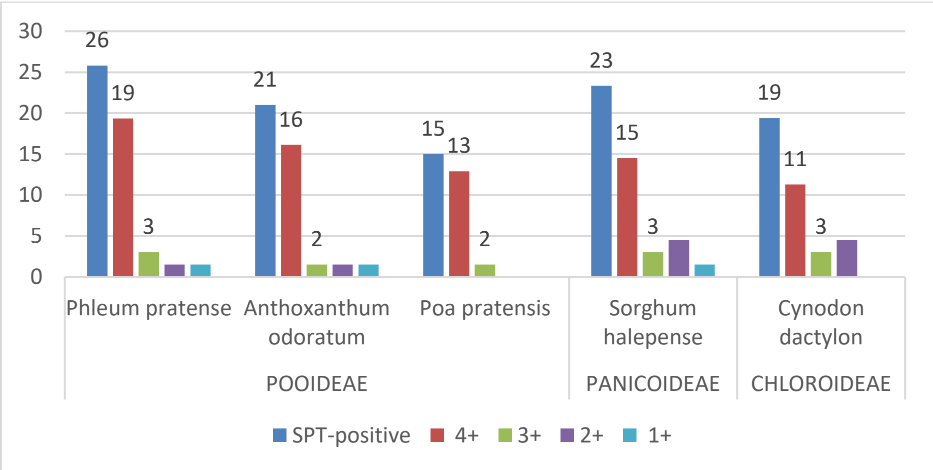

Fig. 4 Strength of reaction to Poaceae allergens across sensitized subjects (4+ the strongest, 1+ the weakest)

Tree allergen sensitization. *Fraxinus excelsior* was the main tree allergen (5 %), followed by *Corylus avellana*, *Olea europaea* and *Morus alba,* which sensitized each 6.7 % of the participants. *Juniperus ashei*, *Quercus rubra*, *Betula alba* and *Acer negundo* sensitized 3.3 %. One participant was polysensitized to all Fagales. Taxa from the Betulaceae, *Corylus*, *Betula* and *Alnus* reached a total sensitization rate of 11.7 %, the order Fagales 15 % (Tab. 4). Polysensitization within the order Fagales was significant (Tab. 6). We did not detect a significant correlation between *Olea* and *Fraxinus*. Tree taxa did not correlate with the total symptom score.

Tab. 6 Correlations of sensitizations to Fagales allergens

		Corylus	Betula	Alnus	Quercus
Corylus	r	1,000	.322*	.478**	.316*
Betula	r	.322*	1	.713**	.491**
Alnus	r	.478**	.713**	1,000	.701**
Quercus	r	.316*	.491**	.701**	1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

In Istanbul *Morus* trees are common and with an APIn of 169 able to sensitize 7 % of the atopic individuals in our sample. *Morus* was significantly correlated with genetically *Celtis occidentalis*, which are both located within the order Rosales (Zhang et al., 2011). Sensitizations further correlated significantly with other unrelated tree species and *Sorghum* (Tab. 7).

Tab. 7 Correlations of sensitizations to *Morus alba*

		Morus	Celtis	Populus	Quercus	Acer	Olea	Sorghum
Morus	r	1,000	.469**	.469**	.335**	.341**	.446**	.269*

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Weed allergen sensitization. *Rumex crispus* was the main sensitizer among weeds. 12.9 % of the participants reacted to it. The mean level of the wheal and flare reaction was 1.83. We detected a correlation with symptoms and problems related to the eyes ($p < 0.01$). *Rumex* sensitization was significantly correlated with grass sensitization (Tab. 8).

Tab. 8 Correlations of sensitizations to *Rumex crispus* and Poaceae allergens

	Rumex	Cynodon	Sorghum	Poa	Anthoxanthum	Phleum
Rumex R	1,000	.322*	.235	.367**	.278*	.428**

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

With respect to Asteroideae pollen allergens 5 % of the participants reacted to both *Artemisia vulgaris* and *Ambrosia artemisiifolia*, while 3.3 % reacted to *Xanthium strumarium*. None of the participants was polysensitized to *Ambrosia*, *Artemisia*, or *Xanthium*. *Xanthium* sensitization, however, correlated significantly with other weed species (Tab. 9). *Ambrosia* induced a grade 4, *Artemisia* a level 3 and *Xanthium* a grade 2 reaction. Asteraceae allergens did not correlate significantly with the total symptom score and neither with the total medical score. *Ambrosia* positive tested participants complained about eye and nose symptoms, though.

The sensitization rate to *Amaranthus retroflexus* (grade 1 and 2) was 3.2 %. It was the main sensitizer among the Amaranthaceae family. Only one person reacted to *Salsola kali* (grade 1), *Chenopodium album* (grade 1), and *Atriplex canescens* (grade 2). The remainder of weed allergens, *Plantago lanceolata* sensitized 3.4 % of the participants displaying a grade 1 reaction, while we did not detect any sensitization to *Parietaria judaica.* In terms of multiple sensitizations, we found a significant correlation between *Rumex*, Amaranthaceae, and *Xanthium*. Besides, *Plantago* sensitization correlated significantly with other weed species (Tab. 9).

Tab. 9 Correlations of sensitizations to weeds

		Artemisia	Salsola	Ambrosia	Chenopo-dium	Plantago	Xanthium	Amaran-thus	Rumex	Atriplex
Artemisia	r	1	-0,03	-0,053	-0,03	.376**	-0,043	-0,043	-0,09	-0,03
Chenopodium	r	-0,03	-0,017	-0,03	1	.689**	.689**	.689**	.293*	-0,017
Plantago	r	.376**	-0,024	-0,043	.689**	1	.466**	.466**	0,168	-0,024
Xanthium	r	-0,043	-0,024	-0,043	.689**	.466**	1	1.000**	.450**	-0,024
Amaranthus	r	-0,043	-0,024	-0,043	.689**	.466**	1.000**	1	.450**	-0,024
Rumex	r	-0,09	-0,051	-0,09	.293*	0,168	.450**	.450**	1	.337**

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Fungal sensitization: 3.2 % were sensitized to *Alternaria alternata*. Sensitization to *Alternaria* did not correlate with the total symptom score, only the correlation with the total nose score was a significant ($p < 0.05$).

Animal sensitization: American house dust mite (*Dermatophagoides farinae*) was the second most relevant allergen in the panel and sensitized 16.1 % of participants. *Dermatophagoides pteronyssinus*, the European dust mite, on the other hand, sensitized 12.9 %. Dust mite sensitization did not correlate with the symptom score, however there was a significant correlation ($p < 0.01$) of polysensitization between the two species ($r = 0.482$). Only one person reacted to cockroach and cat hair and no-one to dog hair.

1.4. Discussion

For the scope of this observational study to recommend a SPT panel to use in Istanbul and possibly Turkish Thrace, 34 allergens were tested on atopic adult patients. To estimate sensitization rates of inhalant allergens we used the ESP as reference and added species from grasses, woody plants, and weeds according to cross reactivity patterns and local relevance. We are aware that allergen extracts are biological mixtures of different macromolecules with characteristic epitopes, which may hinder exact comparison of results between studies using extracts from different manufacturers (Heinzerling et al., 2013). Yet, we can delineate sensitization trends with the proposed methodology, as this is the current practice until recombinant extracts will have replaced biological ones (Ansotegui et al., 2020).

The limitation of our work is the sample size. However, we found our results generally in alignment with larger studies in terms of prevalence of sensitization and symptom development.

Grass pollen sensitization and allergy. Grasses were the most important sensitizers and confirm grass pollen as leading allergens worldwide (García-Mozo, 2017). It was taken care to include representative allergens from three grass subfamilies – all found in the environs of Istanbul and its hinterland. Ersoy (2018) presented an inventory of 238 Poaceae species belonging to 92 genera for Istanbul through literature and field surveys. Among them we included Johnson grass (*Sorghum halepense*) and Bermuda grass (*Cynodon dactylon*) upon the assumption that they may extend the grass pollen season into autumn. The cross reactivity among grass species was observed in our study as expected with reference to the literature used (Ansotegui et al., 2020; Matricardi et al., 2016; Gangl et al., 2013; Weber, 2008, 2007). From the inclusion of several subfamilies of the Poaceae we understood that Panicoideae can be relevant sensitizers in the Istanbul region. Global warming might facilitate future relevance of invasive *Sorghum halepense* as an aeroallergen, as growth conditions will become suitable in higher latitudes (Follak & Essl, 2013). Most relevant in terms of sensitizations were taxa from the Pooideae subfamily, the ones comprised in the ESP (Heinzerling et al., 2013) and known for extensive cross reactivity (Matricardi et al., 2016). Hereby it proved to be meaningful to include *Phleum pratense*, the strongest sensitizer to which other grass allergens were strongly correlated and mostly associated with symptoms of rhino-conjunctivitis. It is the prevailing sensitizer among grass allergens in Europe (Matricardi et al., 2016). *Phleum pratense*, as reviewed in Gangl et al. (2013), could be used as single allergen in immunotherapy against Pooidae allergy. The genetic sequence of the major Phl p1 allergen from *Phleum pratense*, in fact, is reported to have 90 % homology to allergens from other Pooideae-subfamily species. Such homology is the source for cross-sensitization and explains the high prevalence of polysensitization among grass sensitized individuals (Matricardi et al., 2016; Gangl et al., 2013; Weber, 2008). *Sorghum halepense* showed to be a weaker sensitizer than Pooideae species. Yet, monosensitization to this grass species indicate the need for inclusion in test panels. *Cynodon dactylon* seemed to play a minor role in our study sample because we did not detect any monosensitized individuals to this grass. All in all, representative species from the Pooideae subfamily play a prominent role in grass pollen sensitization, as mostly *Poa pratensis* and *Phleum pratense* seem to be responsible for symptoms related to eyes and nose and the consumption of medication.

Analysing previous studies concerning our study area, the results regarding grass sensitization (18-24 %) are in alignment with figures reported in an epidemiological study on inhalant allergen sensitization using SPT performed by Aydin et al. (2009) on the Asian side of Istanbul, about 55 km ESE from our trap location. This retrospective study included 1500 patients suffering from allergic rhinitis in the district of Kartal, between 2002 and 2006. The panel consisted of 15 allergens, including a five grass-mix, a four cereal-mix, Betulaceae, Compositae (Asteraceae), fungal spores (*Alternaria* and others), mites (*Dermatophagoides farinae*, *D. pteronyssinus*), cat hair, dog hair, feather-mix, and cockroach. Among the pollen allergens the cereal-mix caused a skin reaction in 25.2 %, and the grasses-mix in 22.9 % of tested subjects. The study concludes cereal pollen to be the most important sensitizer in the atopic population of Istanbul. It is worthy to discuss also grass sensitization rates reported for Edirne, located in the Thrace region, where our study area stretches into. Yazicioglu et al. (2004) investigated sensitization trends in 539 children and adolescents suffering from allergic asthma, rhinitis or both living in rural and urban areas. The researchers used an extensive panel of single allergens, including comparable allergens to the ones used in this study. Since, to our knowledge, there are no other studies on sensitization rates in adults available for Thrace, we decided to discuss it here, although the subject matter were atopic children and young adolescents. Also, in that study cultivated wheat (*Triticum* vulgare) was reportedly the most significant allergen sensitizing 21.5 % of all participants. Yazicioglu et al. (2004) reported also the sensitization rate on maize (*Zea mays*) (11.3 %), a member of the Panicoideae subfamily. The range of sensitization rates of wild grasses was between 9.1 % (*Phleum pratense*) and 20.2 % (*Lolium perenne*). All in all, grass sensitization rates were slightly lower than in our study, but qualitatively the most important allergens.

Advancements in molecular allergology have shown that including cereals in test panels along with wild species is redundant. Cereals are wild species derived and, hence, carry homologue allergens (Gangl et al., 2013; Hrabina et al., 2008). Due to their polyploid nature, however, they may have increased allergen content and induce stronger reactions in sensitized patients. Most cereals produce lower pollen concentrations per anther, and larger pollen than anemophilous wild grass species due to self-pollination. In routine pollen monitoring cereal pollens appear rarely in the samples, as they do not become airborne easily due to their weight and size. In fact, cereal pollen mostly stays in the surroundings of the fields so that cereal allergy is considered as an occupational type of allergy, concerning mainly field workers who are in direct contact with this crop (Damialis & Konstantinou, 2011).

In the light of these deliberations, a grass panel to use in routine SPT testing in Istanbul should include at least *Phleum pratense* and possibly *Poa pratensis* from the Pooideae subfamily and *Sorghum halepense* from the Panicoideae subfamily; the latter, especially, if symptoms of late summer and autumn grass pollinosis occur.

Woody plant pollen sensitization and allergy: The most important sensitizer among tree pollen was *Fraxinus excelsior*, to which 8.3 % of our patients reacted. Exactly the same sensitization frequency was reported for Edirne (Yazicioglu et al., 2004). In 2015, *Fraxinus* pollen was airborne in low concentrations. The low APIn (91 pollen/m^3) at our location could be explained by annual fluctuations in pollen concentrations, due to masting phenomena (Tapper, 1992), which we can support with our own data (see Chapter 2). Moreover, concentrations at regional level may not reflect exposure at local level, because *Fraxinus* is a common tree in Istanbul's public space. Concentrations monitored in highly urbanized Central Istanbul, where *Fraxinus* was a main pollen contributor between spring 2005-2006 (Celenk et al., 2010) support our deliberations.

Within the Oleaceae, *Olea europapea* sensitized 6.7 % of our participants, while 8.9 % of children and adolescents were sensitized in Edirne (Yazicioglu et al., 2004). For Mediterranean areas *Olea europapea* is considered an important allergen and recommended in the ESP (Heinzerling et al., 2013). In Thessaloniki (Greece), for example, 26 % of patients (n = 168) with allergic rhinitis were sensitized to *Olea* (Katotomichelakis et al., 2015). Moreover, in Bursa, located about 110 km south east from our trap location across the Marmara Sea, *Olea* sensitization was reported to be

33.2 % in 545 adult patients diagnosed with asthma and or allergic rhinitis (Ediger et al., 2020). We explain lower sensitization rates in Istanbul and Edirne by the fact, that there are no olive cultivars in the area and, therefore, allergen exposure needed for sensitization is simply limited. As a matter of fact, *Olea* pollen concentrations in Bursa were reported to have an average APIn of 997 in two years of observations (Bicakci et al., 2003), which is four times higher than what we observed in Istanbul. We did not detect any co-sensitization between *Fraxinus excelsior* and *Olea europaea*, both members of the Oleaceae family, in spite of assumed cross-reactivity between both species due to homology of major allergens (Weber, 2007). However, we found multiple sensitizations between *Fraxinus* and *Corylus*. Cross-reactivity between those two species has been demonstrated in the literature (Weber, 2007).

Aydin et al. (2009) found that a Betulaceae mix (without specification of species included) sensitized 23.5 % of the atopic adult population in Asian Istanbul. However, in our investigation, only 12 % of participants were sensitized to Betulaceae pollen. This figure compares to results reported from Edirne, where 14.2 % of participants reacted to Betulaceae (*Corylus* and *Alnus*) allergens. Also, in this case, less allergen exposure compared to Asian Istanbul may play a role. We found significant correlations in sensitizations within all species of the order of Fagales (Tab. 6) indicating multiple sensitizations within this order with *Corylus avellana* as lead allergen. Correlations imply a cross-sensitizing action. Cross-reactivity between species of the Fagales order, and more prominently within Betulaceae members, is well known and linked to shared major allergens (Bet v 1) (Matricardi et al., 2016; D'Amato et al., 2007; Weber, 2007). The main pollen contributors from this order in our study area were *Quercus* and *Carpinus* (the latter not tested). This might explain why we detected important sensitization rates to *Corylus* and *Betula*, despite comparably low concentrations in the air.

Morus sensitization rates were comparable to *Corylus* and *Olea* and is possibly linked to a pan-allergen sensitization due to non-specific lipid transfer protein such as Mor n 3. Pan-allergens appear to be relevant in polysensitized patient profiles (McKenna et al., 2016). We found, in fact, *Morus* sensitization in connection with polysensitization to other mostly unrelated tree species and *Sorghum* (Tab. 7).

The sensitization rate of 3 % to *Juniperus ashei* (Cupressaceae) in our study is in alignment with findings on adult patients (n = 54) with seasonal allergic rhinitis in Ankara, of which 3.6 % were sensitized to *Cupressus* (Dursun et al., 2008). Again in Ankara, *Cupressus arizonica,* also a Cupressaceae member, sensitized 7.5 % (n = 350) of atopic children and adolescents (Cavkaytar et al., 2015). It is clear, that different allergen extracts tested on different study subjects lead to different results. We observed low sensitizing potential of this extract. Sensitization to *Juniperus ashei* was associated with polysensitization to weeds (*Amaranthus retroflexus* and *Xanthium strumarium*) and cat allergen Panallergen sensitization to Calcium binding proteins may play a role. The polcalcin Jun o 4 isolated from *Juniperus oxycedrus* occurs also in *Ambrosia artemisiifolia* (Amb a 9) and *Artemisia vulgaris* (Art v 5) (McKenna et al., 2016) to which *Xanthium strumarium* is related and cross reactivity is possible (Weber, 2007). Recently, a polcalcin (Ama r 3) has been also isolated from *Amaranthus retroflexus* (Moghaddam et al., 2019). A monosensitized patient to *Juniperus* claimed symptoms in eyes, nose and lungs, whereby, in this case, nose problems were most prominent. Rhinoconjunctivitis has been associated with Cupressaceae (*Cupressus sempervirens* and *Juniperus communis*) sensitization, and also co-sensitization with grass and tree allergens (Sposato & Scalese, 2013). The sensitization rate that we found mirrors the rate reported for the general European population (D'Amato et al., 2007).

Weed pollen sensitization and allergy. All *Rumex* sensitized individuals were polysensitized either to grasses, other weeds or cockroach except for one who reacted to *Juniperus*. All skin reactions to *Rumex* were weak. The results do not indicate a genuine sensitization but rather imply a pan-allergen sensitization. Specific *Rumex* allergens were not found in the WHO/IUIS allergen nomenclature database (http://www.allergen.org/). *R. acetosa* sensitization was previously reported by Yazicioglu et al. (2004) in 5.8 % of children with diagnosed allergic rhinitis or asthma. Cavkaytar et al. (2015) reported 4.4 % sensitization to *Rumex* in atopic children in Ankara, while Dursun et al. (2008) did not observe sensitization to this allergen in a sample of atopic adults. Airborne concentrations of *Rumex* were limited, which also supports the involvement of pan-allergens connected with *Rumex* sensitization.

All in all, we detected multiple sensitizations within all weed allergen extracts, regardless of inter-family relationships. *Amaranthus retroflexus* best represented the Amaranthaceae allergens, which is plausible also in terms of pollen exposure. Low skin reactions connected with this allergen imply that it is not a strong sensitizer and the association with symptom development is low.

Plantago pollen was airborne from spring till late autumn in moderate concentrations. Some species of this genus are ubiquitous. Sensitized individuals were not co-sensitized with grasses and no association with symptom development was found. Our sensitization rate for plantain (3.3 %) reflects the rates (3.5 %) reported by Şahiner et al. (2012) in atopic children. In central Northern-Europe plantain pollinosis appears to be more relevant (Matricardi et al., 2016) than in Turkey.

Parietaria judaica is known as important allergen in the Mediterranean and a significant contributor of allergenic pollen in our study area. As the English name wall pellitory implies, it grows extensively on old stone walls and represents the main species on Istanbul`s walls (Altay et al., 2010). However, we did not detect any sensitisation to *Parietaria*. In this case the sample size may have been too small to detect sensitized individuals. A problem with the allergen extract cannot be excluded. Sensitization in Thessaloniki was 24.4 % (Katotomichelakis et al., 2015). In Izmir, on the Turkish Aegean coast, on the other hand, prevalence reached 52 % (n = 131) in patients with a history of seasonal rhinitis and/or asthma (Terzioğlu et al., 1989). Yazicioglu et al. (2004) in Turkish Thrace found wall pellitory to sensitize 10.9 % of children in a rural and 1.9 % in an urban setting. In central Anatolia, on the other hand, the prevalence of *Parietaria officinalis* sensitization in an atopic adult population (n = 7492) was reported to be 2.4 % in a retrospective analysis (Comert et al., 2014). Due to proven evidence on the sensitization potential of *Parietaria* in the literature, this allergen could have relevance in this region, although we did not confirm it in our study.

Ambrosia was the strongest sensitizer among all tested weeds bearing the potential to become more relevant in Istanbul, if not managed. Known stands in Istanbul happened to lay within the study area (Ozaslan et al., 2016). Besides, there is evidence that *Ambrosia* pollen incurs through long distant transport from across the Black Sea, especially the Ukraine and the Crimean peninsula into the Marmara and the Black Sea region (Zemmer et al., 2012; Alan et al., 2019). *Ambrosia* sensitization rates in the Greek population are reported to be up to 14 % (Konstantinopoulos et al., 2014), but does not seem to play an important role in Thessaloniki (Katotomichelakis et al., 2015). The prevalence in susceptible children and adolescents in Ankara is 16 % (Cavkaytar et al., 2015). This is not surprising, because *Ambrosia* pollen has been reported form the 1990s with increasing concentrations over the years in Ankara. Already in 1999 airborne pollen concentrations were above 10 p/m^3 on 14 days, and 50 p/m^3 on two days (Kaplan et al., 2003). In Istanbul *Ambrosia* concentrations in 2015 were higher than 10 p/m^3 on 13 days and over 40 pollen/m^3 on four days. Our results prove that the allergic population of Istanbul has started to become sensitized to this neophyte. Thus, the prevalence of *Ambrosia* allergy in Istanbul is likely to increase, if the spread of this weed is not managed.

Artemisia is a weed allergen potentially cross-reacting with *Ambrosia*. Figures on sensitization rates in Turkey vary. So were 9.1 % of children with history of allergy sensitized to it in Edirne (Yazicioglu et al., 2004) and 6.7 % in Ankara (Comert et al., 2014). *Xanthium* yielded similar sensitization rates as *Artemisia*, but has shown to be connected with polysensitized individuals.

Alternaria sensitization was low in our study sample, yet there was some clinical indication with respect to nose symptoms connected to *Alternaria* sensitization. This spore type prevails in summer months when temperatures are high. Our results do not reflect the 30 % *Alternaria* sensitization reported by Aydin et al. (2009) for Istanbul. Fungal spore allergens may be used as in the ESP.

Animal dander and fur sensitization: Dust mites were a significant sensitizer. Sensitization rates, though, are smaller than results reported by Aydin et al. (2009) for Istanbul. Atopy including dust mite sensitization is bound to an array of factors, such as socioeconomical ones (Sipahi et al., 2017). We recruited our participants mainly on a university campus. Therefore, we assume they have a middle-high socio-economic background. Sensitization to cat or dog hair (actually their saliva) was negligible. Animal allergens may be used as in the ESP.

Impact of allergy: Sensitization indicates atopy and is a risk factor for allergic disease. When combined with data on symptoms, SPT can be associated with clinical relevance (Ansotegui et al., 2020). In our patient sample 65 % reacted positively to SPT, which is comparable to 60.9 % obtained in Istanbul by Aydin et al. (2009) on 1552 adults with allergic rhinitis, as well as to Pan-European figures (68.2 %) reported by Bousquet et al. (2009). We found a significant relationship between atopy and allergic complying with the results of a multi-centre-cross sectional study involving over 25.000 study subjects in Turkey (Kurt et al., 2009). In terms of allergens, grass pollen sensitization is linked the development of symptoms and the use of medication in our subjects mostly due to the development of nose and eye symptoms.

1.5 Conclusion

Positive and negative inclusion criteria for allergen testing panels is essential to make sure all allergens with sensitizing and pathogenic potential are selected. It is also important not to test for non-relevant extracts that have minimal, if any, clinical significance, especially those comprising mixture extracts. Our recommendation towards pollen allergens to use in Istanbul and possibly Turkish Thrace confirm the ESP with the following emphasis: three grass allergen extracts: *Phleum pratense, Poa pratensis* and *Sorghum halepense*; tree allergens: *Fraxinus excelsior*, *Corylus avellana*; weed allergens: *Ambrosia artemisiifolia, Artemisia vulgaris*. *Parietaria judaica* was not confirmed as sensitizing allergen in our sample but may be relavant at population level. *Rumex crispus* can be an indicator for pan-allergen sensitization. We recommend including *Ambrosia* in case of late summer hay fever and refrain from the use of cereal pollen extracts when testing patients with a diagnosis of grass pollinosis. It is further recommended to refer to local pollen calendars to facilitate an informed selection of potential allergens. Such finding is important in the light of costly population-based studies.

Chapter 2 The Pollen Season in Istanbul

This study is a comprehensive analysis of 3½ year aerobiological data from Büyükçekmece, Istanbul.

Research Paper

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The duration and severity of the allergenic pollen season in Istanbul, and the role of meteorological factors

Abstract

Information on the allergenic pollen season provides insight on the state of the environment of a region and facilitates allergy symptom management. We present a retrospective analysis of the duration and severity of the allergenic pollen season and the role of meteorological factors in Istanbul, Turkey.

Aerobiological sampling from January 2013 – June 2016, pollen identification and counting followed current standard methodology. Pollen seasons were defined according to 95 % of the Annual Pollen Integral (APIn) and the season start date was compared with the first day of five consecutive non-zero records. Generalized Additive Models (GAMs) were created to study the effect of meteorological factors on flowering.

Main pollen contributors were taxa of temperate and Mediterranean climates, and *Ambrosia*. Cupressaceae, Poaceae, Pinaceae, *Quercus* and *Ambrosia* had the greatest relative abundance. The pollen season defined on 95 % of the APIn was adequate for our location with total APIns around 10.000. Woody taxa had generally shorter seasons than herbaceous taxa. In trees, we see precipitation as the main limiting factor for assimilate production prior to anthesis. A severe tree pollen season in 2016 suggests intense synchronous flowering across taxa and populations triggered by favourable water supply in the preceding year.

GAM Models can explain the effect of weather on pollen concentrations during anthesis. Under the climatic conditions over the study period, temperature had a negative effect on spring flowering trees, and a positive one on summer flowering weeds. Humidity, atmospheric pressure and precipitation had a negative effect on weeds. Our findings contribute to environmental and allergological knowledge in Southern Europe and Turkey with relevancy in the assessment of impacts of climate change and the management of allergic disease.

Key words: Allergenic pollen seasons; Pollen severity; Allergy management; Generalized Additive Models; Meteorologicfal factors; Masting

2.1. INTRODUCTION

Information on allergenic pollen seasons and its severity provides insight on the state of the environment of a region and facilitates allergy symptom management (Karatzas et al., 2019; Gehrig et al., 2018). Both aspects are closely connected in aerobiological research. Climate change gives rise to increased respiratory allergy, due to its impact on the start and the duration of the pollen season, the flowering intensity, and the presence of allergen content (D'Amato et al., 2020; Eguiluz-Gracia et al., 2020; Damialis et al., 2019; Katelaris & Beggs, 2018; Jenerowicz et al., 2012). Furthermore, it supports health practitioners in the choice of allergen extracts to test (Zemmer et al., 2021; Ansotegui et al., 2020; Heinzerling et al., 2013), in the timing for implementation of clinical trials (Bastl et al., 2019; Pfaar et al., 2017) and in the sound interpretation of clinical results (Werchan et al., 2018). Pollen types detected in aerobiological sampling differ in their clinical relevance between regions (Karatzas et al., 2019; D'Amato et al., 2007). Within a region, relevance may also change over time. In Milan, for example, Cupressaceae only recently have become important with regard to allergy (Asero et al., 2020). Regular updates on local pollen information are, thus, essential (Ansotegui et al., 2020). In the interpretation of pollen information cross-reactivity patterns of taxa must be considered (Ansotegui et al., 2020). Some of the major proteins causing allergy are panallergens, common to many flowering plants (McKenna et al., 2016). The major birch allergen Bet v 1, for example, belonging to the protein family PR10, has homologues in *Corylus* (Cor a 1) and *Alnus* (Aln g 1) within the families Betulaceae and Fagaceae, for instance *Fagus* (Fag s 1) in the order Fagales (Matricardi et al., 2016; Weber, 2008) and also in non-related taxa, such as *Malus domestica* (Mal d 1) (McKenna et al., 2016). Therefore, when presenting pollen information, you can address clinical needs by grouping taxa above species, genus, and even family level (Hoffmann et al., 2020).

In environmental terms, the local pollen spectrum depends on natural vegetation, land use, ornamental flora in green urban spaces, and ruderal urban flora (Werchan et al., 2018; Monroy-Colín et al., 2018; McInnes et al., 2017; Tosunoglu et al., 2015; Carinanos et al., 2010; Fernández-Rodríguez et al., 2014; Çeter et al., 2012), as well as on prevailing air currents (Alan et al., 2019; Rojo et al., 2015; Damialis et al., 2005). It can reveal the arrival of invasive plants, such as ragweed, when the presence of local sources is not yet known (Zemmer et al., 2012). Meteorological factors influence the flowering phenology, and, thus, the duration and intensity of the pollen flow in anemophilous species (Dahl et al., 2013). Accumulated temperature during the period when flowers or male catkins are initiated and formed can affect the amount of pollen released during flowering (Bogdziewicz et al., 2017; Dahl & Strandhede, 1996). Air humidity impacts pollen release. Dry air favours anther dehiscence in many anemophilous species (Dahl et al., 2013; Martin et al., 2010; Pacini, 2000; Bianchi et al., 1959). When air is humid, pollen grains absorb water and get heavier; some types like grass pollen will even burst as they cannot adjust to humidity (Dahl et al., 2013; Pacini, 2000). In dryer climates, water availability is a limiting factor affecting plant biomass accumulation (Szymczak et al., 2020) and flowering intensity (Velasco-Jiménez et al., 2020; Galán et al., 2016; García-Mozo et al., 2010; Peñuelas et al., 2004). The Mediterranean region including Southern Europe will face increased arid conditions due to climate change (Hoegh-Guldberg et al., 2018). In Istanbul this trend has become evident in the shift from a moist-subhumid towards dry subhumid climate during the last twenty years (Turoğlu, 2014). The result may be spatial and temporal shifts of allergenic pollen sources (Damialis et al., 2019). Reports on phenology and flowering intensity of wind pollinated taxa of a region provide, therefore, important information on environmental changes over time (Velasco-Jiménez et al., 2020; Galán et al., 2016).

In this paper we analyse atmospheric pollen monitored in Istanbul from the environmental perspective. The aims are to i) define pollen seasons ii) report on the intensity of pollen concentrations iii) and on the effect of meteorological factors on the main taxa.

2.2. Methodology

Study area

The monitoring was performed in Büyükçekmece at the western fringes of Greater Istanbul. The climate of Istanbul corresponds to the Köppen Geiger Csa-type (Turoğlu, 2014) that is somewhat between temperate and Mediterranean (Climate-Data.org, 2020). The Bosporus and the south of the city are influenced by the Marmara Sea, while the climate in the northern and western hinterland is more continental, as it is influenced by the Black Sea and the Balkans. The study area is, thus, characterized by a transitional climate from cool and wet conditions northwards into the Istranca mountain range, to a dry and warm situation in the south and around Bosporus (Baser, 2011; Fig. 1). The topography is hilly and the highest elevation is about 300 m above sea level. North-easterly winds prevail. The vegetation changes according to the climatic north-south gradient. In plant geographical terms, two floral regions blend here into one another: the South-Eurosiberian and the Mediterranean (Schroeder, 1998). Most of the wood cover is situated upwind the trap in the Belgrad Forest, and features the following wind-pollinated deciduous thermophilus trees: *Quercus frainetto*, *Q. petrea*, *Q. robur*, *Q. cerris*, *Fagus orientalis*, *Castanea sativa*, *Corylus avellana*, *Carpinus betulus*, *C. orientalis*, *Alnus glutinosa*, *Fraxinus angustifolia*, *F. ornus*, *Populus tremula*, *Erica arborea*, *E. manipuliflora, Ulmus minor, Salix caprea* and *S. cinerea* (Çoban et al., 2016; Baser, 2011; Kavgaci et al., 2010). There are also areas of *Pinus nigra* plantations. Amidst patches of pseudomaquis with *Quercus coccifera*, *Juniperus communis*, *Paliurus spina-christi* and *Pistacia terebinthus*, grasslands turning dry in summer, and agricultural lands, villages emerge. The agricultural land is used for the growth of annual crops (sunflower, canola, cereals). The area has four watersheds, some of them functioning as fresh water reserves of the city with restrictions on urban development. Anemophilous urban flora on walls, wastelands and coasts feature herbaceous plants pertaining to Poaceae, for instance *Digitaria sanguinalis*, *Setaria* ssp., *Echinochloa* ssp., *Sorghum halepense*, Asteraceae, for instance *Artemisia vulgaris*, *A. annua*, *A. absinthium*, *Xanthium spinosum*, *X. strumarium*, Urticaceae, for instance *Urtica dioica*, *U. membranacea*, *U. pilulifera*, *U. urens*, *Parietaria judaica*, Amaranthaceae, for instance *Amaranthus* ssp., *Chenopodium* ssp., *Atriplex* ssp., *Salicornia* ssp., Polygonaceae (*Rumex* ssp.), Plantaginaceae, for instance, *Plantago lanceolata*, *P. major*, *P. coronopus*, *P. afra* (Bakis et al., 2020; Altay et al., 2010). Common anemophilous ornamental trees are *Cupressus sempervirens*, *Cedrus libani*, *Plantanus x acerifolia*, *P. orientalis*, *Thuja orientalis*, *Morus ssp.*, *Salix babylonica*, *Fraxinus angustifolia*, *Ulmus* ssp., *Celtis australis*, *Ailanthus altissima*, *Acer negundo*, *Pinus* ssp. (Baser, 2011) and *Olea europaea.*

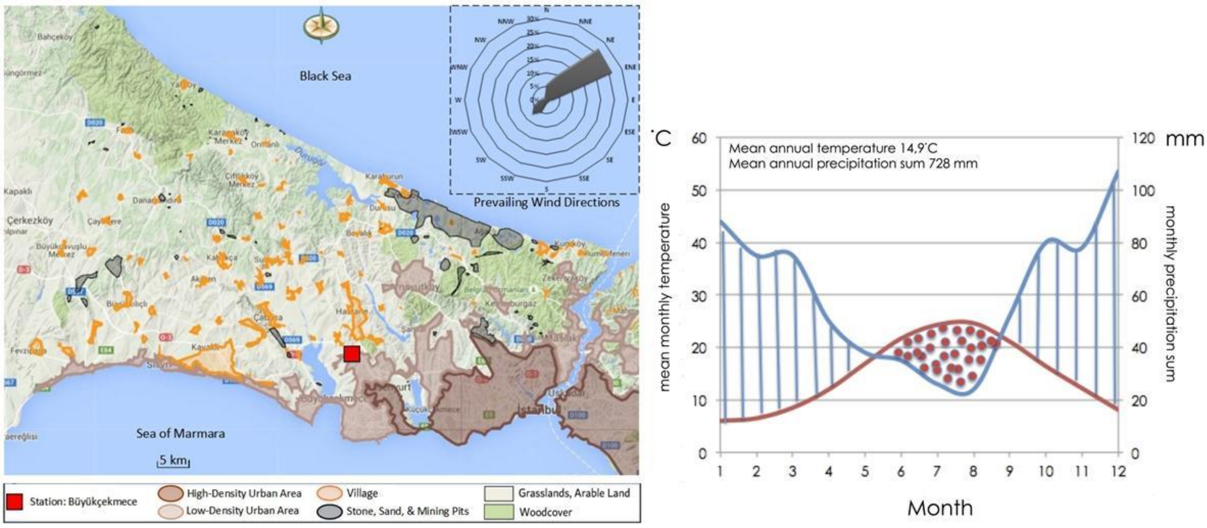

Fig. 1 Study area (left) and climate diagram of Istanbul (right). The striped areas in the climate diagram denote humid conditions, and the dotted areas show when the conditions are arid (constructed according to Walter & Lieth, 1960).

POLLEN MONITORING

Pollen data include a 3 ½ year period from 28th January 2013 to 26th June 2016 according to the current standard methodology with a 7-day volumetric Hirst-type pollen sampler (Hirst, 1952). Pollen monitoring, identification and counting followed the minimum requirements of the European Society of Aerobiology (EAS) (Galán et al., 2014) and normed by EN 16868 (2019). Daily mean pollen concentrations are expressed in pollen/m^3 air.

On 21st April 2014 the position of the trap had to be moved from 3 m to 10 m height (ca. 200 m ENE from the original position) due to a construction project. We did not alter the master data set for the period prior to the change, based on findings that the height of the trap does not influence the time series significantly (Rojo et al., 2019; Fernández-Rodríguez et al., 2014; Galán et al., 1995). We verified this assumption following the theory that most pollen is shed in the vicinity of the trap (Adams-Groom et al., 2017). To do so, we studied the distribution of pollen concentrations of anemophilous plants growing in the immediate surrounding of the trap (Poaceae and *Plantago*) and of *Olea* and Pinaceae growing at about 40 m distance. These taxa had conspicuously higher Seasonal Pollen Integrals (SPIns) and peak values in 2013 compared to the following years studied but other spring flowering taxa had not. We chose to compare the pollen concentrations during their peak flowering period in May (Fig. 2), which, in case of *Plantago,* coincided with the pre-peak period (Tab. 2 and 3).

Meteorological data

Meteorological data on temperature, humidity and pressure were obtained from the open source www.weatherunderground.com, recorded at 41.14 °N, 28.46 °E Istanbul Hezarfen Airfield Station located 6 km northwest of the trap location. Precipitation data was purchased from the State Meteorological Service (www.mgm.gov.tr). We tested the effect of maximum (Tmax) and minimum daily temperature (Tmin), mean daily humidity (HumMean), mean daily pressure (PressMean) and precipitation (Prec) on selected pollen taxa (Tab. 1). Precipitation of the year 2012 was included to study the effect of this factor on trees a year ahead of anthesis when flowers are initiated (Dahl & Strandhede, 1996).

Tab. 1 Meteorological factors recorded during the study period. Precipitation data of 2012 were included for the interpretation of the effects on the flowering of woody taxa.

	Parameter/Month	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Annual Precipitation**
2012	Precipitation (mm)	41	111	40	115	54	27	2	46	18	33	40	131	658
2013	Max. mean temperature (°C)	10	12	14	18	25	27	30	31	26	19	14	10	
	Min. mean temperature (°C)	4	6	7	10	16	20	22	23	18	6	11	4	
	Mean rel. humidity (%)	76	74	68	66	63	60	54	59	54	73	73	72	
	Mean pressure (hPa)	1021	1012	1009	1012	1009	1009	1010	1010	1011	1012	1016	1025	
	Precipitation (mm)	94	68	66	32	21	22	7	11	8	52	62	50	494
2014	Max. mean temperature (°C)	12	12	15	19	22	26	30	30	25	20	15	13	
	Min. mean temperature (°C)	6	6	7	11	15	19	22	23	19	14	10	8	
	Mean rel. humidity (%)	80	78	71	71	70	68	63	65	68	78	75	81	
	Mean pressure (hPa)	1015	1016	1012	1010	1009	1010	1008	1008	1010	1014	1017	1115	
	Precipitation (mm)	41	24	73	44	77	64	12	72	122	40	71	97	736
2015	Max. mean temperature (°C)	10	10	12	15	23	26	30	31	27	20	17	11	
	Min. mean temperature (°C)	5	5	7	8	15	19	22	24	21	15	12	6	
	Mean rel. humidity (%)	72	76	71	66	64	66	59	60	68	72	72	72	
	Mean pressure (hPa)	1015	1028	1016	1014	1010	1010	1003	1010	1011	1016	1016	1026	
	Precipitation (mm)	108	153	41	82	5	25	1	3	56	61	51	12	596
2016	Max. mean temperature (°C)	9	13	14	20	22	28							
	Min. mean temperature (°C)	4	8	8	12	15	21							
	Mean rel. humidiy (%)	74	74	68	59	65	60							
	Mean pressure (hPa)	1013	1016	1011	1010	1009	1009							
	Precipitation (mm)	158	78	86	29	42	24							417 (½ year)

DATA ANALYSIS

The dataset was homogenised to obtain equally 31 taxa for all years by removing pollen taxa with negligible Annual Pollen Integral (APIn) (Arecaceae, *Mercurialis, Typha, Iva*). Single pollen out of season attributed to resuspension or flowering out of season were also removed. Next, a quality check was performed to identify missing data. In 2013 and 2015 sampling was discontinued during January for technical reasons. We interpolated the missing data with the moving mean function, because it adapted better to our data than linear regression did in preliminary tests. We confirm that for aerobiological data, the moving mean interpolation performs better as compared to other methods as suggested by Picornell et al. (2021).

As our data comprise less than five years, we present the weekly average of our studied years, not considering this presentation a pollen calendar (Galán et al., 2017). For this figure, we chose a scale of 10 colour gradients from light to dark: light colour standing for low and dark for higher pollen concentrations.

We used descriptive statistics for the analysis of phenology, represented by the timing of pollen season, and the flowering intensity, represented by the SPIn of single taxa. APIns of each year studied are provided. For the retrospective analysis of our aerobiological data the percent method was deemed appropriate (Bastl et al., 2018). The pollen seasons were, thus, defined according to the 95 % method (Andersen, 1991): the season starts when 2.5 % of the APIn is reached and ends on the day with 97.5% of the APIn. The result thereof can be used to calculate SPIn. To assess the validity of this method for the season start, the first day of five non-zero records was determined for the main taxa and compared with the start day at 2.5% of the APIn. The standard deviations of the differences in days between the season start days of both methods were calculated. Aerobiological data were mainly analysed with the computational tool for aerobiological data AeRobiology package (Rojo, et al., 2019) in RStudio version 3.6.3.

To analyse the effect of meteorological factors on pollen seasons, we run Generalized additive models (GAMs) based on the assumption of a non-linear relationship between meteorological factors and pollen concentrations. These models estimate the effect on the change of pollen concentrations with each added unit of independent variable

(Ravindra et al., 2019). The model can automatically fit non-linear dependencies and is adequate for short term and long-term time series (Ravindra et al., 2019). For the model we used the quasi-Poisson function and a spline function on time. GAMs were created with the nlme package (Pinheiro et al., 2021) in R.

2.3. RESULTS

TRAP POSITION CHANGE

Herbaceous Poaceae and *Plantago* displayed pronouncedly higher pollen concentrations in May 2013 compared to the other years, and peak concentrations were over three times higher. In woody *Olea* and Pinaceae, the bulk of the data was similar over the studied years. However, peak concentrations were twice higher in 2013 than in the other years. Effects on pollen intensities of other taxa were mostly negligible (compare SPIn and peak values in Tab. 2 and 3). When reading diagrams this bias should be considered.

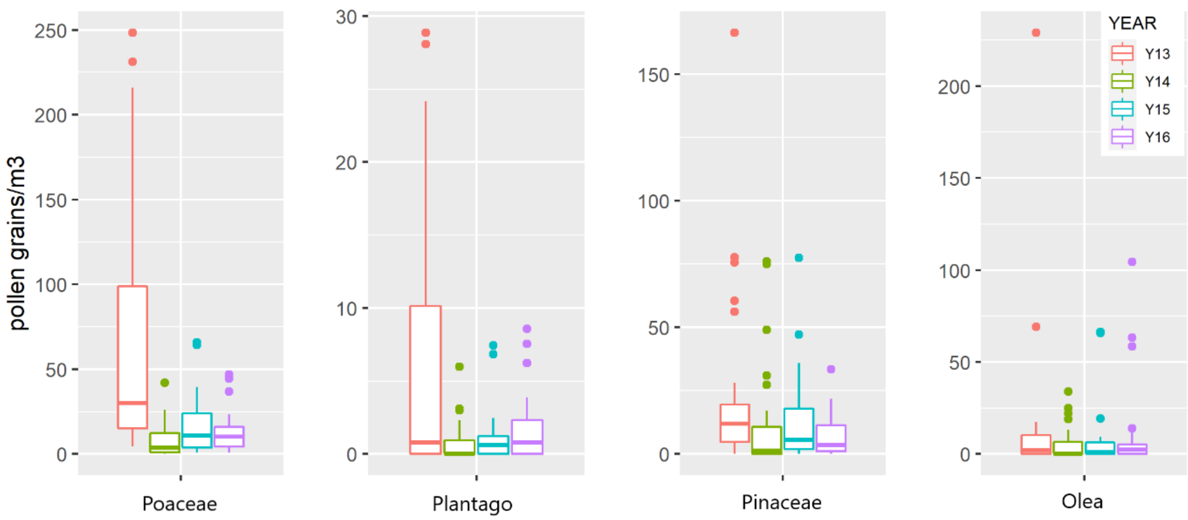

Fig. 2 Pollen concentrations of taxa growing in the surrounding of the trap during May in a four-year comparison. The boxes include 50 % of the data, the interquartile range (IQR) between the first quartile (Q1) and the third quartile (Q3); the line in the box is the median, the whiskers show the data range (Q1 – 1.5* IQR, Q3 + 1.5*IQR); dots are outliers and indicate peak concentrations

FLOWERING PHENOLOGY

We calculated numerical details on seasonal parameters of 31 woody and herbaceous taxa (Tab. 2 and 3).

The start of the pollen seasons reflecting the flowering of anemophilous plants varied between years (Fig. 3, Tab. 2 and 3). The longest seasons were generally observed in herbaceous plants, i.e., Urticaceae (> 60 days difference between years), Amaranthaceae and Poaceae, but also in *Carpinus* (about 50 days difference between years). Variations for the end date of the seasons was pronounced in herbaceous *Plantago*, Amaranthaceae and Urticaceae, and for some trees, especially in Pinaceae (over 100 days difference between years), *Carpinus*, and to a lesser extent in *Fraxinus*. The differences in the season start dates were low also for *Olea* (except in 2016) and *Betula* (except in 2013).

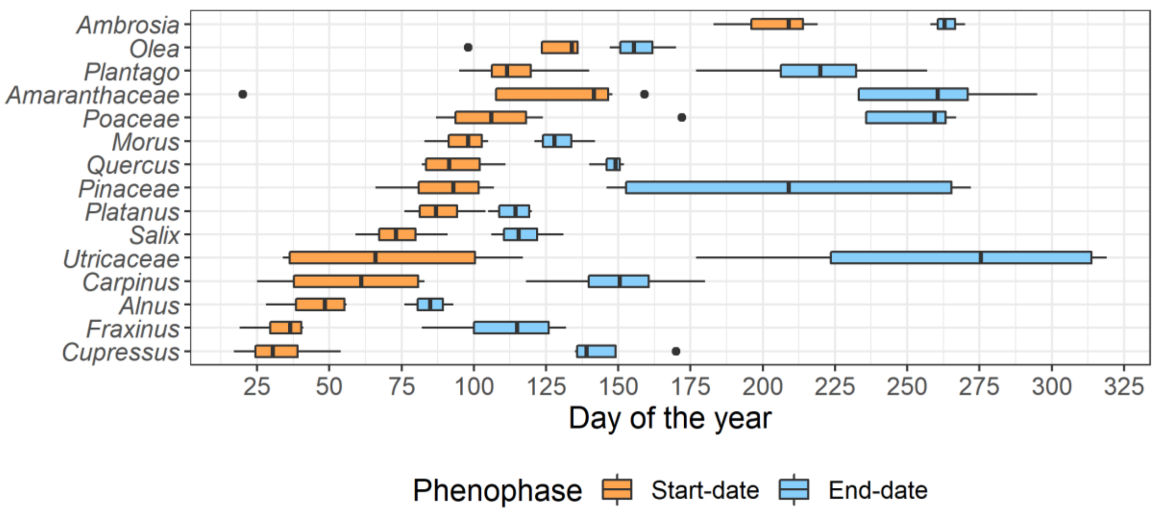

Fig. 3 Variability in the pollen season start and end dates of 15 main pollen taxa monitored in Büyükçekmece between January 2013 and June 2016

Tab. 2 Annual pollen seasons (PS), start and end date, duration, peak value and day and the Seasonal Pollen Integral (SPIn) of woody taxa. For main pollen contributing taxa the first day of 5 consecutive non-zero records, the days difference between the methods, and the standard deviation (Sd) of four seasons. Empty cells imply not applicable calculations or data deficiency.

Taxon	Year	95% PS start	PS end	Duration [days]	SPIn	Peak Value	Peak Date	1st day of 5 non-zero record date	Sd	Days difference
Pinaceae	2013	10/04/2010	29/09/2013	173	963	166	22/05/2013	10/04/2013	2.7	0
	2014	27/03/2014	20/09/2014	178	581	76	14/05/2014	04/04/2014		8
	2015	17/04/2015	04/06/2015	49	836	99	24/04/2015	21/04/2015		4
	2016	06/03/2016	25/05/2016	81	1100	124	03/04/2016	13/03/2016		6
Salix	2013	11/03/2013	22/04/2013	43	208	56	13/04/2013	01/04/2013	7.6	21
	2014	17/03/2014	29/04/2014	44	321	41	24/03/2014	17/03/2014		0
	2015	01/04/2015	11/05/2015	41	92	11	30/04/2015	14/04/2015		13
	2016	28/02/2016	15/04/2016	48	104	21	28/03/2016	14/03/2016		15
Ulmaceae	2013	08/03/2013	31/03/2013	24	34	11	19/03/2013			
	2014	19/01/2014	28/03/2014	69	94	11	12/02/2014			
	2015	24/02/2015	08/04/2015	44	9	2	26/02/2015			
	2016	04/02/2016	17/03/2016	43	25	4	11/02/2016			
Populus	2013	07/03/2013	23/04/2013	48	54	9	31/03/2013			
	2014	04/03/2014	11/04/2014	39	48	11	21/03/2014			
	2015	23/03/2015	14/04/2015	23	34	19	14/04/2015			
	2016	27/02/2016	30/03/2016	33	38	10	28/03/2016			
Corylus	2013	02/02/2013	21/03/2013	48	30	8	13/02/2013			
	2014	16/01/2014	01/04/2014	76	67	9	16/02/2014	22/01/2014		6
	2015	12/02/2015	26/03/2015	43	33	5	05/03/2015			
	2016	24/01/2016	07/03/2016	44	20	4	11/02/2016			

Taxon	Year	95% PS start	PS end	Duration [days]	SPIn	Peak Value	Peak Date	1st day of 5 non-zero record date	Sd	Days difference
Alnus	2013	25/02/2013	03/04/2013	38	76	13	09/03/2013	09/03/2013	6.6	12
	2014	28/01/2014	29/03/2014	61	223	76	12/03/2014	24/01/2014		-4
	2015	24/02/2015	23/03/2015	28	60	7	25/02/2015	01/03/2015		5
	2016	11/02/2016	16/03/2016	35	75	12	20/02/2016	23/02/2016		12
Olea	2013	16/05/2013	19/06/2013	35	431	229	22/05/2013	13/05/2013	12.2	-3
	2014	12/05/2014	01/06/2014	21	159	34	15/05/2014	12/05/2014		0
	2015	16/05/2015	08/06/2015	24	225	66	27/05/2015	13/05/2015		-3
	2016	08/04/2016	26/05/2016	49	392	105	09/05/2016	04/05/2016		26
Quercus	2013	25/03/2013	28/05/2013	65	275	48	29/04/2013	05/05/2013	18.7	41
	2014	09/04/2014	01/06/2014	54	209	26	14/05/2014	15/04/2014		36
	2015	21/04/2015	30/05/2015	40	627	62	29/04/2015	16/04/2015		25
	2016	22/03/2016	19/05/2016	59	1516	232	04/04/2016	15/03/2016		-7
Platanus	2013	01/04/2013	30/04/2013	30	116	17	10/04/2013	01/04/2013	3.8	0
	2014	24/03/2014	20/04/2014	28	160	24	24/03/2014	01/04/2014		8
	2015	14/04/2015	29/04/2015	16	342	80	26/04/2015	14/04/2015		0
	2016	16/03/2016	14/04/2016	30	773	108	05/04/2016	14/03/2016		-2
Fraxinus	2013	02/02/2013	23/03/2013	50	228	19	21/03/2013	06/02/2013	2.5	0
	2014	19/01/2014	04/05/2014	106	206	16	23/04/2014	22/01/2014		3
	2015	09/02/2015	12/05/2015	93	89	7	10/02/2015			
	2016	10/02/2016	15/04/2016	66	528	70	01/03/2016	08/02/2016		-2

Taxon	Year	95% PS start	PS end	Duration [days]	SPIn	Peak Value	Peak Date	1st day of 5 non-zero record date	Sd	Days difference
Carpinus	2013	21/03/2013	27/05/2013	68	40	6	10/04/2013	NA		
	2014	25/01/2014	29/06/2014	156	57	8	08/04/2014	24/01/2014	9.5	-1
	2015	24/03/2015	03/06/2015	72	234	66	14/05/2015	13/04/2015		20
	2016	11/02/2016	27/04/2016	77	429	126	27/03/2016	29/02/2016		18
Cupressaceae	2013	03/02/2013	19/06/2013	137	2274	309	13/03/2013	31/01/2013		-3
	2014	17/01/2014	16/05/2014	120	2180	183	19/01/2014	12/01/2014	0.8	-5
	2015	23/02/2015	22/05/2015	89	1760	422	26/02/2015			
	2016	27/01/2016	14/05/2016	109	3471	729	19/02/2016	23/01/2016		-4
Betula	2013	01/04/2013	09/05/2013	39	75	11	01/05/2013	29/04/2013		28
	2014	27/03/2014	18/05/2014	53	37	6	09/04/2014	01/04/2014	9.9	5
	2015	09/04/2015	06/05/2015	28	19	4	14/04/2015	13/04/2015		4
	2016	20/03/2016	13/05/2016	55	109	20	28/03/2016	27/03/2016		7
Ericaceae	2013	10/03/2013	05/11/2013	241	26	4	10/03/2013			
	2014	15/03/2014	03/10/2014	203	24	5	24/03/2014			
	2015	05/03/2015	14/09/2015	194	31	4	25/03/2015			
	2016	23/02/2016				6	17/03/2016			
Celtis	2013	18/03/2013	27/04/2013	41	20	6	03/04/2013			
	2014									
	2015	21/02/2015	21/03/2015	29	2	1	21/03/2015			
	2016	14/03/2016	17/03/2016	4	7	4	16/03/2016			
Moraceae	2013	12/04/2013	11/05/2013	30	119	24	06/05/2013	01/05/2013		19
	2014	24/03/2014	05/05/2014	43	44	7	24/03/2014		6.2	
	2015	15/04/2015	22/05/2015	38	165	17	29/04/2015	20/04/2015		5
	2016	03/04/2016	30/04/2016	28	169	29	04/04/2016	10/04/2016		7

Tab. 3 Annual pollen seasons (PS), start and end date, duration, peak value and day and the Seasonal Pollen Integral (SPIn) of herbaceous taxa. For main pollen contributing taxa the first day of 5 consecutive non-zero records, the days difference between the methods, and the standard deviation (Sd) of four seasons. Empty cells imply not applicable calculations or data deficiency.

Taxon	Year	PS start	PS end	Duration	SPIn	Peak Value	Peak Date	1st day of 5 non-zero record date	Sd	Days difference
Poaceae	2013	4/5/13	14/9/13	134	2426	248	21/5/13	1/5/13	2.3	1
	2014	6/4/14	24/9/14	172	482	42	12/5/14	6/4/14		0
	2015	26/4/15	19/9/15	147	869	66	22/5/15	21/4/15		-5
	2016	27/3/16				47	9/5/16	25/3/16		-2
Atremisia	2013	31/7/13	28/9/13	60	107	12	1/8/13			
	2014	13/7/14	8/11/14	119	45	3	10/8/14			
	2015	15/7/15	3/10/15	81	78	7	27/8/15			
	2016									
Amaranthaceae	2013	26/5/13	15/9/13	113	202	28	9/8/13			
	2014	17/5/14	22/10/14	159	93	6	5/9/14			
	2015	28/5/15	20/9/15	116	247	31	27/8/15	24/7/15		57
	2016									
Rumex	2013	16/4/13	25/5/13	40	14	4	7/5/13			
	2014	9/4/14	13/7/14	96	35	6	31/5/14			
	2015	17/4/15	21/7/15	96	45	7	17/5/15			
	2016									
Ambrosia	2013	3/7/13	15/9/13	75	471	51	19/8/13	6/8/13		35
	2014	28/7/14	20/9/14	55	539	89	5/9/14	9/8/14	14.9	12
	2015	7/8/15	27/9/15	52	600	138	31/8/15	6/8/15		-1
	2016									
Plantago	2013	20/5/13	4/8/13	77	209	29	23/5/13	20/5/13		0
	2014	20/4/14	14/9/14	148	97	7	20/6/14	NA	0.5	
	2015	23/4/15	12/8/15	112	108	8	6/6/15	22/4/15		-1
	2016									

Taxon	Year	PS start	PS end	Duration	SPIn	Peak Value	Peak Date	1st day of 5 non-zero record date	Sd	Days difference
Apiaceae	2013	9/5/13	8/9/13	123	27	6	23/5/13			
	2014	12/5/14	14/8/14	95	28	2	26/5/14			
	2015	19/5/15	31/8/15	105	81	10	9/7/15			
	2016	28/4/16	25/6/16	59	86	11	20/5/16			
Urticaceae	2013	27/4/13	15/11/13	203	179	22	13/8/13	31/7/13	49.9	95
	2014	3/2/14	8/11/14	279	281	12	23/7/14	6/6/14		123
	2015	5/4/15	27/8/15	145	280	18	31/5/15	17/5/15		42
	2016	6/2/16			247	16	19/6/16	30/1/16		-7
Xanthium	2013	1/8/13	22/9/13	53	43	7	1/9/13			
	2014	5/8/14	23/9/14	50	34	7	2/9/14			
	2015	10/8/15	13/9/15	35	28	4	23/8/15			
	2016									
Cannabaceae	2013	1/8/13	15/9/13	46	68	9	2/8/13			
	2014	23/7/14	10/9/14	50	32	4	3/9/14			
	2015	17/7/15	4/9/15	50	27	3	15/8/15			
	2016									
Brassicaceae	2013	25/9/13	25/9/13	1	1	1	25/9/13			
	2014	5/4/14	19/9/14	168	44	7	13/5/14			
	2015	3/4/15	18/7/15	107	25	4	27/4/15			
	2016	4/4/16			95	21	15/4/16			

We compared the 95 % method to define the season start with the date of the first day of five non-zero records for the main pollen contributing taxa to assess the feasibility of this method at our location. Cupressaceae, Pinaceae, *Fraxinus*, *Platanus*, Poaceae, and *Plantago* had a standard deviation <5 in the start dates of the season during the study period.

Pollen intensity

The relative abundance of 15 main pollen types based on their APIn (Rojo et al., 2019) is reported in Fig. 4. The summarization of Betulaceae and Fagaceae in the analysis is made to follow the rationale of cross-reactions in the Fagales order (Matricardi et al., 2016). *Alnus* and *Carpinus* were the main pollen contributors within Betulaceae and *Quercus* in the Fagaceae.

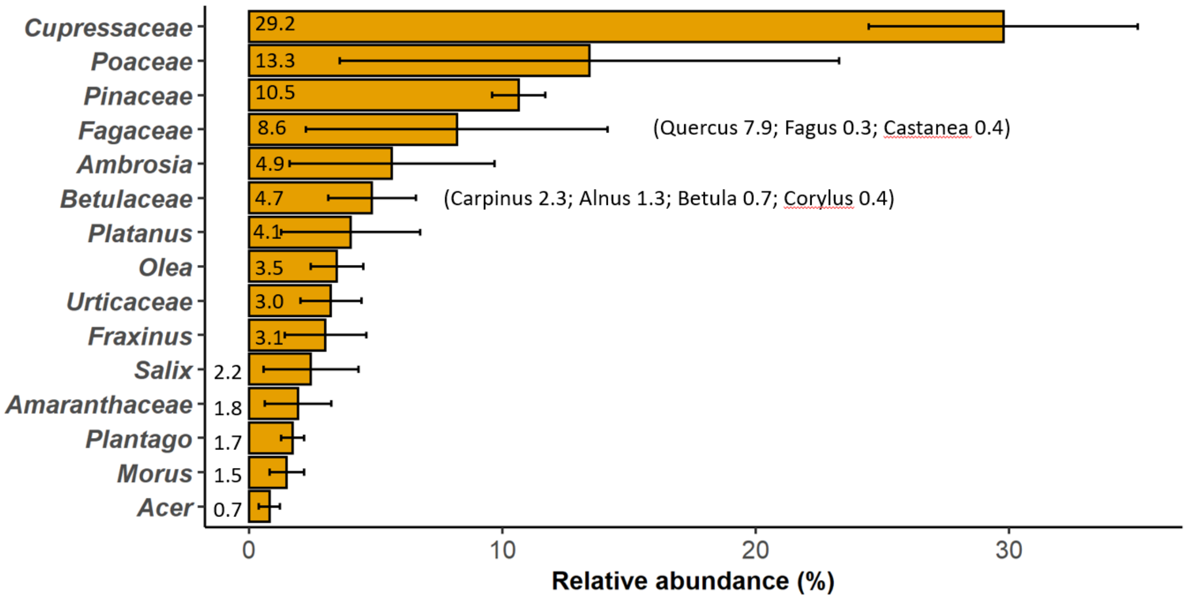

Fig. 4 Top: Relative abundance of the 15 main pollen taxa during the study period based on APIns. The error bars reflect the annual variability. *Alnus*, *Corylus*, *Carpinus* and *Betula* are grouped into Betulaceae; *Quercus*, *Fagus* and *Castanea* into Fagaceae

The details on the phenology and relative abundancies of all taxa pertaining to the Fagales including *Corylus*, *Fagus* and *Castanea sativa,* all three with < 0.5 % of the APIn, are shown as violin plots in Fig. 5. Violin plots can show the entire distribution of the data emphasising peak periods. Wider parts in the plot contain observations with higher density than in thinner parts (Hintze & Nelson, 1998). *Corylus*, *Quercus* and *Castanea* show a shorter pre-peak period than *Alnus*, which can be inferred from the positive skewness of the distribution. *Alnus* displays a short and intense flowering period. *Fagus* and *Betula* show a more uniform distribution without a pronounced peak.

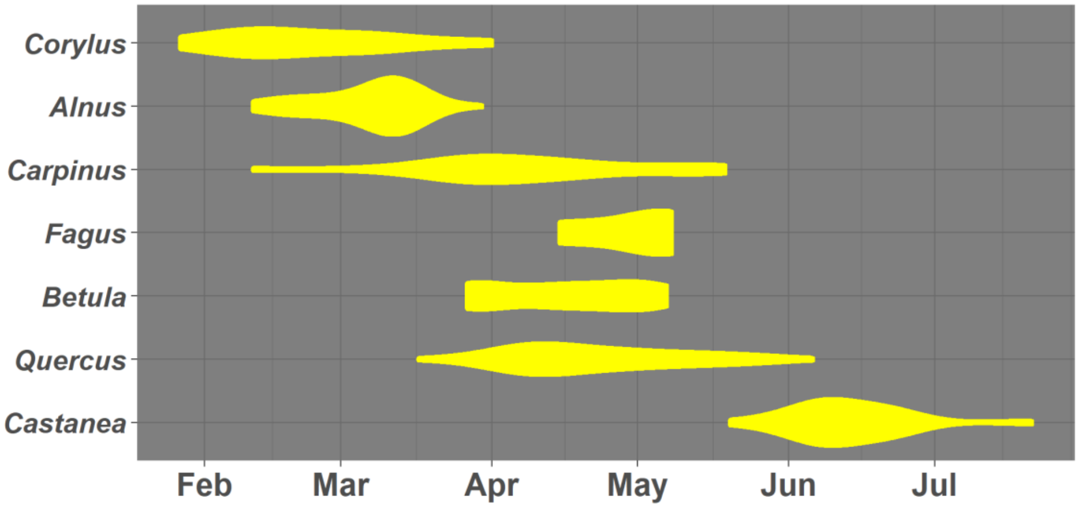

Fig. 5 The phenology and relative intensity of Fagales pollen taxa (January 2013- June 2016) calculated on the base of the density of daily mean pollen concentration records of each taxon without scales and units

Variation in the interannual flowering intensity was pronounced for most species. We observed remarkable differences in the SPIn of single years (Tab. 2 and 3) and Fig. 4. In 2013, the SPIn of Poaceae was about four times and of *Plantago* two times higher, than the mean concentrations of the other three years observed (see pollen curves in Annex II). *Olea* had the highest SPIn and peak value in 2013. Comparably higher SPIns and peak concentrations were observed also in *Salix* both in 2013 and 2014. In 2014, the SPIn of *Corylus* and *Alnus* was higher (more than two times higher, and three times higher than the mean). The SPIn of *Carpinus* was five times higher in 2015 (234 p/m^3) and nine times higher in 2016 (429 p/m^3) than the mean SPIn of the two preceding years 2013 and 2014 (48 p/m^3). All in all, seven woody taxa (Pinaceae, *Platanus*, *Fraxinus*, Cupressaceae, *Betula*, *Quercus* and *Castanea*) out of 20 showed an obvious increase in the SPIn in 2016. The SPIn of *Ambrosia* increased steadily from 476 p/m^3 in 2013, to 539 p/m^3 in 2014 and 600 p/m^3 in 2015 (see pollen curves in Annex II).

Weekly mean pollen concentrations of the 15 main pollen contributing species recorded in Büyükcekmece are shown in Fig. 6. The highest pollen concentrations were found in the months April and May. Tab. 4 provides an overview of taxa with concentrations >=100 p/m^3. Over the entire study period the highest pollen loads were recorded in April 2016. The APIn in 2013 was 9591 p/m^3; in 2014: 6695 p/m^3; in 2015: 7458 p/m^3. Interestingly, till the 26th of June 2016 already more pollen (10790) was shed than in all the other full years monitored.

Tab. 4 The frequency of daily pollen concentrations >= 100 p/m^3, >= 200 p/m^3, >= 400 p/m^3 recorded over the study period

		Concentration (p*m-3)		
Year	**Taxon**	**>=100**	**>=200**	**>=400**
2013	Cupressaceae	7	3	0
	Olea	1	1	0
	Pinaceae	1	0	0
	Poaceae	8	3	0
2014	Cuperssaceae	3	0	0
2015	Ambrosia	1	0	0
	Cupressaceae	4	2	1
2016	Carpinus	1	0	0
	Cupressaceae	10	3	2
	Pinaceae	1	0	0
	Platanus	1	0	0
	Quercus	3	1	0

Fraxinus and Pinaceae displayed pronounced bimodal flowering periods. Low pollen concentrations of Cupressaceae were also recorded irregularly in autumn, mostly in October and November and in the last week of December. *Urtica* and *Plantago* pollen were not continuously recorded during the season.

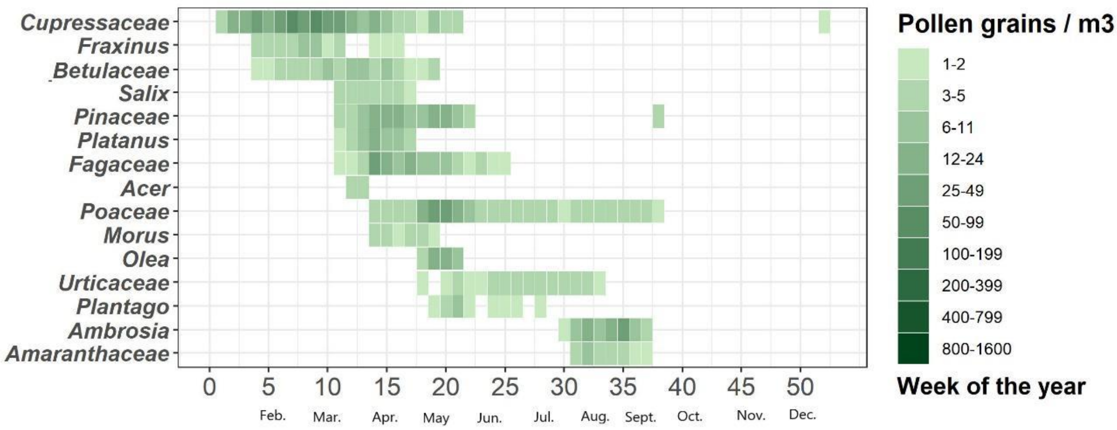

Fig. 6 Phenology and intensity of pollen based on mean weekly airborne pollen concentrations on a 10 scaled-colour gradient of the main pollen taxa monitored in Büyükçekmece. Weekly means < 1 are not considered in the plot, so that staggered occurrence is eliminated

The effect of meteorological factors

We observed an increased flowering intensity in spring flowering trees in 2015 and even more so in 2016. This was most likely elicited by abundant and evenly distributed rains throughout spring 2014 and the second half of 2014 including the winter months, which continued into spring 2015 (Tab. 1). As a result, the APIn in 2015 was higher than in 2014. In Istanbul's winters, humidity is higher than it is in summer (Tab. 1) with pronounced daily fluctuations due to frequent precipitation events during this season. Water was sufficiently available during the vegetative growth period in winter and early spring during the years studied. The dry period in Istanbul lasts from May to September (Fig. 1).

We investigated the effect of meteorological factors over the study period on the 15 main woody and herbaceous taxa with generalized additive models (GAMs). In the GAMs meteorological factors were used as independent variables, and pollen concentrations of pollen taxa as the dependent ones.

Tab. 5 Results of GAMs

Pollen Taxon	**Tmax**	**Tmin**	**HumMean**	**PressMean**	**Prec**
Cupressaceae	*** (-)	***(-)	**		.(-)
Fraxinus	***(-)	***(-)	*	**(-)	
Betulaceae	***(-)	***(-)	***		
Salix	***(-)	***(-)	**		
Pinaceae	*(-)	***(-)			
Acer	***(-)	***(-)			
Platanus	***(-)	***(-)			.(-)
Fagaceae	***(-)	***(-)	**		
Morus	***(-)	***(-)			
Olea				**(-)	
Poaceae		.(-)		***(-)	**(-)
Urticaceae	***	***	*** (-)	*** (-)	*(-)
Amaranthaceae	***	***	*** (-)	*** (-)	*** (-)
Ambrosia	***	***	*** (-)	*(-)	

Signif. Codes <0.001 '***' <0.01 '**'< 0.05 '*' '.'<1 ' '

We observed the following patterns (Tab. 5): The effect of temperature on pollen concentrations was significantly negative for tree taxa mainly flowering in winter and spring until the end of April (Tab. 2 and Fig. 6). On Poaceae and *Olea*, with peak flowering in May, the effect faded. For the weeds with flowering in summer (*Plantago*, Amaranthaceae and *Ambrosia*), the effect of temperature turned to be the opposite. This applies also to Urticaceae, which have their peak in summer (Tab. 3, Fig. 6). Relative humidity had a positive effect on winter and spring flowering Cupressaceae, *Fraxinus*, Betulaceae and *Salix*, and in Fagaceae. Significances for relative humidity, pressure, and precipitation were mostly negative for taxa with a pollen season in summer.

2.4. Discussion

Flowering phenology

Airborne pollen can be used to infer the timing of flowering of taxa that are relevant in allergological terms (Velasco-Jiménez et al., 2020; Grundström et al., 2019; Karatzas et al., 2019; Jato et al., 2015). The intensity of the pollen season depends on the magnitude of the flowering, as well as on weather and climate (Dahl et al., 2013). The duration of the season, however, is bound to how it is defined. The 95 % method based on the APIn (Andersen, 1991), can be suitable for low pollen concentrations (Bastl et al., 2018), as it is the case at the trap location in Büyükcekmece/Istanbul with APIns < 10.000 p/m^3 during each year studied. To compare, Kastamonu, located in the mountainous Turkish Black Sea Region with dense forest cover, has APIns >100.000 p/m^3 (Çeter et al., 2012); Antalya, on the Mediterranean coast on the foot of the Taurus mountains has >20.000 p/m^3 (Tosunoglu et al., 2015). The disadvantage of the percentual method is that the delineation of seasons depends on the flowering intensity, on missing data in the database, and can only be done retrospectively (Grundström et al., 2019; Bastl et al., 2018). The inherent bias for an earlier start of the season for years with lower intensities was seen, for example, in Poaceae and *Ambrosia*. Alternatively, with the 1st day of 5 non-zero records, the start date of the season was generally delayed, except for taxa with high pollen production, such as Cupressaceae or Poaceae. For *Fraxinus* and *Platanus*, but also Pinaceae, Poaceae, and *Plantago* the season start date using both methods was very similar. In South Spain the pollen season start for *Fraxinus* and *Populus* was defined with 1 p/m^3 plus 5 non-zero counts (Velasco-Jiménez et al., 2020) suggesting that this method could possibly work also in Istanbul for those taxa, which had a standard deviation <5 days of the difference in the season start dates. The five consecutive non-zero pollen counts are not suitable for all taxa at our location because the start date would fall far into the main flowering phase of the taxon and even trespass the first peak. Grundström et al (2019), who analysed several methods to calculate the oak pollen season across Europe, found that the cumulative sum method of three consecutive days with 5 p/m^3 delayed the start 10-12 days into the oak season as compared to the cumulative sum of 50 pollen grains. Further investigation targeted to individual taxa would be needed to delineate the most suitable method to define the pollen season in Istanbul. Other factors determine the duration of the pollen season. First, the taxonomic level of identification of a taxon under the light microscope, whether it consists of a species, for instance *Olea europaea;* a genus, for instance *Quercus* or a family (Cupressaceae, Poaceae). Second, if conspecific plants flower at the same time (synchronically) or continuously staggered (asynchronically) and how many flowers are open per day (the flowering rate). The third important factor is weather conditions (Dahl et al., 2013). The pollen season start and end of herbaceous plants, for example, Urticaceae, showed considerable variations over three pollen seasons and had a long flowering duration. In Urticaceae we can observe asynchronous flowering with the contribution of *Parietaria judaica* to the pollen spectrum of this family. *Parietaria judaica*, a perennial herb, grows and flowers all year long in shaded, ruderal urban (wall) communities of the city (Altay et al., 2010). Continuous growth ensures reproduction when availability of resources is unpredictable (Fotiou et al., 2011). The course of the Urticaceae pollen season in Istanbul was comparable to the Urticaceae season in Thessaloniki (Fotiou et al., 2011), with continuous records from May to August and irregular ones stretching into November. Conversly, in Krakow, in an analysis of pollen season dynamics on a 17-year long data series, pollen seasons of herbaceous plants were reported to have generally low variability with regard to the season start and end date (Myszkowska, 2014). We explain this difference with summer drought in Mediterranean regions forcing herbaceous plants to an opportunistic flowering behaviour (Dahl et al., 2013). In Krakow, a typical city with warm temperate Cfb climates of Europe, June, July and August are the wettest months (climate-data.org, 2020), while they are the driest in Istanbul. The pollen season start and end dates were less variable for grasses. The onset of the grass pollen season in Istanbul is preceded by regular equinoctial (winter) rains facilitating growth during the developmental phase (Dahl et al., 2013). This leads to rather short and intense flowering peaks. When abundant rains occur during anthesis, the grass pollen season can be prolonged (Dahl et al., 2013). We observed this phenomenon during 2014, when grass flowering coincided with persistent rainfalls in spring.

Woody taxa had generally shorter pollen seasons than herbaceous ones, which was also observed by Werchan et al. (2018) and Myszkowska et al. (2011). The season end of Pinaceae, however, was prolonged due to the bi-modal flowering of this taxon. Tosunoglu et al. (2015) identified a distinct pollen season for *Cedrus* in Antalya, from the end of September to mid-December, with highest concentrations in October. It could be envisioned to record *Cedrus*-pollen separately from the rest of the Pinaceae family also in Istanbul, to better delineate the pollen season of this taxon. Cupressaceae, *Carpinus* and *Fraxinus* had long seasons, too. In the case of *Fraxinus*, for example, *Fraxinus angustifolia* flowers in early spring, but *Fraxinus ornus* flowers in April. While *Fraxinus* *angustifolia* is a common ornamental tree, *F. ornus* grows in the submediterranean woods in the hinterland of the city. The consequence is an *Fraxinus* pollen season that can possibly stretch from the last week of January into the beginning of May. We observed pollen of *Carpinus* rather irregularly, with two months difference in the start and the end date of pollen seasons in four years of observations. The *Carpinus*/*Ostrya* type in Split (Croatia) varied also six weeks in the season start (Puljak et al., 2016). Eurosiberian vegetation is found mostly about 80 km upwind and at ca. 15 km downwind of our trap location. Temporal shifts in the beginning of flowering due to temperature differences at the different growth locations, and atmospheric transport can explain the long season of this taxon.

Flowering intensity

Relative abundances and the SPIn of pollen are measures of flowering intensity. Differences in the magnitudes should be discussed in the light of annual variations and the bias of the trap height for some taxa in 2013 and early 2014. Annual variation depends on meteorological conditions and species inherent physiological flowering dynamics in woody perennial plants. The weather determines the amount of resources (assimilates in form of carbohydrates, the resorption of nutrients) that a plant can accumulate (Bogdziewicz et al., 2017; Dahl et al., 2013; Dahl & Strandhede, 1996). In 2016, conspicuously high intensities of Cupressaceae, *Quercus*, *Fraxinus*, Pinaceae, *Platanus* and *Carpinus* (Tab. 4) seem to be the result of favourable environmental conditions for the accumulation of resources acting across families. We suspect a masting phenomenon across taxa and populations. Masting, in fact, does not occur independently of environmental factors (Bogdziewicz et al., 2017). At time intervals plant populations in a wider area spend these resources in highly synchronous mass reproduction (Koenig et al., 2015). Masting is typical for many wind-pollinated woody species (Pearse et al., 2016) including *Betula*, *Quercus*, and *Fraxinus* (Bogdziewicz et al., 2020; Dahl et al., 2013; Dahl & Strandhede, 1996; Tapper, 1992). The correlation of weather with synchronous fluctuations in pollination success of isolated populations (known as pollination Moran effect) acts on individuals of the same taxon but also across taxa and ecosystems (Hansen et al., 2020; Pearse et al., 2016). Interestingly, in 2016 the highest birch pollen concentrations since the start of the records were reported for Poland and connected to long distance transport from Southeast Europe early in the birch pollen season in April (Myszkowska et al., 2021).

An increased magnitude of pollen with pronouncedly higher peak values of Poaceae and *Plantago* growing within 10 m of the trap location was detected when the trap was positioned at 3 m height, than when it was positioned at 10 m height. Also, higher peak values of *Olea* and possibly Pinaceae in 2013 could result from stands in the vicinity. Conversely, several *Platanus* trees grow about 250 m (N)NE of the original trap location but pollen concentrations were not increased in comparison to other years. Pollen concentration, in fact, is highest in the immediate vicinity of the source (Adams-Groom et al., 2017). All in all, the change of the trap height did not constitute an apparent effect on most pollen type concentrations monitored, which is consistent with Rojo et al. (2019).

The main contributing pollen taxa in Istanbul during the study period are very similar to the taxa reported for Thessaloniki (in order of magnitude: Cupressaceae, *Quercus*, Urticaceae, Oleaceae, Pinaceae, Poaceae, *Platanus*, *Corylus*, Amaranthaceae, Asteraceae, *Populus* and Plantaginaceae) (Gioulekas et al., 2004). The city is located at similar geographical latitude as Istanbul and has warm-temperate Cfa climate with more precipitation in summer (Climate-Data.org, 2020). Cupressaceae typically domineers the pollen spectrum of Mediterranean cities in the winter and spring (Puljak et al., 2016; Tosunoglu et al., 2015 Martínez-Bracero et al., 2015, Gioulekas et al., 2004). Cupressaceae pollen can be responsible for pollinosis early in the year (Asero et al., 2020; Charpin et al., 2019; Sposato & Scalese, 2013). However, sensitization to Cupressaceae allergen in western Istanbul seems to be low (3 %) (Zemmer et al., 2021). Comparing data from our trap location in the western suburbs with data from Central Istanbul obtained between 2005 and 2006 (Celenk et al., 2010), relative abundances for Cupressaceae (34 %), Fraxinus (3 %), and Moraceae (2 %) were similar. Abundances of Poaceae, Pinaceae, *Quercus*, *Olea* and *Salix* pollen, in contrast, were considerably higher at our site, while *Platanus* (24 %) and Urticaceae pollen (13 %) appeared to be more important in the city centre. In highly urbanised Central Istanbul ornamental species like *Platanus* and *Fraxinus* are frequent. In the historic peninsula there are still many

Platanus trees originating from the Ottoman Empire (Baser, 2011) which are very large in size. Besides, *Parietaria judaica* is common on the ancient stone walls of the city (Altay et al., 2010). In contrast, the hinterland of Istanbul features grass- and woodlands. Pollen from taxa of the Fagales order reached considerable concentrations at our study site, due to north-easterly air currents. Despite the environmental focus of this study, the health impact should not be neglected. We considered Fagales pollen as a group for allergological reasons. Practitioner as well as self-empowered patients can swiftly identify periods of increased incidence of this allergenic pollen taxon when grouped at order level. Highly allergenic *Ambrosia* pollen has become the main weed pollen contributor in western Istanbul with proven clinical relevance (Zemmer et al., 2021). A clinical pollen season (Pfaar et al., 2017) of *Ambrosia* identified on the base of pollen data from 2018 (Hoffmann et al., 2020), endorses this finding.

Meteorological factors

An important limiting factor for flowering intensity in the Mediterranean is precipitation (Galán et al 2016; Dahl et al., 2013). Prior to anthesis, in the example of *Betula*, catkin initiation depends on the availability of assimilates during the vegetation period previous to flowering (Dahl & Strandhede, 1996). In summer-dry Istanbul, water availability plays an important role in this process.

The flowering intensity of herbaceous plants such as grasses and weeds is influenced by meteorological conditions during vegetative growth (Dahl et al., 2013). Relatively even grass seasons during the years studied is explained with constant water availability necessary for the induction of flowering shoots during vegetative growth (Dahl et al., 2013). Drought, as a limiting growth factor in the study area, might have affected the intensity of the Urticaceae pollen season in 2013. Urticaceae have the peak period in summer, but flower all year so that water deprivation might have reduced the vigour of the plants and the ability to produce flowers in 2013.

To assess the effect of meteorological factors during anthesis we ran GAMs. In aerobiological studies, the relationship between meteorological factors and pollen concentrations is often discussed on the base of correlations (Kluska et al., 2020; Bruffaerts et al., 2018) and regressions (Gioulekas et al., 2004) assuming a linear dependency (Le, 2003). The effects of meteorological factors studied in a GAM provide insight on flowering behaviour and can be part of predictive models (Cordero et al., 2021). Significances in the GAMs reflected direct impacts of meteorological factors on the pollen curve. If the pollen curve follows the factor units, the effect will be positive, otherwise negative. Temperature models were significantly positive when pollen concentrations rose with rising temperatures, as in the case of weeds. Summer rains – often torrential - reduce temperatures. As temperatures rise again and humidity deceases when skies clear, dehiscence occurs (Dahl et al., 2013). In the case of spring flowering trees, at a certain point in time, there is no longer pollen left in the anthers, and thus the curve goes down although it is getting warmer as summer is approaching. As a result, the temperature effect turns negative. Poaceae and *Olea* flower at the onset of summer when temperatures increase and precipitation becomes less frequent. In this case, the GAM model did not show a significant effect in temperature.

Humidity models were positive for early spring (winter) flowering trees, such as Cupressaceae and *Fraxinus*. Rising pollen curves must have coincided with rising mean humidity levels, when dehiscence can still occur at sunny spells during a day. In 2015, mean humidity was higher in February than in January and may have had an effect on the global model. Negative humidity signals for summer flowering weeds could either be linked to both short term precipitation effects as in Amaranthaceae or *Ambrosia* or to a seasonal decrease in humidity after the peak period in the course the pollen seasons, as in *Plantago*.

In weeds, the signals of atmospheric pressure were the same as in the humidity models. The moist parameters humidity, pressure and precipitation had a similar negative effect, as they are interconnected (Brenner, 2004). This was not evident in spring flowering trees, due to daily weather fluctuations.

Precipitation generally lowers pollen concentrations (Paschalidou et al., 2020). This phenomenon occurs due to wash out (Tormo-Molina et al., 2010), whereby 5 mm/h are necessary for a pronounced effect (Kluska et al., 2020) and has been shown also in correlation analysis by Bruffaerts et al. (2018). In Poaceae, for example, an extended period of rain during the last week of April and first ten days of May in 2014 may have caused a decrease in airborne pollen concentrations and explain the negative effect in the GAM.

GAMs run over a time series of several years provide a more general picture on the effect of the factors studied on the pollen seasons, than seasonal or annual GAMs. A higher resolution of the effects on the pollen curve can be obtained with short term GAMs and used in forecasts (Cordero et al., 2021; Ravindra et al., 2019).

2.5 Conclusion

Main pollen contributing taxa were Cupressaceae, Poaceae, Pinaceae, *Quercus, Carpinus, Olea, Fraxinus,* Urticaceae*, Salix*, Amaranthaceae, *Plantago, Morus, Acer*, *Platanus* and the neophyte ragweed. The definition of the pollen season with the 95 % method seems adequate due to low concentrations of airborne pollen. For Pinaceae, *Platanus*, *Fraxinus*, *Olea,* as well as for Poaceae and *Plantago,* the method of consecutive 5 non-zero daily records could be an alternative to the percent-method. Further investigations on single taxa are needed to define a pollen season start detached from the percentual method. High pollen concentrations observed in 2016 indicate that weather cues prior to anthesis can trigger intense flowering across tree populations. We possibly witnessed a masting phenomenon. Further research on pollen seasons of neighbouring regions in 2016 would provide insight on the truth of this hypothesis. A change in how high the pollen trap was positioned mainly affected pollen concentrations of taxa growing in the immediate surrounding. Differences in the importance of pollen contributing taxa between the city centre and the suburbs suggest that one trap in the city centre and one each at the western and eastern outskirts of the city would assure representable pollen information for Istanbul. GAMs provide causative explanations on the effect of weather on pollen concentrations during anthesis. Meteorological factors showed contrasting effects on spring and summer flowering taxa.

CHAPTER 3 THRESHOLD LEVELS AND RELATIVE RISK

This study shows seasonal dose-response relationships of Poaceae, Fagales and *Ambrosia* pollen on symptoms and medicine use in the allergic population. Pollen thresholds were derived by visual interpretation of the dose-response curves. In cooperation with the Biosense Institute, Serbia, pollen thresholds based on crowd sourced data from pollendiary.com have been studied for the first time in Southeast Europe. The methodology is practicable and may be used for improvements of public pollen warnings in countries where pollen information is developing.

THRESHOLD LEVELS OF AIRBORNE POLLEN CONCENTRATIONS AND THE RELATIVE RISK FOR HAY FEVER MORBIDITY BASED ON CROWD SOURCED DATA IN TWO REGIONS OF SOUTHEAST EUROPE

ABSTRACT

Threshold levels necessary to induce pollen allergy symptoms are not universal. For Southeast Europe pollen thresholds, the risk for allergy symptoms, and the need for medicine use have not been investigated, yet. The aim of this study was to identify pollen threshold levels and the relative risk for respiratory allergy symptoms and medication use in western Istanbul, Turkey, and the region Yuvojvod, Serbia.

The effects of airborne pollen sampled with a Hirst type trap on symptom and medicine scores obtained from crowed sourced data between 2014 and 2016 were analysed with generalized additive models (GAMs). Maximum daily temperature, time and PM10 were used as confounders. Thresholds were visually identified on a dose response curve. The incseased relative morbidity risk (RR) at 10 p/m^3 of Poaceae, *Ambrosia*, Betulaceae/Fagales was estimated.

In western Istanbul and Yuvojvod, Poacaeae and *Ambrosia* were the most important pollen allergens. In Istanbul the effect of Poaceae pollen on symptoms and medicine use began at 4-5 p/m^3, and reached saturation after 17-20 p/m^3. The RR for symptom development and medicine use was 1.18 and 1.17. *Ambrosia* pollen had a linear effect on symptoms with a RR of 1.10. The effect of Fagales pollen on medicine use started at 10 p/m^3 with a RR of 1.16 and was highest at 70 p/m^3. Total pollen had a marginal effect on symptoms (RR 1.02) and medicine use (RR 1.04).

In Yuvojvod Poaceae induced medicine use from 1 p/m^3 onwards. At an increase of 10 p/m^3, the RR was 1.20. The RR was 1.08. The effect of *Ambrosia* began at 2 p/m^3, and reached saturation at 15-17 p/m^3. The RR for symptoms and medication use was 1.21 and 1.14. The symptom threshold for Betulaceae was light up to 18 p/m^3 and severe after 30 p/m^3. The RR was 1.14. Medicine use increased linearly after 20 p/m^3. The effect of Total pollen on symptoms and medicine use began at 45 p/m^3 and 50 p/m^3. The RR was 1.05 and 1.02.

Conclusion: Pollen threshold levels and the RR were assessed for Poaceae, *Ambrosia*, Fagales/Betulaceae, and Total pollen in two cities in Southeast Europe to support public pollen information, and to aid health practitioners.

Key words: Pollen thresholds; Electronic pollen diary; Non-linear trends; Medication scores, Symptom scores; Aerobiology

3.1. Introduction

Public pollen information is useful for the allergy management of allergic patients who seeks relief from symptoms (Gehrig et al., 2018; Geller-Bernstein et al., 2019). Online tools, where pollen information is linked to personal symptoms, make it now possible to issue individual forecasts (Bastl et al., 2020; Bastl et al., 2018; Berger et al., 2011, 2013; Kmenta et al., 2014). Allergy symptoms, on the other hand, can be used to improve classical pollen warnings (Šukienė et al., 2021) compared to warnings solely grounded on airborne pollen concentrations of allergenic taxa and weather (Bucher et al., 2017).

Symptom data are often presented according to the scales for pollen warnings, as for example in Switzerland, Austria or Spain. The number of pollen causing allergy risk varies between taxa and it is usually translated to several classes: “low”, “moderate”, “high” (Galán et al., 2007), and “very high” pollen loads (Gehrig et al., 2018).

Threshold levels of pollen concentrations necessary to induce allergy symptoms have been investigated in several European countries and reviewed (Becker et al., 2021; de Weger et al., 2013; Kitinoja et al., 2020). It has been shown that universal thresholds for the development of adverse health effects do not exist (Becker et al., 2021; de Weger et al., 2013). One reason is the varying pollen allergenicity of botanical taxa. Moreover, the amount of allergenic pollen varies between geographical areas (D’Amato et al., 2007), as does sensitivity to one or more taxa of atopic people at population and individual level. Furthermore, sensitivity may change over the course of the pollen season (Caillaud et al., 2012), as well as does the weather driven allergen content of the air, independently of the pollen load (Alan et al., 2019; Galan et al., 2013). Plants under stress can produce more allergen per pollen as a strategy to ensure pollination (Fernández-González et al., 2020; Rodríguez-Rajo et al., 2011).

Air pollutants like PM10, ozone (O_3) and nitrogen dioxide (NO_2) add to the health burden (Lam et al., 2021) and, thus, may affect symptom driven threshold values (Caillaud et al., 2014).

There is a clear need for regional scale assessments on the pollen thresholds that induce symptoms in the local allergic population (Šukienė et al., 2021; Pfaar et al., 2020; Berger et al., 2013). Pollen forecasts improve in quality, if symptom data is included.

A standardized method for threshold assessments has not yet been proposed. Methods, as reviewed by (de Weger et al., 2013), comprise clinical studies with pollen chambers in which patients are exposed to controlled pollen levels or park studies with pollen samplers in the vicinity. Another method is to ask patients to keep a symptom diary (Caillaud, 2014) that would reflect real life exposure. To assess personal exposure of patients in the real life is almost impossible and would always include population samples with more or less limited size. Approximations of exposure based on data from pollen samplers on rooftop is a practical option that has been used in threshold assessment studies (Šukienė et al., 2021; Caillaud et al., 2014; Caillaud et al., 2012). When combined with online tools to collect crowd sourced data on hay fever symptoms and medicine use from the allergic population, approximations towards pollen thresholds for symptom development and medicine use are feasible (de Weger et al., 2013).

The Pannonian part of Serbia borders the northern Balkans, while Istanbul lays at the south-eastern endpoint of the Balkan Peninsula. Poaceae, Betulaceae and *Ambrosia* are important allergen sources in Southeast Europe (Zemmer et al., 2021; Leru et al., 2019; Katotomichelakis et al., 2015; Peternel et al., 2008; Yazicioglu et al., 2004), for which – to our knowledge – threshold levels for symptom development and medication have not been investigated.

Incidence of allergy morbidity is linked to pollen allergen exposure in relation to spatial and temporal occurrence. Caillaud et al. (2013, 2012) have shown non-linear effects of Poaceae and *Betula* pollen concentrations on hay fever symptoms and derived thresholds for the onset of symptoms.

The aim of this paper is to issue threshold values of pollen concentrations that lead to allergic symptoms and the use of medication for western Istanbul, Turkey and the Pannonian part of Serbia along with the relative risk for symptom development and medicine consumption.

3.2. Methodology

Crowed sourced data

For the analysis of manifestation of pollen allergy, crowd sourced data from the Patient's Hay-fever Diary (PHD), pollendiary.com (Berger et al., 2013, 2011) logged between 01.01.2014 and 30.06.2016 were used. The structure of the data logging interface is described in detail by Karatzas et al. (2014).

In Istanbul the PHD was promoted via online (Facebook) and offline tools (flyers) to people with respiratory allergy in western Istanbul who had lived, worked or studied within 30 km of the trap (Kiotseridis et al., 2013; Caillaud et al., 2012) for at least two years. About half of participants were patients from the clinical panel study - the Istanbul Pollen Allergen Sensitization Study (IPASS) – who were instructed to keep the PHD for the duration of the study period (Zemmer et al., 2021). In the Pannonian part of Serbia, the PHD was promoted by means of the regular pollen information service and occasionally over public media.

From the raw data set retrieved from pollendiary.com the default parameter “Overall Symptoms Total” was used; the “Total Medicine Score” was obtained by adding scores of medication categories (eye drops, nose drops, tablets, homeopathic, and other). The range of the Overall Symptom Total Score in Istanbul was 0-19, that of the Total Medicine Score 0-2; in Juvojvod the scores were and 0-4 and 0-25, respectively. N= 725 person days were used in the GAMs for Istanbul, and n = 5982 for Yuvojvod.

ALLERGENIC POLLEN AND CONFOUNDING VARIABLES

Allergenic pollen was monitored according to the standard for sampling and analysis of airborne pollen grains with the volumetric Hirst method (EN 16868, 2019). Poaceae, *Ambrosia* and Betulaceae/Fagales were selected for the analysis. Due to low pollen concentrations in Istanbul (Chapter 2), Betulaceae and Fagaceae were grouped at Fagales order level. In Yuvojvod, the family level Betulaceae was chosen. This selection is justified with the high cross-reactivity of the Bet v 1 pollen allergen in species pertaining to this order (Biedermann et al., 2019; Marticardi, 2016). The sampling device was positioned at rooftop. Data was sent to the European Aeroallergen Network (EAN) (https://ean.polleninfo.eu/Ean/) once a week, and made available for the PHD. As confounding variables maximum daily temperature was retrieved from weatherunderground.com (Istanbul, Hazerfen Airfield) and air pollution data (PM10) from https://sim.csb.gov.tr/, İstanbul-Esenyurt-MTHM. Both stations lay within 10 km from the trap location. Pollen data and confounding variables from Yuvojvod were provided by the Biosense Institute, Serbia.

DATA ANALYSIS

Generalized additive models (GAMs) can be used to estimate the increment of risk for a health impact, linked to the increment of a unit of airborne particle (Bell et al., 2004). The advantage of using GAMs for the assessment of the effects of airborne particles on health, in our case hay fever symptoms and medicine use, lays in the visualization of the shape of the response curves. Such has been proposed by Caillaud et al. (2012) to assess effects of pollen intensities on symptom development.

GAMs are non-linear regression models, in which confounders (independent variables other than pollen that may have an effect on the dependent one) can be added. The confounders by default in our models were “time”, “maximum daily temperature” and “PM10”. We chose the quasi-Poisson distribution with a log link suitable for highly dispersed not normally distributed data (Ravindra et al., 2019). The number of degrees of freedom (k) was chosen not to overfit the model. Diagnostic plots obtained with the gam.check() function helped to understand the goodness of fit.

Work up:

- Identify significant symptom/medicine signals of an allergic taxon by means of GAMs
- Include additional taxa in the model, if necessary
- Extract thresholds with spline on taxon
- Extract relative risk (RR) from estimate of taxon analysed

Firstly, we used plots to visually assess threshold levels for symptom development. We analysed the curve obtained by applying the smoothing spline to the independent variable (pollen taxon). The curve location *t* with the lowest error sum of squares can be the start-threshold (Benedetti et al., 2009), and the thresholds, when morbidity has reached moderate or highest levels, is indicated by changes in the direction of the regression curve.

Secondly, we used the model estimate to calculate the relative risk (RR) for developing hay fever symptoms and the need for mitigating medication as compared to the risk when a pollen taxon or other pollutant is not airborne. The RR can be derived from the GAM's effect estimate. For example, a GAM where the daily Poaceae pollen concentration is used as an independent variable, and the dependent variable is the Overall Symptoms Total score, gives the estimate 0.017 (see example of GAMs in supplementary material). The RR is generally reported as denominator to 1 (Andrade, 2015). So, at a concentration of 10 p/m^3 of Poaceae the rate to develop symptoms will be 1.17, a 117 % percent increase in symptoms compared to the situation when there is no pollen in the air.

3.3. Results

General trends of symptom development and medicine consumption were calculated based on crowd sourced data. The effect of confounding factors for seasonality "Time" and maximum daily temperature "MaxT" were significant in all models. PM10 was significant in the models of Yuvojvod.

Thresholds and RR in Istanbul

Poaceae pollen caused the strongest signal for hay fever symptoms that lead to an increase in medical score. From Fig. 1 we can infer the beginning of symptom development at a concentration of 4 p/m^3 and increased linearly till 10 p/m^3. When Poaceae pollen concentrations reached 10 p/m^3, the RR to develop symptoms in people allergic to Poaceae pollen in Istanbul was rounded 118 %, and the RR to need medication was 117 % as compared to the situation when no Poaceae pollen is airborne (Tab. 1). This risk estimation is valid as long as the curve of the predictor is linear. Moderate symptoms were observed between 10-17 p/m^3. The increase of magnitude on the *y*- axis by unit *x* slowed down from 10 to 17 p/m^3 to reach a plateau after 17 p/m^3 until a concentration of about 50 p/m^3. About 17 p/m^3 mark the threshold concentration that leads to severe hay fever symptoms at the beginning of the Poaceae pollen season. Concentrations >= 60 p/m^3 may further increase the symptoms. The standard error increases with increasing pollen concentrations. Medicine use followed a similar course. Medicine use started at 5 p/m^3, and between 21 p/m^3 and 30 p/m^3 most patients used medicine. After that it decreased to remain at a moderate level at >= 60 p/m^3.

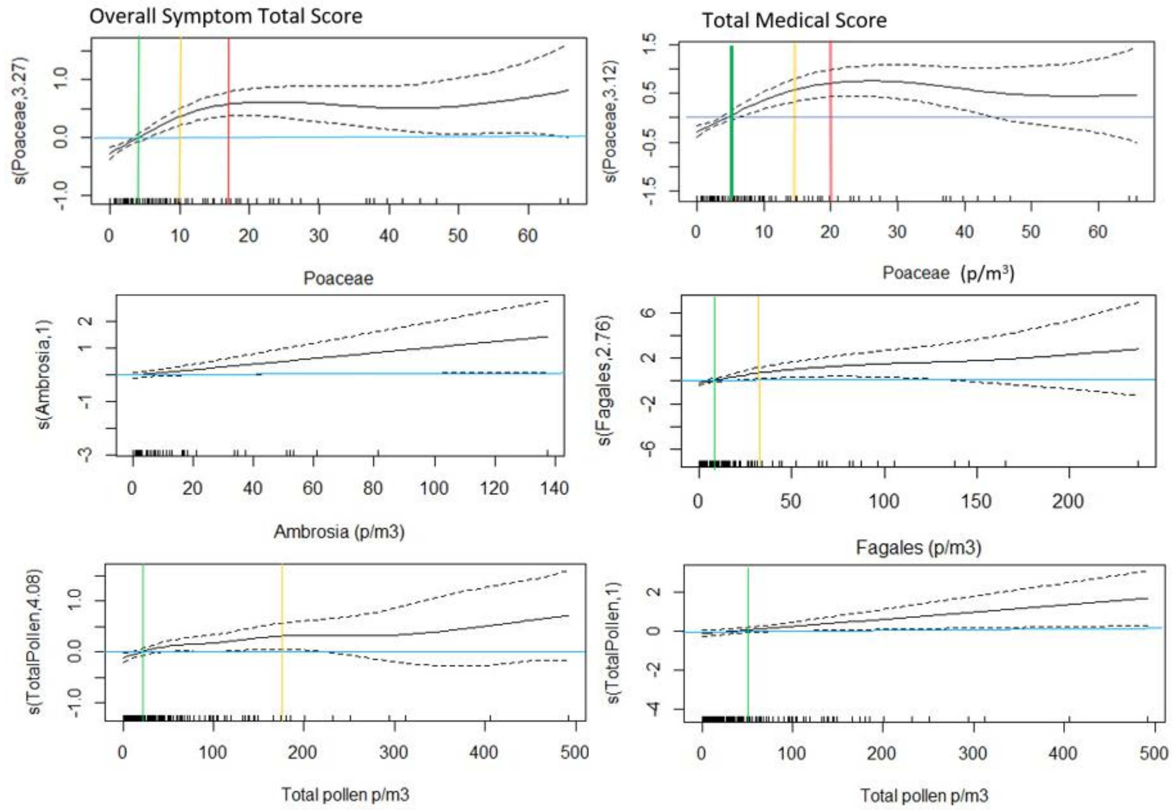

Fig. 1 The effect curves of pollen concentrations in Istanbul (2014-June 2016) on symptoms (left) and medicine use (right) with thresholds. The green line denotes the start of a health effect in unit increase in the log (*y*) abundance by unit increase in *x*. The yellow line denotes the threshold for moderate to severe symptoms. The red line marks the saturation threshold. The blue line marks zero effect. The legend on the *y*-axis refers to the effective degrees of freedom of the independent variable. Note the relationship between the confidence intervals and the data logs.

Ambrosia pollen had a significant linear effect on symptoms but not on medicine use (Fig. 1). The RR to have hay fever symptoms at an *Ambrosia* pollen concentration of 10 p/m^3 was 111 % as compared to when *Ambrosia* pollen was not airborne. At 20 p/m^3 the RR would be 121 % due to the linear increase of the effect. At higher concentrations than that the uncertainty increases along with the standard error.

Due to thin data logs and non-significant results on Betulaceae, including the taxa *Corylus*, *Alnus*, *Carpinus* and *Betula*, we ran models at the higher taxonomic level. The Fagales order comprises taxa of the Fagaceae family, *Quercus*, *Fagus* and *Castanea* and the Betulaceae family. Fagales pollen had a significant positive effect on the total medicine score, but not on the overall total symptom score. At about 10 p/m^3 of Fagales, medication scores started to increase linearly up to about 30 p/m^3. After that, the increase in magnitude of the medicine score was minimal and the threshold for the highest score connected with Fagales pollen was around 70 p/m^3, which we infer from a slight change in the slope of the curve. The RR to use medication in people allergic to Fagales was 116 % at a concentration of 10 p/m^3. In the Fagales model Poaceae and Total pollen were included as additives besides PM10, maximum daily temperature and time.

The effect of Total pollen on the development of symptoms started at about 25 p/m^3. The *x* unit effect on the magnitude of *y* was small and reached a plateau at about 170 p/m^3. Above 300 p/m^3 there might be a further increase in the effect on symptom development with a high level of uncertainty. The effect of Total pollen on the medicine score increased linearly after a concentration of 50 p/m^3. In the case of Total pollen, the RR to have symptoms was 18 % at 10 p/m^3. The effect of Total pollen lost its significance on both dependent variables, when Poaceae pollen was added to the model (Annex III).

Tab. 1: RR for health effects in Istanbul and Yuvojvod at 10 units of the independent factor based on model estimates with standard errors (se) and significance codes

	ISTANBUL		YUVOJVOD	
Independent factor	**RR symptoms with se**	**RR medicine use with se**	**RR symptoms with se**	**RR medicine use with se**
Poaceae	1.176 +-0.004 ***	1.167+-0.005***	NS	1.203+-0.010*
Ambrosia	1.105 +-0.005*	NS	1.214+-0.007**	1.14+-0.007*
Betulaceae	NS	NS	1.140+0.002***	1.08 +-0.003*
Fagales	NS	1.16+-0.006**	--	--
Total pollen	1.018 +-0.001**	1.037+-0.002*	1.005 +-0.001***	1.02+-0.001*
PM10	NS	NS	1.02+-0.001*	1.03+-0.001***
Signif. codes:	0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1			

Thresholds and RR in Yuvojvod, Serbia

In the region Yuvojvod GAMs with Poaceae as independent factor did not provide significant signals of effects on the Overall Symptoms Total, but on the Total Medical Score. Up to about 5 p/m^3 the increase was linear, then the effect of unit *x* increased only marginally the magnitude of *y*, reaching a plateau at 8 p/m^3 (Fig. 2). The RR for medicine use was 120 % for allergic people when Poaceae pollen was airborne (Tab. 1). In contrast to Istanbul, in Yuvojvod GAMs on symptoms and medicine use related to Betulaceae pollen were significant. Therefore, a model on Fagales was deemed redundant and omitted.

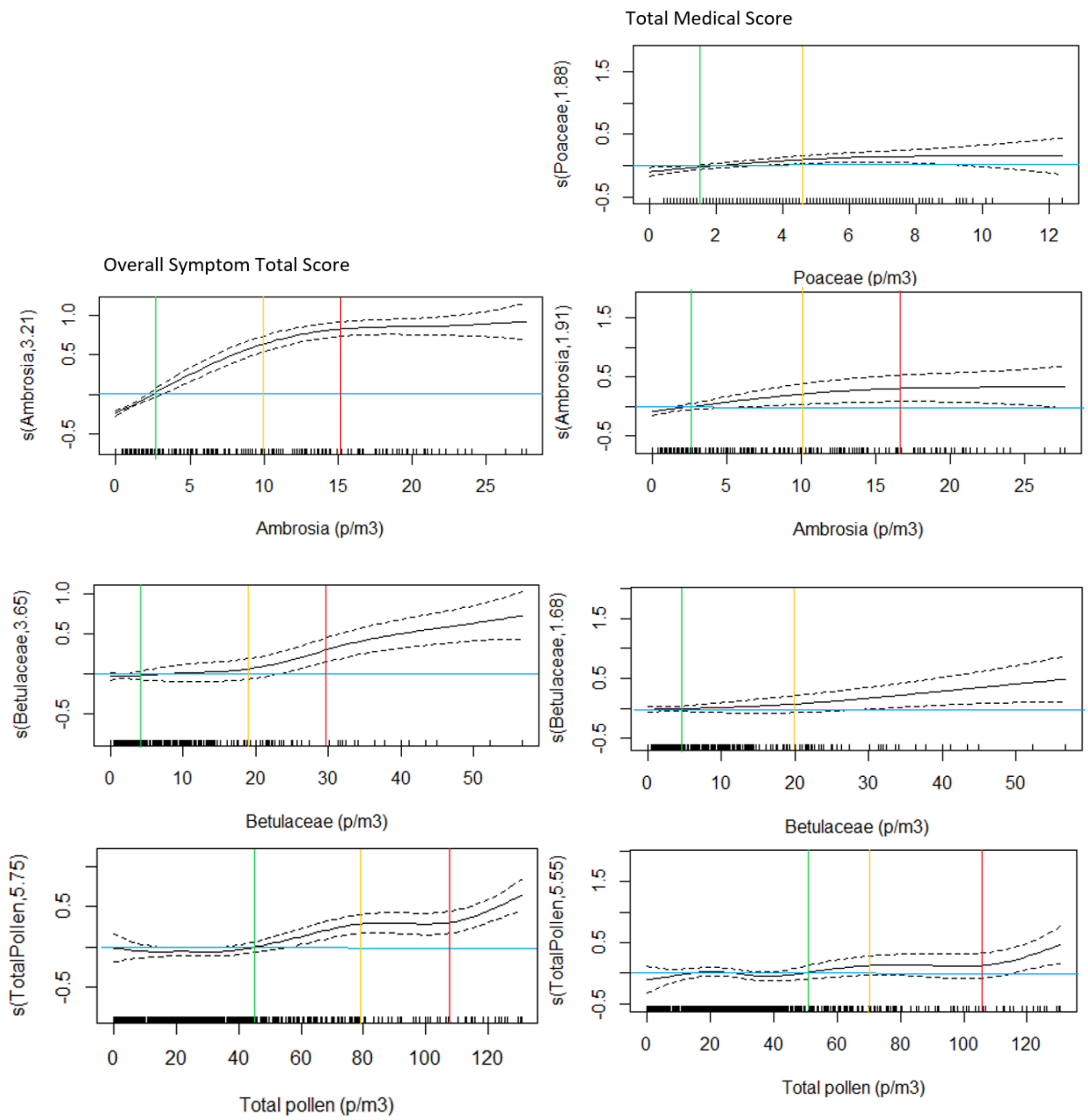

Fig. 2 The effect curves of pollen concentrations in Yuvojvod (2014-2016) on symptoms (left) and medicine use (right) with thresholds. The green line denotes the start of a health effect in unit increase in the log (*y*) abundance by unit increase in *x*. The yellow line denotes the threshold for moderate to severe symptoms. The red line marks the saturation threshold. The blue line marks zero effect. The legend on the *y*-axis refers to the effective degrees of freedom of the independent variable. Note the relationship between the confidence intervals and the data logs.

Between three and 18 p/m^3 of Betulaceae symptoms were light, between 19 and about 30 p/m^3 moderate, and from 30 p/m^3 onwards severe. The effect on medicine use due to Betulaceae was pronounced, starting at 20 p/m^3 with a linear increase. The RR for people allergic to Betulaceae to have symptoms at 10 p/m^3 was 140 % compared to non-allergic people, while the RR to need medication was 80 %. This means that not all the people with Betulaceae allergy used medication. From Betulaceae, *Carpinus* had a significant effect on the total symptom score and the total medicine score. The risk to develop symptoms was 37 % increased when *Carpinus* was airborne, while the risk to consume medicine increased by 18 %. *Betula* increased the risk to develop symptoms by 11 % but did not have a significant effect on medicine consumption (Annex III).

The strongest effect on symptoms was observed in connection to *Ambrosia* pollen. Symptoms started at a concentration of 2 p/m^3 and increased steeply up 10 p/m^3. After that concentration the slope inclination diminished to reach a plateau at about 15 p/m^3. Thus, we derived the threshold for moderate at 10 p/m^3, and for severe symptoms at 15 p/m^3. Medicine use began at the onset of symptoms. The magnitude increased linearly till 10 p/m^3. The slope inclination diminished gradually up to a concentration of about 17 p/m^3 and remained somewhat steady after this concentration. The RR to experience symptoms due to *Ambrosia* pollen was 121 %, the RR for medicine use 114 %.

The effect of Total pollen in the magnitude of symptoms started at 45 p/m^3 and increased linearly till a concentration of about 80 p/m^3. This would be the threshold for moderate symptoms due to Total pollen. After about 110 p/m^3 we observed a further increase in the slope of the curve – the threshold for severe symptoms. Medicine use began at 50 p/m^3, reached at a plateau at about 70 p/m^3, and increased again at around 110 p/m^3. The RR Total pollen added to the burden of hay fever sufferers was 5 % with respect to total symptoms and 20 % in terms of medicine use.

Besides pollen, the pollutant PM10 had a significant effect both on the symptoms and the medicine use. Ten units of PM10 contributed to the RR for symptom development and medicine with 20 % and 30 %, respectively.

AEROBIOLOGICAL PARAMETERS

Pollen threshold levels and the RR in both locations depend on the pollen exposure, which can be inferred by aerobiological data. Data of 2016 for Poaceae in Istanbul and

Ambrosia in both locations were available till 30th of June, so that pollen parameters involving summer and autumn seasonality are not presented. Pollen intensities were comparably lower in Istanbul than in Yuvojvod (Tab. 2). Clinical pollen seasons as proposed by Pfaar et al. (2017) occurred exclusively in Yuvojvod for Betulaceae and *Ambrosia* consistently in all three years of the study period, and for Poaceae in 2015.

Tab. 2 Aerobiological parameters in Istanbul and Yuvojvod

Location	**Taxon**	**Year**	**Clinical HS start date**	**Clinical HS end date**	**Days >100 p/m³**	**APIn**	**Peak Value**	**Peak Date**
Istanbul	Poaceae	2014	NA	NA	0	504	42	12/05/2014
		2015	NA	NA	0	913	66	22/05/2015
		2016	NA	NA	0	NA	47	09/05/2016
Yuvojvod		2014	NA	NA	0	2196	79	19/05/2014
	Poaceae	2015	18/05/2015	21/05/2015	4	2574	194	19/05/2015
		2016	NA	NA	2	NA	221	24/06/2016
Istanbul	Ambrosia	2014	NA	NA	0	566	89	05/09/2014
		2015	NA	NA	1	628	138	31/08/2015
Yuvojvod	Ambrosia	2014	14/08/2014	12/09/2014	26	7670	639	02/09/2014
		2015	26/08/2015	16/09/2015	18	8047	1171	02/09/2015
Istanbul	Fagales	2014	NA	NA	0	652	76	12/03/2014
		2015	NA	NA	0	1.072	69	14/05/2015
		2016	NA	NA	0	2.353	237	04/04/2016
Yuvojvod	Betulaceae	2014	18/03/2014	05/04/2014	22	16475	2951	22/03/2014
		2015	10/04/2015	17/04/2015	14	7599	1506	12/04/2015
		2016	18/03/2016	18/04/2016	35	17627	2792	31/03/2016

3.4 Discussion

We delineated pollen thresholds and the RR for symptom development and medicine use related to airborne Poaceae, *Ambrosia*, Betulaceae/Fagales, and average daily pollen concentrations (Total pollen) for Istanbul and Yuvojvod. Additionally, the RR emerging from exposure to PM10 was defined from the global dataset. PM10, maximum daily temperature and time were used as confounders in all models. Our results on thresholds are based on a visual interpretation of the shape of dose-response curves of pollen on symptom scores and medicine scores. Thereof, several levels of morbidity due to pollen allergy were derived.

Istanbul and Yuvojvod mark the two end-points on a NW-SE gradient across Southeast Europe. Yuvojvod is located in the Pannonian Plain at the verge of the Balkan Peninsula. In both regions Poaceae, *Ambrosia*, Betulaceae/Fagales pollen allergens are an important cause for pollinosis (Burbach et al., 2009; D'Amato et al., 2007).

In our preliminary Poaceae models, taxa with overlapping seasons, for example *Olea*, *Quercus*, Urticaceae in Istanbul; *Carpinus* and *Betula* in Yuvojvod, were included to control their effect on the outcome. De Weger et al. (2011) included *Betula* in linear regression models to study a possible effect on the symptoms of seasonal allergic rhinitis during the Poaceae pollen season. Symptoms and medicine use in Istanbul were mainly connected to Poaceae pollen, despite lower pollen exposure than in Yuvojvod. Several panel studies on inhalant allergen sensitization in Istanbul and Turkish Trace have reported Poaceae as the main sensitizers in allergic subjects (Zemmer et al., 2021; Aydin et al., 2009; Yazicioglu et al., 2004). With this study, we link sensitization to actual symptom development by means of PHD data from hay-fever sufferers. A few airborne grass pollen (4 p/m^3) marked the onset of symptoms in Istanbul. Similarly, in France 2-4 p/m^3 was the threshold for beginning symptoms, 5-35 p/m^3 for moderate symptoms, and > 35 p/m^3 for high symptoms (reviewed in de Weger et al., 2013). In Istanbul, the majority of PHD users recorded symptoms below 20 p/m^3. At higher concentrations no increase in the symptoms occurred as can be inferred from the plateau in the effect curve (Caillaud et al., 2012). We took this concentration as threshold for severe symptoms. It might be argued, that continued medicine use suppresses symptoms. There is evidence, that the use of medication did not have an effect on the Poaceae symptom scores and that patients consuming medicine had stronger symptoms than patients who did not (de Weger et al., 2011). This may explain why the curve of the medicine scores was similar to the symptom curve. A review on the thresholds of Poaceae pollen and its health impacts yielded a threshold for medicine consumption starting at 14 p/m^3 following a non-linear trend (Becker et al., 2021). This concentration was the cut off for moderate medicine use in Istanbul. Non-linear effect curves are linked to the frequency of data logs. Days with very high Poaceae concentrations are rare (as compared to those with low and moderate concentrations), and thus the number of data logs are. However, also hyposensitization may play a role. Few studies elaborate on this topic. In the Netherlands it has been found that symptoms scores at the beginning of the Poaceae pollen season were more pronounced than in the late season (de Weger et al., 2011). The authors indicate a "natural potential to downregulate their allergic response after repeated allergen exposure". In immunologic terms this mechanism involves a receptor down-regulation and the emergence of Treg cells as the season progresses. This phenomenon would explain similar effects from other allergenic pollen than Poaceae, for instance from *Ambrosia* in Yuvojvod. Interestingly, threshold levels of Poaceae pollen based on pollen loads only (but verified whit symptoms proposed for Lithuania) coincide with those in Istanbul (Šukienė et al., 2021).

In Yuvojvod, the effect of Poaceae on medicine use was lower than in Istanbul, despite higher pollen concentrations. Poaceae pollen is possibly less important than the other allergens investigated in this region. This is reflected by non-significant symptom models. However, medicine scores at Poaceae concentrations < 10 p/m^3 underline that those low airborne concentrations lead to morbidity. Similar to Istanbul, the medicine score curve was non-linear with few logs after a concentration of 10 p/m^3. All in all, grass pollen concentrations starting with 10-12 p/m^3 can cause detrimental symptoms that may even lead to emergency department visits and hospital admissions (Becker et al., 2021).

In the population of Yuvojvod, *Ambrosia* pollen was the strongest inducer of respiratory allergy. In Yuvojvod the effect on both scores was non-linear. Symptoms and medicine scores reached saturation at 15-17 p/m^3. As previously discussed, morbidity caused by *Ambrosia* allergy may naturally decrease over the season due to hyposensitization (de Weger et al., 2011). Very high pollen concentrations of *Ambrosia* matched the criteria for clinical seasons lasting for about three weeks. The saturation in symptoms, however, occured at 15 p/m^3. The Pannonian Plain is source for *Ambrosia* pollen incursions into the southern Balkans (Šikoparija et al., 2009) which may add to the allergic burden by local stands. Incidence of *Ambrosia* allergy in Eastern Europe is reported to be 50 % in Hungary (Burbach et al., 2009). On a south-eastern gradient reported sensitization rates to *Ambrosia* allergen are 20 % in Romania (Leru et al., 2019) and 6 % in Istanbul (Zemmer et al., 2021). In Istanbul, where the *Ambrosia* pollen load is much less than in Yuvojvod, the use of medicine followed a linear course with an associated significant RR of 1.1. Thin data logs in Istanbul may be the reason for the linear increase. In both regions detrimental effects started at the beginning of the season. Linearity of effects was found in France on 60 *Ambrosia* sensitized patients (Caillaud et al., 2014).

Balkan vegetation with deciduous woods of the class Querco-Fagetea stretch into the geographical area of Istanbul (Çoban et al., 2016; Kavgaci et al., 2010). Fagales include taxa with shared allergens (Biedermann et al., 2019; Matricardi et al., 2016), so that sensitized subjects may be primed by *Corylus* and *Alnus* pollen (Biedermann et al., 2019) flowering early in the year and suffer from hay fever from spring to early summer following the flowering phenology of *Carpinus*, *Ostrya*, *Betula*, *Quercus*, *Fagus* and *Castanea* (Grewling et al., 2014; D'Amato et al., 2007). In Yuvojvod, influenced by the vegetation of the upper Balkans, *Betula* and *Carpinus* are important pollen sources, while in Istanbul the *Ostrya*-*Carpinus* pollen type prevails. Greatest abundances of Fagales pollen are, however, related to *Quercus* pollen (Chapter 2). Symptoms at the onset of the season in Yuvojvod were induced by Betulaceae pollen concentrations of 4-20 p/m^3, while severe symptoms occurred from 30 p/m^3 onwards. This was the threshold reported for *Betula* pollen by Caillaud et al. (2014) for symptoms of the eyes, and the nose, which increased linearly up to 70-110 p/m^3 with subsequent saturation. In Yuvojvod the increase in symptom scores was stepwise: a slow increase in symptoms at the beginning of the season and a nearly linear increase at > 30 p/m^3. The slow increase at the start of the season could be linked to daily pollen concentrations when *Corylus* and *Alnus* pollen are airborne as compared to *Betula* and *Carpinus* pollen. In Istanbul, Fagales had a pronounced effect on medicine scores (RR 1.16) at the beginning of the season, when Betulaceae are airborne. High pollen intensities when *Quercus* flowers may increase the use of medication. The associated RR of 1.14 at an increase of 10 p/m^3 for symptoms in Yuvojvod was comparable to the odds ratio of 1.07-1.17 reported by Caillaud et al. (2014) for nasal, ocular and bronchial symptoms. In Hungary, the closest country to Serbia, *Betula* sensitization with clinical relevance was 16.2 % in a European study (Burbach et al., 2009). In Istanbul the sensitization rate to Fagales was 15 % in a panel of 60 patients (Zemmer et al., 2021).

It has been argued by (Kitinoja et al., 2020) to consider average daily total pollen to assess thresholds for symptom development due to the difficulty to assess for confounding factors. Based on our findings, Total pollen can provide a small additive effect to the risk of symptom development, but cannot be an indicator for symptoms caused by taxa with varying allergenicity and seasonality.

3.5 Conclusion

The strength of our study is the use of crowd sourced data to show dose-response relationships of main pollen allergens in two locations delineating a NE-SW gradient through Southeast Europe. We run global models of the study period to obtain a general picture on thresholds and the RR for an increase in symptom and medicine scores of important allergen sources that lead to morbidity in the investigated regions. For Poaceae pollen in Istanbul and *Ambrosia* pollen in Yuvojvod, we found that symptoms and medicine scores did not increase after a threshold was reached. This finding is important, if pollen warnings shall include symptom data. Further, clinical trials or immunotherapy treatments can be timed accordingly. Limitations include the lack of controlling for patient characteristics as specific allergen sensitizations, compliance with the use of the diary, and the small sample size in Istanbul. Thresholds and related risks may be further refined in future studies by discerning time periods of specific pollen seasons. Seasonal models (Caillaud, et al. 2014; Caillaud et al. 2012; de Weger et al. 2011) may provide a clearer picture on the effects of an investigated pollen taxon. Additional pollutants may be included into the models.

OVERALL CONCLUSIONS

1) A total of 36 single allergens were selected based on the European Standard Panel for skin prick testing, on local occurrence and cross-reactivity patterns of allergens.

2) We confirm the European Standard Panel for Istanbul and possibly Turkish Thrace based on a patient sample with the emphasis to use these grass allergen extracts: *Phleum pratense*, *Poa pratensis* and *Sorghum halepense*; tree allergens: *Fraxinus excelsior*, *Corylus avellana*; weed allergens: *Ambrosia artemisiifolia, Artemisia vulgaris*. *Parietaria judaica* was not confirmed as sensitizing allergen in our sample but may be relavant at population level. *Rumex crispus* can be an indicator for pan-allergen sensitization. Such finding is important in the light of costly population-based studies.

3) Cupressaceae (29 %) and Poaceae (13 %) are the main contributors to pollen in the atmosphere of the city. Important are also in order of magnitude: Pinaceae (11 %), Fagaceae (9 %), *Ambrosia* (5 %), Betulaceae (5 %), *Platanus* (4 %), *Olea* (4 %), Urticaceae (3 %), *Fraxinus* (3 %), *Salix* (2 %), Amaranthaceae (2 %), *Plantago* (2 %), *Morus* (2 %) and *Acer* (1 %).

4) To define the pollen season with the 95 % method is adequate, due to low concentrations of airborne pollen. For *Pinaceae*, *Platanus*, *Fraxinus*, *Olea*, and for Poaceae *and Plantago*, the method of consecutive 5 non-zero daily concentrations could be an alternative to the percent-method. Further investigations on single taxa are needed to define a pollen season start detached from the percentual method.

6) At two meters trap height, only pollen concentrations of taxa growing in the immediate surrounding of the trap are higher than what they are at 10 meters height.

7) Flowering across tree populations was extraordinarily intense in 2016. We possibly witnessed a masting phenomenon in Southeast Europe. This needs to be confirmed in further research.

8) One pollen trap in the city center and one each at the western and eastern outskirts of the city would assure representative pollen information for Istanbul.

9) Generalized Additive Models of meteorological factors on pollen concentrations had contrasting effects on spring and summer flowering taxa.

10) We defined pollen concentration thresholds for allergy morbidity of main pollen allergens in two locations delineating a NE-SW gradient through Southeast Europe for the first time. To do so, the shape of dose-response curves of pollen on symptom scores and medicine scores were visually interpreted.

11) For Poaceae pollen in Istanbul and *Ambrosia* pollen in Yuvojvod (Serbia), we found that symptoms and medicine scores did not increase after a threshold was reached.

12) These local threshold levels improve the quality of pollen warnings and are important for the interpretation of pollen allergy. Clinical trials or immunotherapy treatments can be timed accordingly.

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REFERENCES

Abbas, A. K., Lichtman, A. H., & Pillai, S. (2012). *Cellular and molecular immunology* (7th ed). Elsevier/Saunders.

Adams-Groom, B., Skjøth, C. A., Baker, M., & Welch, T. E. (2017). Modelled and observed surface soil pollen deposition distance curves for isolated trees of Carpinus betulus, Cedrus atlantica, Juglans nigra and Platanus acerifolia. *Aerobiologia*, *33*(3), 407–416. https://doi.org/10.1007/s10453-017-9479-1

Alan, Ş., Sarışahin, T., Acar Şahin, A., Kaplan, A., & Pınar, N. M. (2019). An assessment of ragweed pollen and allergen loads in an uninvaded area in the Western Black Sea region of Turkey. *Aerobiologia*. https://doi.org/10.1007/s10453-019-09620-z

Altay, V., Özyiğit, II., Yarci, C. (2010). Urban ecological characteristics and vascular wall flora on the Anatolian side of Istanbul, Turkey. *Maejo Int J Sci Technol*.;4(03):483-495.

Andersen, T. B. (1991). A model to predict the beginning of the pollen season. *Grana*, *30*(1), 269–275. https://doi.org/10.1080/00173139109427810

Andrade, C. (2015). Understanding Relative Risk, Odds Ratio, and Related Terms: As Simple as It Can Get: (Clinical and Practical Psychopharmacology). *The Journal of Clinical Psychiatry*, *76*(07), e857–e861. https://doi.org/10.4088/JCP.15f10150

Ansotegui, I. J., Melioli, G., Canonica, G. W., Caraballo, L., Villa, E., Ebisawa, M., Passalacqua, G., Savi, E., Ebo, D., Gómez, R. M., Luengo Sánchez, O., Oppenheimer, J. J., Jensen-Jarolim, E., Fischer, D. A., Haahtela, T., Antila, M., Bousquet, J. J., Cardona, V., Chiang, W. C., … Zuberbier, T. (2020). IgE allergy diagnostics and other relevant tests in allergy, a World Allergy Organization position paper. *World Allergy Organization Journal*, *13*(2), 100080. https://doi.org/10.1016/j.waojou.2019.100080

Asam, C., Hofer, H., Wolf, M., Aglas, L., & Wallner, M. (2015). Tree pollen allergens-an update from a molecular perspective. *Allergy*, *70*(10), 1201–1211. https://doi.org/10.1111/all.12696

Asero, R., Ceriotti, V., & Bonini, M. (2020). Cypress pollen allergy in Milan: The story of an ongoing growth. *European Annals of Allergy and Clinical Immunology*, *online first*. https://doi.org/10.23822/EurAnnACI.1764-1489.155

Aydin, S., Hardal, U., & Hakki, A. (2009). *An Analysis of Skin Prick Test Reactions in Allergic Rhinitis Patients in Istanbul, Turkey*. *27*, 19–25.

Bakis, Y., Babac, M., & Uslu, E. (2020, April 30). *Turkish Plants Data Service (TÜBİVES)*. http://www.tubives.com/

Baldacci, S., Maio, S., Cerrai, S., Sarno, G., Baïz, N., Simoni, M., Annesi-Maesano, I., & Viegi, G. (2015). Allergy and asthma: Effects of the exposure to particulate matter and biological allergens. *Respiratory Medicine*, *109*(9), 1089–1104. https://doi.org/10.1016/j.rmed.2015.05.017

Baser, B. (2011). Natural and cultural traces of the flora of Istanbul. *Turkish Academy of Sciences Journal of Cultural Inventory*, *9*, 155–170.

Bastl, K., Bastl, M., Bergmann, K.-C., Berger, M., & Berger, U. (2020). Translating the Burden of Pollen Allergy Into Numbers: 10-Year Observational Study of Electronically Generated Symptom Data From the Patient's Hayfever Diary in Austria and Germany. *Journal of Medical Internet Research*, *22*(2), e16767. https://doi.org/10.2196/16767

Bastl, K., Bastl, M., Bergmann, K.-C., & Berger, U. (2019). How to do a clinical trial? Recommendations from the aerobiological point of view. *World Allergy Organization Journal*, *12*(4), 100020. https://doi.org/10.1016/j.waojou.2019.100020

Bastl, K., Kmenta, M., Berger, M., & Berger, U. (2018). 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*, *11*, 24. https://doi.org/10.1186/s40413-018-0203-6

Bastl, K., Kmenta, M., & Berger, U. E. (2018). Defining Pollen Seasons: Background and Recommendations. *Current Allergy and Asthma Reports*, *18*(12), 73. https://doi.org/10.1007/s11882-018-0829-z

Becker, J., Steckling-Muschack, N., Mittermeier, I., Bergmann, K.-C., Böse-O'Reilly, S., Buters, J., Damialis, A., Heigl, K., Heinrich, J., Kabesch, M., Mertes, H., Nowak, D., Schutzmeier, P., Walser-Reichenbach, S., Weinberger, A., Korbely, C., Herr, C., Heinze, S., & Kutzora, S. (2021). Threshold values of grass pollen (Poaceae) concentrations and increase in emergency department visits, hospital admissions, drug consumption and allergic symptoms in patients with allergic rhinitis: A systematic review. *Aerobiologia*, *37*(4), 633–662. https://doi.org/10.1007/s10453-021-09720-9

Bell, M. L., Samet, J. M., & Dominici, F. (2004). Time-Series Studies of Particulate Matter. *Annual Review of Public Health*, *25*(1), 247–280. https://doi.org/10.1146/annurev.publhealth.25.102802.124329

Benedetti, A., Abrahamowicz, M., Leffondré, K., Goldberg, M. S., & Tamblyn, R. (2009). Using Generalized Additive Models to Detect and Estimate Threshold Associations. *The International Journal of Biostatistics*, *5*(1). https://doi.org/10.2202/1557-4679.1172

Berger, U. E., Jäger, S., & Bergman, K.-C. (2011). PHD, the electronic patient's hayfever diary. *European Respiratory Journal*, *38*, 3213.

Berger, U. E., Karatzas, K., Jaeger, S., Voukantsis, D., Sofiev, M., Brandt, O., Zuberbier, T., & Bergmann, K. C. (2013). Personalized pollen-related symptom-forecast information services for allergic rhinitis patients in Europe. *Allergy*, *68*(8), 963–965. https://doi.org/10.1111/all.12181

Bianchi, D. E., Schwemmin, D. J., & Wagner, W. H. (1959). Pollen Release in the Common Ragweed (Ambrosia artemisiifolia). *Botanical Gazette*, *120*(4), 235–243. JSTOR.

Bicakci, A., Tatlidil, S., Sapan, N., Malyer, H., & Canitez, Y. (2003). Airborne pollen grains in Bursa, Turkey, 1999–2000. *Ann Agric Environ Med*, *10*, 31–36.

Biedermann, T., Winther, L., Till, S. J., Panzner, P., Knulst, A., & Valovirta, E. (2019). Birch pollen allergy in Europe. *Allergy*, all.13758. https://doi.org/10.1111/all.13758

Bignardi, D., Comite, P., Mori, I., Ferrero, F., Fontana, V., Bruzzone, M., Mussap, M., & Ciprandi, G. (2019). Allergen-specific IgE: Comparison between skin prick test and serum assay in real life. *Allergologie Select*, *3*(01), 9–14. https://doi.org/10.5414/ALX01891E

Bogdziewicz, M., Fernández-Martínez, M., Bonal, R., Belmonte, J., & Espelta, J. M. (2017). The Moran effect and environmental vetoes: Phenological synchrony and drought drive seed production in a Mediterranean oak. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1866), 20171784. https://doi.org/10.1098/rspb.2017.1784

Bousquet, P.-J., Burbach, G., Heinzerling, L. M., Edenharter, G., Bachert, C., Bindslev-Jensen, C., Bonini, S., Bousquet-Rouanet, L., Demoly, P., Bresciani, M., Bruno, A., Gjomarkaj, M., Canonica, G. W., Darsow, U., Durham, S., Fokkens, W. J., Giavi, S., Gramiccioni, C., Papadopoulos, N. G., … Zuberbier, T. (2009). GA2LEN skin test study III: Minimum battery of test inhalent allergens needed in epidemiological studies in patients. *Allergy*, *64*(11), 1656–1662. https://doi.org/10.1111/j.1398-9995.2009.02169.x

Brenner, I. S. (2004). The Relationship between Meteorological Parameters and Daily Summer Rainfall Amount and Coverage in West-Central Florida. *Weather and Forecasting*, *19*(2), 286–300. https://doi.org/10.1175/1520-0434(2004)019<0286:TRBMPA>2.0.CO;2

Bruffaerts, N., De Smedt, T., Delcloo, A., Simons, K., Hoebeke, L., Verstraeten, C., Van Nieuwenhuyse, A., Packeu, A., & Hendrickx, M. (2018). Comparative long-term trend analysis of daily weather conditions with daily pollen concentrations in Brussels, Belgium. *International Journal of Biometeorology*, *62*(3), 483–491. https://doi.org/10.1007/s00484-017-1457-3

Bucher, E., Bottarelli, L., De Gironimo, V., Verardo, P., Anelli, P., Ivaldi, C., Moretti, O., Gottardini, E., Nardelli, V., Onorari, M., Pellegrini, E., Peana, I., Stenico, A., Tassan, F., & Lessi, S. (2017). *POLLnet—Linee guida per il monitoraggio aerobiologico* (Vol. 151). ISPRA. http://www.pollnet.it/pubblicazioni.asp

Burbach, G. J., Heinzerling, L. M., Edenharter, G., Bachert, C., Bindslev-Jensen, C., Bonini, S., Bousquet, J., Bousquet-Rouanet, L., Bousquet, P. J., Bresciani, M., Bruno, A., Canonica, G. W., Darsow, U., Demoly, P., Durham, S., Fokkens, W. J., Giavi, S., Gjomarkaj, M., Gramiccioni, C., … Zuberbier, T. (2009a). GA ^{2} LEN skin test study II: Clinical relevance of inhalant allergen sensitizations in Europe. *Allergy*, *64*(10), 1507–1515. https://doi.org/10.1111/j.1398-9995.2009.02089.x

Caillaud, D., Martin, S., Segala, C., Besancenot, J.-P., Clot, B., & Thibaudon, M. (2012). Nonlinear short-term effects of airborne Poaceae levels on hay fever symptoms. *Journal of Allergy and Clinical Immunology*, *130*(3), 812-814.e1. https://doi.org/10.1016/j.jaci.2012.04.034

Caillaud, D., Martin, S., Segala, C., Besancenot, J.-P., Clot, B., & Thibaudon, M. (2014). Effects of Airborne Birch Pollen Levels on Clinical Symptoms of Seasonal Allergic Rhinoconjunctivitis. *International Archives of Allergy and Immunology*, *163*(1), 43–50. https://doi.org/10.1159/000355630

Caillaud, D., Thibaudon, M., Martin, S., Ségala, C., Besancenot, J. P., Clot, B., François, H., & French Aerobiology Network. (2014). Short-term effects of airborne ragweed pollen on clinical symptoms of hay fever in a panel of 30 patients. *Journal of Investigational Allergology & Clinical Immunology*, *24*(4), 249–256.

Carinanos, P., Alcázar, P., … de la Corte Zamorano, E. (2010). *Aerobiology and Biogeography of Genus Artemisia in Andalucia*. 9th International Congress on Aerobiology, Buenos Aires.

Cavkaytar, O., Buyuktiryaki, Betul, Sag, Erdal, Soyer, Ozge, & Sekerel, Bulent E. (2015). What we miss if standard panel is used for skin prick testing? *Asian Pacific Journal of Allergy and Immunology*. https://doi.org/10.12932/AP0564.33.3.2015

Cebrino, J., Portero de la Cruz, S., Barasona, M. J., Alcázar, P., Moreno, C., Domínguez-Vilches, E., & Galán, C. (2017). Airborne pollen in Córdoba City (Spain) and its implications for pollen allergy. *Aerobiologia*, *33*(2), 281–291. https://doi.org/10.1007/s10453-016-9469-8

Cecchi, L., Domeneghetti M. Paola, Crisci Alfonso, Onorari Marzia, & Orlandini Simone. (2006). Long distance transport of ragweed pollen as a potential cause of allergy in central Italy. *Annals of Allergy, Asthma & Immunology*, *96*(1), 86–91. https://doi.org/10.1016/S1081-1206(10)61045-9

Celenk, S., Bicakci, A., Tamay, Z., Guler, N., Altunoglu, M. K., Canitez, Y., Malyer, H., Sapan, N., & Ones, U. (2010). Airborne pollen in European and Asian parts of Istanbul. *Environmental Monitoring and Assessment*, *164*(1–4), 391–402. https://doi.org/10.1007/s10661-009-0901-1

Çeter, T., Pinar, N. M., Güney, K., Yildiz, A., Aşcı, B., & Smith, M. (2012). A 2-year aeropalynological survey of allergenic pollen in the atmosphere of Kastamonu, Turkey. *Aerobiologia*, *28*(3), 355–366. https://doi.org/10.1007/s10453-011-9240-0

Charpin, D., Pichot, C., Belmonte, J., Sutra, J.-P., Zidkova, J., Chanez, P., Shahali, Y., Sénéchal, H., & Poncet, P. (2019). Cypress Pollinosis: From Tree to Clinic. *Clinical Reviews in Allergy & Immunology*, *56*(2), 174–195. https://doi.org/10.1007/s12016-017-8602-y

Climate-Data.org. (2020). *Climate data for cities worldwide*. https://en.climate-data.org/

Çoban, S., Bayraktar, S., & Akgül, M. (2016). Forest Vegetation Maps and Its Development in Turkey: A Case Study From İstanbul-Belgrade Forest. *For. Review*, *47*(7), 7–16.

Comert, S., Demir, A. U., Karakaya, G., & Kalyoncu, A. F. (2014). Minimum prick test panel for adult patients with asthma and rhinitis in Ankara, Turkey. *Journal of Asthma*, *51*(4), 417–422. https://doi.org/10.3109/02770903.2013.878846

Cordero, J. M., Rojo, J., Gutiérrez-Bustillo, A. M., Narros, A., & Borge, R. (2021). Predicting the Olea pollen concentration with a machine learning algorithm ensemble. *International Journal of Biometeorology*, *65*(4), 541–554. https://doi.org/10.1007/s00484-020-02047-z

Dahl, Å., Galan, C., Hajkova, L., & Pauling, A. (2013). The onset, course and intensity of the pollen season. In *Allergenic pollen: A review of the production, release, distribution and health impacts* (pp. 29–70). Springer.

Dahl, Å., & Strandhede, S.-O. (1996). Predicting the intensity of the birch pollen season. *Aerobiologia*, *12*(2), 97–106. https://doi.org/10.1007/BF02446601

D’Amato, G., Cecchi, L., Bonini, S., Nunes, C., Annesi-Maesano, I., Behrendt, H., Liccardi, G., Popov, T., & van Cauwenberge, P. (2007). Allergenic pollen and pollen allergy in Europe. *Allergy*, *62*(9), 976–990. https://doi.org/10.1111/j.1398-9995.2007.01393.x

D’Amato, G., Chong-Neto, H. J., Monge Ortega, O. P., Vitale, C., Ansotegui, I., Rosario, N., Haahtela, T., Galan, C., Pawankar, R., Murrieta-Aguttes, M., Cecchi, L., Bergmann, C., Ridolo, E., Ramon, G., Gonzalez Diaz, S., D’Amato, M., & Annesi-Maesano, I. (2020). The effects of climate change on respiratory allergy and asthma induced by pollen and mold allergens. *Allergy*, *75*(9), 2219–2228. https://doi.org/10.1111/all.14476

Damialis, A., Gioulekas, D., Lazopoulou, C., Balafoutis, C., & Vokou, D. (2005). Transport of airborne pollen into the city of Thessaloniki: The effects of wind direction, speed and persistence. *International Journal of Biometeorology*, *49*(3), 139–145. https://doi.org/10.1007/s00484-004-0229-z

Damialis, A., & Konstantinou, G. N. Cereal pollen sensitisation in pollen allergic patients: to treat or not to treat? *Eur Ann Allergy Clin Immunol*. 2011;43(2):36-44

Damialis, A., Traidl-Hoffmann, C., & Treudler, R. (2019). Climate Change and Pollen Allergies. In M. R. Marselle, J. Stadler, H. Korn, K. N. Irvine, & A. Bonn (Eds.), *Biodiversity and Health in the Face of Climate Change* (pp. 47–66). Springer International Publishing. https://doi.org/10.1007/978-3-030-02318-8_3

de Weger, L. A., Beerthuizen, T., Gast-Strookman, J. M., Plas, D. T., Terreehorst, I., Hiemstra, P. S., & Sont, J. K. (2011). Difference in symptom severity between early and late grass pollen season in patients with seasonal allergic rhinitis. *Clinical and Translational Allergy*, *1*(1), 18. https://doi.org/10.1186/2045-7022-1-18

de Weger, L., Bergmann, K.-C., Rantio-Lehtimäki, A., Dahl, Å., Buters, J., Déchamp, C., Belmonte, J., Thibaudon, M., Cecchi, L., Besancenot, J.-P., Galan, C., & Waisel, Y. (2013). Impact of Pollen. In *Allergenic pollen: A review of the production, release, distribution and health impacts*. Springer.

Dursun, A. B., Celik G.E., Pinar M., Mungan D., & Misriligil Z. (2008). Regional pollen load: Effect on sensitisation and clinical presentation of seasonal allergic rhinitis in patients living in Ankara, Turkey. *Allergologia et Immunopathologia*, *36*(6).

Ediger, D., Günaydin, F. E., & Seker, Ü. (2020). Trends of sensitization to aeroallergens in patients with allergic rhinitis and asthma in the city of Bursa, South Marmara Sea Region of Turkey. *Turkish Journal of Medical Sciences*, *50*, 330–336.

Eguiluz-Gracia, I., Mathioudakis, A. G., Bartel, S., Vijverberg, S. J. H., Fuertes, E., Comberiati, P., Cai, Y. S., Tomazic, P. V., Diamant, Z., Vestbo, J., Galan, C., & Hoffmann, B. (2020). The need for clean air: The way air pollution and climate change affect allergic rhinitis and asthma. *Allergy*, *75*(9), 2170–2184. https://doi.org/10.1111/all.14177

EN 16868. (2019). *Ambient air—Sampling and analysis of airborne pollen grains and fungal spores for networks related to allergy—Volumetric Hirst method*. CEN. https://www.en-standard.eu/

Ersoy, Y. (2018). *Floristic observations on the familiy Poaceae Barn naturally grown in Istanbul* [MS Thesis, Namık Kemal University]. http://acikerisim.nku.edu.tr:8080/xmlui/bitstream/handle/20.500.11776/2606/0053103.pdf?sequence=1&isAllowed=y

Fernández-González, M., González-Fernández, E., Fernández-González, D., & Rodríguez-Rajo, F. J. (2020). Secondary Outcomes of the Ole e 1 Proteins Involved in Pollen Tube Development: Impact on Allergies. Frontiers in Plant Science, 11, 974. https://doi.org/10.3389/fpls.2020.00974

Fernández-Rodríguez, S., Skjøth, C. A., Tormo-Molina, R., Brandao, R., Caeiro, E., Silva-Palacios, I., Gonzalo-Garijo, Á., & Smith, M. (2014). Identification of potential sources of airborne Olea pollen in the Southwest Iberian Peninsula. *International Journal of Biometeorology*, *58*(3), 337–348. https://doi.org/10.1007/s00484-012-0629-4

Ferreira, M. A. R., Matheson, M. C., Tang, C. S., Granell, R., Ang, W., Hui, J., Kiefer, A. K., Duffy, D. L., Baltic, S., Danoy, P., Bui, M., Price, L., Sly, P. D., Eriksson, N., Madden, P. A., Abramson, M. J., Holt, P. G., Heath, A. C., Hunter, M., … Hopper, J. L. (2014). Genome-wide association analysis identifies 11 risk variants associated with the asthma with hay fever phenotype. *Journal of Allergy and Clinical Immunology*, *133*(6), 1564–1571. https://doi.org/10.1016/j.jaci.2013.10.030

Follak, S., & Essl, F. (2013). Spread dynamics and agricultural impact of *Sorghum halepense*, an emerging invasive species in Central Europe. *Weed Research*, *53*(1), 53–60. https://doi.org/10.1111/j.1365-3180.2012.00952.x

Fotiou, C., Damialis, A., Krigas, N., Halley, J. M., & Vokou, D. (2011). Parietaria judaica flowering phenology, pollen production, viability and atmospheric circulation, and expansive ability in the urban environment: Impacts of environmental factors. *International Journal of Biometeorology*, *55*(1), 35–50. https://doi.org/10.1007/s00484-010-0307-3

Galán, C., Alcázar, P., Oteros, J., García-Mozo, H., Aira, M. J., Belmonte, J., Diaz de la Guardia, C., Fernández-González, D., Gutierrez-Bustillo, M., Moreno-Grau, S., Pérez-Badía, R., Rodríguez-Rajo, J., Ruiz-Valenzuela, L., Tormo, R., Trigo, M. M., & Domínguez-Vilches, E. (2016). Airborne pollen trends in the Iberian Peninsula. *Science of The Total Environment*, *550*, 53–59. https://doi.org/10.1016/j.scitotenv.2016.01.069

Galán, C., Alcázar-Teno, P., Domínguez-Vilches, E., Villamandos de la Torre, F., & Garcia-Pantaleon, F. I. (1995). Airborne pollen grain concentrations at two different heights. *Aerobiologia*, *11*(2), 105–109. https://doi.org/10.1007/BF02738275

Galán, C., Antunes, C., Brandao, R., Torres, C., Garcia-Mozo, H., Caeiro, E., Ferro, R., Prank, M., Sofiev, M., Albertini, R., Berger, U., Cecchi, L., Celenk, S., Grewling, Ł., Jackowiak, B., Jäger, S., Kennedy, R., Rantio-Lehtimäki, A., Reese, G., … The HIALINE working group. (2013). Airborne olive pollen counts are not representative of exposure to the major olive allergen Ole e 1. *Allergy*, *68*(6), 809–812. https://doi.org/10.1111/all.12144

Galán, C., Ariatti, A., Bonini, M., Clot, B., Crouzy, B., Dahl, A., Fernandez-González, D., Frenguelli, G., Gehrig, R., Isard, S., Levetin, E., Li, D. W., Mandrioli, P., Rogers, C. A., Thibaudon, M., Sauliene, I., Skjoth, C., Smith, M., & Sofiev, M. (2017). Recommended terminology for aerobiological studies. *Aerobiologia*, *33*(3), 293–295. https://doi.org/10.1007/s10453-017-9496-0

Galán, C., Cariñanos, P., Alcázar, P., & Domínguez Vilches, E. (2007). *Spanish Aerobiology Network (REA): Management and Quality Manual*. Argos Impresores S.L. http://www.uco.es › raa › infor_raa › manual_eng

Galán, C., Smith, M., Thibaudon, M., Frenguelli, G., Oteros, J., Gehrig, R., Berger, U., Clot, B., & Brandao, R. (2014). Pollen monitoring: Minimum requirements and reproducibility of analysis. *Aerobiologia*, *30*(4), 385–395. https://doi.org/10.1007/s10453-014-9335-5

Gangl, K., Niederberger, V., & Valenta, R. (2013). Multiple grass mixes as opposed to single grasses for allergen immunotherapy in allergic rhinitis. *Clinical & Experimental Allergy*, *43*(11), 1202–1216. https://doi.org/10.1111/cea.12128

García-Mozo, H. (2017). Poaceae pollen as the leading aeroallergen worldwide: A review. *Allergy*, *72*(12), 1849–1858. https://doi.org/10.1111/all.13210

García-Mozo, H., Mestre, A., & Galán, C. (2010). Phenological trends in southern Spain: A response to climate change. *Agricultural and Forest Meteorology*, *150*(4), 575–580. https://doi.org/10.1016/j.agrformet.2010.01.023

Gehrig, R., Maurer, F., & Schwierz, C. (2018). Designing new automatically generated pollen calendars for the public in Switzerland. *Aerobiologia*, *34*(3), 349–362. https://doi.org/10.1007/s10453-018-9518-6

Geller-Bernstein, C., Lahoz, C., Cardaba, B., Hassoun, G., Iancovici-Kidon, M., Kenett, R., & Waisel, Y. (2002). Is it ‘bad hygiene’ to inhale pollen in early life? *Allergy*, *57*(s71), 37–40. https://doi.org/10.1034/j.1398-9995.2002.057s71037.x

Geller-Bernstein, C., & Portnoy, J. M. (2019). The Clinical Utility of Pollen Counts. *Clinical Reviews in Allergy & Immunology*, *57*(3), 340–349. https://doi.org/10.1007/s12016-018-8698-8

Ghitarrini, S., Galán, C., Frenguelli, G., & Tedeschini, E. (2017). Phenological analysis of grasses (Poaceae) as a support for the dissection of their pollen season in Perugia (Central Italy). *Aerobiologia*, *33*(3), 339–349. https://doi.org/10.1007/s10453-017-9473-7

Gioulekas, D., Balafoutis, C., Damialis, A., Papakosta, D., Gioulekas, G., & Patakas, D. (2004). Fifteen years’ record of airborne allergenic pollen and meteorological parameters in Thessaloniki, Greece. *International Journal of Biometeorology*, *48*(3), 128–136. https://doi.org/10.1007/s00484-003-0190-2

Göksel, Ö., & Güvensen, A. (2021). İzmir Polen Alerji Bülteni. https://polenalerji.ege.edu.tr/

Grewling, Ł., Bogawski, P., Kryza, M., Magyar, D., Šikoparija, B., Skjøth, C. A., Udvardy, O., Werner, M., & Smith, M. (2019). Concomitant occurrence of anthropogenic air pollutants, mineral dust and fungal spores during long-distance transport of ragweed pollen. *Environmental Pollution*, *254*, 112948. https://doi.org/10.1016/j.envpol.2019.07.116

Grewling, Ł., Jenerowicz, D., Nowak, M., Polańska, A., Jackowiak, B., Czarnecka-Operacz, M., & Smith, M. (2014). Clinical relevance of Corylus pollen in Poznań, western Poland. *Ann Agric Environ Med.*, *21*(1), 64–69.

Grundström, M., Adams-Groom, B., Pashley, C. H., Dahl, Å., Rasmussen, K., de Weger, L. A., Thibaudon, M., Fernández-Rodríguez, S., Silva-Palacios, I., & Skjøth, C. A. (2019). Oak pollen seasonality and severity across Europe and modelling the season start using a generalized phenological model. *Science of The Total Environment*, *663*, 527–536. https://doi.org/10.1016/j.scitotenv.2019.01.212

Hansen, B. B., Grøtan, V., Herfindal, I., & Lee, A. M. (2020). The Moran effect revisited: Spatial population synchrony under global warming. *Ecography*, *43*(11), 1591–1602. https://doi.org/10.1111/ecog.04962

Hanski, I., von Hertzen, L., Fyhrquist, N., Koskinen, K., Torppa, K., Laatikainen, T., Karisola, P., Auvinen, P., Paulin, L., Makela, M. J., Vartiainen, E., Kosunen, T. U., Alenius, H., & Haahtela, T. (2012). Environmental biodiversity, human microbiota, and allergy are interrelated. *Proceedings of the National Academy of Sciences*, *109*(21), 8334–8339. https://doi.org/10.1073/pnas.1205624109

Heinzerling, L., Mari, A., Bergmann, K.-C., Bresciani, M., Burbach, G., Darsow, U., Durham, S., Fokkens, W., Gjomarkaj, M., Haahtela, T., Bom, A. T., Wöhrl, S., Maibach, H., & Lockey, R. (2013). The skin prick test – European standards. *Clinical and Translational Allergy*, *3*(1). https://doi.org/10.1186/2045-7022-3-3

Hintze, J. L., & Nelson, R. D. (1998). Violin Plots: A Box Plot-Density Trace Synergism. *The American Statistician*, *52*(2), 181–184. https://doi.org/10.1080/00031305.1998.10480559

Hirst, J. M. (1952). An automatic volumetric spore trap. *Annals of Applied Biology*, *39*(2), 257–265. https://doi.org/10.1111/j.1744-7348.1952.tb00904.x

Hoegh-Guldberg, O., Jacob, D., Taylor, M., Bindi, M., Brown, S., Camilloni, A., Diedhiou, A., Daljante, R., Ebi, K. L., Engelbrecht, F., & et. al. (2018). Impacts of 1.5ºC Global Warming on Natural and Human Systems. In *Global Warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission athways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty*.

Hoffmann, T. M., Acar Şahin, A., Aggelidis, X., Arasi, S., Barbalace, A., Bourgoin, A., Bregu, B., Brighetti, M. A., Caeiro, E., Caglayan Sozmen, S., Caminiti, L., Charpin, D., Couto, M., Delgado, L., Di Rienzo Businco, A., Dimier, C., Dimou, M. V., Fonseca, J. A., Goksel, O., … Dramburg, S. (2020). “Whole” vs. “fragmented” approach to EAACI pollen season definitions: A multicenter study in six Southern European cities. *Allergy*, *75*(7), 1659–1671. https://doi.org/10.1111/all.14153

Hrabina, M., Jain, K., & Gouyon, B. (2008). Cross-reactivity between pollen allergens from common Pooideae grasses and cultivated cereals. *Clinical & Experimental Allergy Reviews*, *8*(1), 18–20. https://doi.org/10.1111/j.1472-9733.2008.00128.x

Jato, V., Rodríguez-Rajo, F. J., Fernandez-González, M., & Aira, M. J. (2015). Assessment of Quercus flowering trends in NW Spain. *International Journal of Biometeorology*, *59*(5), 517–531. https://doi.org/10.1007/s00484-014-0865-x

Jato, V., Rodríguez-Rajo, F. J., González-Parrado, Z., Elvira-Rendueles, B., Moreno-Grau, S., Vega-Maray, A., Fernández-González, D., Asturias, J. A., & Suárez-Cervera, M. (2010).

Detection of airborne Par j 1 and Par j 2 allergens in relation to Urticaceae pollen counts in different bioclimatic areas. *Annals of Allergy, Asthma & Immunology*, *105*(1), 50–56. https://doi.org/10.1016/j.anai.2010.04.019

Jenerowicz, D., Wojciech, S., Dańczak-Pazdrowska, A., & Polańska, A. (2012). Environmental factors and allergic diseases. *Annals of Agricultural and Environmental Medicine 2012, Vol 19, No 3, 475-481*, *19*(3), 475–481.

Kaplan, A., Sakiyan, N., & Pınar, N. M. (2003). Daily Ambrosia Pollen Concentration in the Air of Ankara, Turkey (1990-1999). *Acta Botanica Sinica*, *45*(12), 1408–1412.

Karatzas, K., Voukantsis, D., Jaeger, S., Berger, U., Smith, M., Brandt, O., Zuberbier, T., & Bergmann, K. Ch. (2014). The patient's hay-fever diary: Three years of results from Germany. *Aerobiologia*, *30*(1), 1–11. https:/Alan, Ş., Sarışahin, T., Acar Şahin, A., Kaplan, A., & Pınar, N. M. (2019). An assessment of ragweed pollen and allergen loads in an uninvaded area in the Western Black Sea region of Turkey. *Aerobiologia*. https://doi.org/10.1007/s10453-019-09620-z

Karatzas, K., Tsiamis, A., Charalampopoulos, A., Damialis, A., & Vokou, D. (2019). Pollen season identification for three pollen taxa in Thessaloniki, Greece: A 30-year retrospective analysis. *Aerobiologia*, *35*(4), 659–669. https://doi.org/10.1007/s10453-019-09605-y

Katelaris, C. H., & Beggs, P. J. (2018). Climate change: Allergens and allergic diseases. *Internal Medicine Journal*, *48*(2), 129–134. https://doi.org/10.1111/imj.13699

Katotomichelakis, M., Nikolaidis, C., Makris, M., Zhang, N., Aggelides, X., Constantinidis, T. C., Bachert, C., & Danielides, V. (2015). The clinical significance of the pollen calendar of the Western Thrace/northeast Greece region in allergic rhinitis: Pollen calendar and allergic rhinitis. *International Forum of Allergy & Rhinology*, *5*(12), 1156–1163. https://doi.org/10.1002/alr.21623

Kavgaci, A., Carni, A., Tecimeni, B., & Özalp, G. (2010). Diversity and ecological differentiation of oak forests in NW Thrace (Turkey). *Archives of Biological Sciences*, *62*(3), 705–718. https://doi.org/10.2298/ABS1003705K

Kavgaci, A., Yalcin, E., & Korkmaz, H. (2015). Numerical classification and ordination of the floodplain forests in the Euxine region of Turkey. *Turkish Journal of Botany*, *39*.

Kiotseridis, H., Cilio, C. M., Bjermer, L., Tunsäter, A., Jacobsson, H., & Dahl, Å. (2013). Grass pollen allergy in children and adolescents-symptoms, health related quality of life and the value of pollen prognosis. *Clinical and Translational Allergy*, *3*(1), 19. https://doi.org/10.1186/2045-7022-3-19

Kitinoja, M. A., Hugg, T. T., Siddika, N., Rodriguez Yanez, D., Jaakkola, M. S., & Jaakkola, J. J. K. (2020). Short-term exposure to pollen and the risk of allergic and asthmatic manifestations: A systematic review and meta-analysis. *BMJ Open*, *10*(1), e029069. https://doi.org/10.1136/bmjopen-2019-029069

Kluska, K., Piotrowicz, K., & Kasprzyk, I. (2020). The impact of rainfall on the diurnal patterns of atmospheric pollen concentrations. *Agricultural and Forest Meteorology*, *291*, 108042. https://doi.org/10.1016/j.agrformet.2020.108042

Kmenta, M., Bastl, K., Berger, U., Kramer, M. F., Heath, M. D., Pätsi, S., Pessi, A.-M., Saarto, A., Werchan, B., Werchan, M., Zetter, R., & Bergmann, K.-C. (2017). The grass pollen season 2015: A proof of concept multi-approach study in three different European cities. *World Allergy Organization Journal*, *10*, 31. https://doi.org/10.1186/s40413-017-0163-2

Kmenta, M., Bastl, K., Jäger, S., & Berger, U. (2014). Development of personal pollen information—The next generation of pollen information and a step forward for hay fever sufferers. *International Journal of Biometeorology*, *58*(8), 1721–1726. https://doi.org/10.1007/s00484-013-0776-2

Konstantinopoulos AP, Karantoumanis D, Psomiadou M, et al. PD02 - Observational follow-up study with the Pan-European standard prick test to determine inhalant allergen sensitisation rates in a Greek population. *Clin Transl Allergy*. 2014;4(S1):P2. doi:10.1186/2045-7022-4-S1-P2

Koenig, W. D., Knops, J. M. H., Carmen, W. J., & Pearse, I. S. (2015). What drives masting? The phenological synchrony hypothesis. *Ecology*, *96*(1), 184–192. https://doi.org/10.1890/14-0819.1

Kurt, E., Metintas, S., Basyigit, I., Bulut, I., Coskun, E., Dabak, S., Deveci, F., Fidan, F., Kaynar, H., Uzaslan, E. K., Onbasi, K., Ozkurt, S., Karakis, G. P., Sahan, S., Sahin, U., Oguzulgen, K., Yildiz, F., Mungan, D., Yorgancioglu, A., … A.Fuat Kalyoncu for the PARFAIT Study of the Turkish Thoracic Society Asthma and Allergy Working Group. (2009). Prevalence and Risk Factors of Allergies in Turkey (PARFAIT): Results of a multicentre cross-sectional study in adults. *European Respiratory Journal*, *33*(4), 724–733. https://doi.org/10.1183/09031936.00082207

Lam, H. C. Y., Jarvis, D., & Fuertes, E. (2021). Interactive effects of allergens and air pollution on respiratory health: A systematic review. *Science of The Total Environment*, *757*, 143924. https://doi.org/10.1016/j.scitotenv.2020.143924

Le, C. T. (2003). *Introductory biostatistics*. Wiley-Interscience.

Leru, P. M., Anton, V. F., & Colentina Clinical Hospital, Bucharest, Romania. (2019). Clinical pattern and risk factors of respiratory allergies due to Ambrosia (ragweed) pollen: Experience of one allergy center from Bucharest. *Archives of the Balkan Medical Union*, *54*(4), 705–711. https://doi.org/10.31688/ABMU.2019.54.4.13

Martin, M. D., Chamecki, M., & Brush, G. S. (2010). Anthesis synchronization and floral morphology determine diurnal patterns of ragweed pollen dispersal. *Agricultural and Forest Meteorology*, *150*(9), 1307–1317. https://doi.org/10.1016/j.agrformet.2010.06.001

Martínez-Bracero, M., Alcázar, P., Díaz de la Guardia, C., González-Minero, F. J., Ruiz, L., Trigo Pérez, M. M., & Galán, C. (2015). Pollen calendars: A guide to common airborne pollen in Andalusia. *Aerobiologia*, *31*(4), 549–557. https://doi.org/10.1007/s10453-015-9385-3

Matricardi, P. M., Kleine-Tebbe, J., Hoffmann, H. J., Valenta, R., Hilger, C., Hofmaier, S., Aalberse, R. C., Agache, I., Asero, R., Ballmer-Weber, B., Barber, D., Beyer, K., Biedermann, T., Bilò, M. B., Blank, S., Bohle, B., Bosshard, P. P., Breiteneder, H., Brough, H. A., … Ollert, M. (2016). EAACI Molecular Allergology User's Guide. *Pediatric Allergy and Immunology*, *27*, 1–250. https://doi.org/10.1111/pai.12563

McInnes, R. N., Hemming, D., Burgess, P., Lyndsay, D., Osborne, N. J., Skjøth, C. A., Thomas, S., & Vardoulakis, S. (2017). Mapping allergenic pollen vegetation in UK to study environmental exposure and human health. *Science of The Total Environment*, *599–600*, 483–499. https://doi.org/10.1016/j.scitotenv.2017.04.136

McKenna, O. E., Asam, C., Araujo, G. R., Roulias, A., Goulart, L. R., & Ferreira, F. (2016). How relevant is panallergen sensitization in the development of allergies? *Pediatric Allergy and Immunology*, *27*(6), 560–568. https://doi.org/10.1111/pai.12589

Moghaddam, M. V., Fallahpour, M., Mohammadi, M., Varaee, F. S. R., Mokhtarian, K., Khoshmirsafa, M., Jafari, R., Shirzad, N., & Falak, R. (2019). Identification of polcalcin as a novel allergen of Amaranthus retroflexus pollen. *Allergologia et Immunopathologia*, *47*(4), 357–364. https://doi.org/10.1016/j.aller.2018.12.006

Monroy-Colín, A., Silva-Palacios, I., Tormo-Molina, R., Maya-Manzano, J. M., Rodríguez, S. F., & Gonzalo-Garijo, Á. (2018). Environmental analysis of airborne pollen occurrence, pollen source distribution and phenology of Fraxinus angustifolia. *Aerobiologia*, *34*(3), 269–283. https://doi.org/10.1007/s10453-018-9512-z

Myszkowska, D. (2014). Predicting tree pollen season start dates using thermal conditions. *Aerobiologia*, *30*(3), 307–321. https://doi.org/10.1007/s10453-014-9329-3

Myszkowska, D., Piotrowicz, K., Ziemianin, M., Bastl, M., Berger, U., Dahl, Å., Dąbrowska-Zapart, K., Górecki, A., Lafférsová, J., Majkowska-Wojciechowska, B., Malkiewicz, M., Nowak, M., Puc, M., Rybnicek, O., Saarto, A., Šaulienė, I., Ščevková, J., Kofol Seliger, A., Šikoparija, B., … Czarnobilska, E. (2021). Unusually high birch (Betula spp.) pollen concentrations in Poland in 2016 related to long-range transport (LRT) and the regional pollen occurrence. *Aerobiologia*, *37*(3), 543–559. https://doi.org/10.1007/s10453-021-09703-w

Ozaslan, C., Onen, H., Farooq, S., Gunal, H., & Akyol, N. (2016). Common ragweed: An emerging threat for sunflower production and human health in Turkey: Ragweed invasion in Turkey. *Weed Biology and Management*, *16*(1), 42–55. https://doi.org/10.1111/wbm.12093

Özer, Z., Önen, H., Tursun, N., & Uygur, F. N. (1999). *Some important weeds of Turkey (Definition and Chemical Management)* (Vol. 16). https://www.researchgate.net/publication/287760089_Bazi_Onemli_Yabanci_Otlari_Tanimlari_ve_Kimyasal_Savasimlari_Some_important_weeds_of_Turkey_Definition_and_Chemical_Management

Pacini, E. (2000). From anther and pollen ripening to pollen presentation. *Plant Systematics and Evolution*, *222*(1/4), 19–43. JSTOR.

Paschalidou, A. K., Psistaki, K., Charalampopoulos, A., Vokou, D., Kassomenos, P., & Damialis, A. (2020). Identifying patterns of airborne pollen distribution using a synoptic climatology approach. *Science of The Total Environment*, *714*, 136625. https://doi.org/10.1016/j.scitotenv.2020.136625

Pearse, I. S., Koenig, W. D., & Kelly, D. (2016). Mechanisms of mast seeding: Resources, weather, cues, and selection. *New Phytologist*, *212*(3), 546–562. https://doi.org/10.1111/nph.14114

Peel, M. C. and Finlayson, B. L. and McMahon, T. A. (2007). Updated world map of the Köppen–Geiger climate classification. *Hydrology and Earth System Sciences,* 11, 1633–1644. https://doi:10.5194/hess-11-1633-2007.

Peñuelas, J., Filella, I., Zhang, X., Llorens, L., Ogaya, R., Lloret, F., Comas, P., Estiarte, M., & Terradas, J. (2004). Complex spatiotemporal phenological shifts as a response to rainfall changes. *New Phytologist*, *161*(3), 837–846. https://doi.org/10.1111/j.1469-8137.2004.01003.x

Peternel, R., Music Milanovic, S., & Srnec, L. (2008). Airborne ragweed (Ambrosia artemisiifolia L.) pollen content in the city of Zagreb and implications on pollen allergy. *Annals of Agricultural and Environmental Medicine*, *15*(1), 125–130.

Pfaar, O., Bastl, K., Berger, U., Buters, J., Calderon, M. A., Clot, B., Darsow, U., Demoly, P., Durham, S. R., Galán, C., Gehrig, R., Gerth van Wijk, R., Jacobsen, L., Klimek, L., Sofiev, M., Thibaudon, M., & Bergmann, K. C. (2017). Defining pollen exposure times for clinical trials of allergen immunotherapy for pollen-induced rhinoconjunctivitis—An EAACI position paper. *Allergy*, *72*(5), 713–722. https://doi.org/10.1111/all.13092

Pfaar, O., Karatzas, K., Bastl, K., Berger, U., Buters, J., Darsow, U., Demoly, P., Durham, S. R., Galán, C., Gehrig, R., Gerth van Wijk, R., Jacobsen, L., Katsifarakis, N., Klimek, L., Saarto, A., Sofiev, M., Thibaudon, M., Werchan, B., & Bergmann, K. (2020). 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*, *75*(5), 1099–1106. https://doi.org/10.1111/all.14111

Picornell, A., Oteros, J., Ruiz-Mata, R., Recio, M., Trigo, M. M., Martínez-Bracero, M., Lara, B., Serrano-García, A., Galán, C., García-Mozo, H., Alcázar, P., Pérez-Badia, R., Cabezudo, B., Romero-Morte, J., & Rojo, J. (2021). Methods for interpolating missing data in aerobiological databases. *Environmental Research*, *200*, 111391. https://doi.org/10.1016/j.envres.2021.111391

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & R Core Team. (2021). *nlme: Linear and Nonlinear Mixed Effects Models* (R package version 3.1-152) [Computer software].

Puljak, T., Mamić, M., Mitić, B., Hrga, I., & Hruševar, D. (2016). First aerobiological study in Mediterranean part of Croatia (Dalmatia): Pollen spectrum and seasonal dynamics in the air of Split. *Aerobiologia*, *32*(4), 709–723. https://doi.org/10.1007/s10453-016-9444-4

Ravindra, K., Rattan, P., Mor, S., & Aggarwal, A. N. (2019). Generalized additive models: Building evidence of air pollution, climate change and human health. *Environment International*, *132*, 104987. https://doi.org/10.1016/j.envint.2019.104987

Ritenberga, O., Sofiev, M., Kirillova, V., Kalnina, L., & Genikhovich, E. (2016). Statistical modelling of non-stationary processes of atmospheric pollution from natural sources: Example of birch pollen. *Agricultural and Forest Meteorology*, *226–227*, 96–107. https://doi.org/10.1016/j.agrformet.2016.05.016

Rodríguez-Rajo, F. J., Jato, V., González-Parrado, Z., Elvira-Rendueles, B., Moreno-Grau, S., Vega-Maray, A., Fernández-González, D., Asturias, J. A., & Suárez-Cervera, M. (2011). The combination of airborne pollen and allergen quantification to reliably assess the real pollinosis risk in different bioclimatic areas. *Aerobiologia*, *27*(1), 1–12. https://doi.org/10.1007/s10453-010-9170-2

Rojo, J., Oteros, J., Pérez-Badia, R., Cervigón, P., Ferencova, Z., Gutiérrez-Bustillo, A. M., 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, M. C., Guerrero, C., Berra, D., … Buters, J. (2019). Near-ground effect of height on pollen exposure. *Environmental Research*, *174*, 160–169. https://doi.org/10.1016/j.envres.2019.04.027

Rojo, J., Picornell, A., & Oteros, J. (2019). AeRobiology: The computational tool for biological data in the air. *Methods in Ecology and Evolution*, *10*(8), 1371–1376. https://doi.org/10.1111/2041-210X.13203

Rojo, J., Rapp, A., Lara, B., Fernández-González, F., & Pérez-Badia, R. (2015). Effect of land uses and wind direction on the contribution of local sources to airborne pollen. *Science of The Total Environment*, *538*, 672–682. https://doi.org/10.1016/j.scitotenv.2015.08.074

Şahiner, U. M., Civelek, E., Yavuz, S. T., Büyüktiryaki, A. B., Tuncer, A., & Şekerel, B. E. (2012). Skin Prick Testing to Aeroallergen Extracts: What Is the Optimal Panel in Children and Adolescents in Turkey? *International Archives of Allergy and Immunology*, *157*(4), 391–398. https://doi.org/10.1159/000329870

Schroeder, F.-G. (1998). *Lehrbuch der Pflanzengeographie*. Quelle u. Meyer.

Šikoparija, B., Smith, M., Skjøth, C. A., Radišić, P., Milkovska, S., Šimić, S., & Brandt, J. (2009). The Pannonian plain as a source of Ambrosia pollen in the Balkans. *International Journal of Biometeorology*, *53*(3), 263–272. https://doi.org/10.1007/s00484-009-0212-9

Sin, B. A., Inceoglu, Ö., Mungan, D., Çelik, G., Kaplan, A., & Misirligil, Z. (2001). Is it important to perform pollen skin prick tests in the season? *Annals of Allergy, Asthma & Immunology*, *86*(4), 382–386. https://doi.org/10.1016/S1081-1206(10)62482-9

Sipahi, S., Nacaroğlu, H. T., Can, D., Günay, İ., Ünsal-Karkıner, C. Ş., Kamalı, H., Özdemir, A., & Günay, T. (2017). Effect of socioeconomic status on allergic diseases and atopy in school children. *The Turkish Journal of Pediatrics*, *59*(6), 670. https://doi.org/10.24953/turkjped.2017.06.008

Skjøth, C. A., Smith, M., Šikoparija, B., Stach, A., Myszkowska, D., Kasprzyk, I., Radišić, P., Stjepanović, B., Hrga, I., & Apatini, D. (2010). A method for producing airborne pollen source inventories: An example of Ambrosia (ragweed) on the Pannonian Plain.

Agricultural and Forest Meteorology, *150*(9), 1203–1210. https://doi.org/10.1016/j.agrformet.2010.05.002

Smith, M., Cecchi, L., Skjøth, C. A., Karrer, G., & Šikoparija, B. (2013). Common ragweed: A threat to environmental health in Europe. *Environment International*, *61*, 115–126. https://doi.org/10.1016/j.envint.2013.08.005

Sposato, B., & Scalese, M. (2013). Prevalence and real clinical impact of Cupressus sempervirens and Juniperus communis sensitisations in Tuscan "Maremma", Italy. *Allergologia et Immunopathologia*, *41*(1), 17–24. https://doi.org/10.1016/j.aller.2011.08.001

Šukienė, L., Šaulienė, I., Dubakienė, R., Rudzevičienė, O., & Daunys, G. (2021). Analysis of allergenic pollen data, focusing on a pollen load threshold statement. *Aerobiologia*, *37*(4), 843–860. https://doi.org/10.1007/s10453-021-09727-2

Szymczak, S., Häusser, M., Garel, E., Santoni, S., Huneau, F., Knerr, I., Trachte, K., Bendix, J., & Bräuning, A. (2020). How Do Mediterranean Pine Trees Respond to Drought and Precipitation Events along an Elevation Gradient? *Forests*, *11*(7), 758. https://doi.org/10.3390/f11070758

Tapper, P.-G. (1992). Irregular fruiting in Fraxinus excelsior. *Journal of Vegetation Science*, *3*(1), 41–46. https://doi.org/10.2307/3235996

Terzioğlu E., Sin A., Kokuludag A., Kirmaz C., Erdem E., Sebik F., & Tabakci T. (1989). Sensitivity to Parietaria pollen in Izmir, Turkey as determined by skin prick and serum specific IgE values. *J Investig Allergol Clin Immunol*, *8*(3), 180–183.

Tormo-Molina, R., Gonzalo-Garijo, M., Silva-Palacios, I., & Muñoz-Rodríguez, A. (2010). General Trends in Airborne Pollen Production and Pollination Periods at a Mediterranean Site (Badajoz, Southwest Spain). *J Investig Allergol Clin Immunol*, *20*(7), 567–574.

Tosunoglu, A., Altunoglu, M. K., Bicakci, A., Kilic, O., Gonca, T., Yilmazer, I., Saatcioglu, G., Akkaya, A., Celenk, S., Canitez, Y., Malyer, H., & Sapan, N. (2015). Atmospheric pollen concentrations in Antalya, South Turkey. *Aerobiologia*, *31*(1), 99–109. https://doi.org/10.1007/s10453-014-9350-6

Turkish Statistical Institute. (2020). *Statistics by Topic*. Turkey Health Survey - Period: 2019. www.tuik.gov.tr

Turoğlu, H. (2014). Detection of Changes on Temperature and Precipitation Features in Istanbul (Turkey). *Atmospheric and Climate Sciences*, *4*, 549–562.

Vega-Maray, A. M., Fernández-González, D., Valencia-Barrera, R., & Suárez-Cervera, M. (2006). Allergenic proteins in Urtica dioica, a member of the Urticaceae allergenic family. *Annals of Allergy, Asthma & Immunology*, *97*(3), 343–349. https://doi.org/10.1016/S1081-1206(10)60799-5

Velasco-Jiménez, M. J., Alcázar, P., Díaz de la Guardia, C., Trigo, M. del M., de Linares, C., Recio, M., & Galán, C. (2020). Pollen season trends in winter flowering trees in South Spain. *Aerobiologia*, *36*(2), 213–224. https://doi.org/10.1007/s10453-019-09622-x

Walter, H., & Lieth, H. (1960). *Klimadiagramm-Weltatlas (Climate Diagram World Atlas)*. Fischer.

Weber, R. W. (2007). *Cross-reactivity of pollen allergens: Impact on allergen immunotherapy*. *99*, 203–212.

Weber, R. W. (2008). Guidelines for using pollen cross-reactivity in formulating allergen immunotherapy. *Journal of Allergy and Clinical Immunology*, *122*(1), 219–221. https://doi.org/10.1016/j.jaci.2008.05.034

Werchan, M., Werchan, B., & Bergmann, K.-C. (2018). German pollen calendar 4.0 – update based on 2011–2016 pollen data. *Allergo Journal International*, *27*(3), 69–71. https://doi.org/10.1007/s40629-018-0055-1

Yazicioglu, M., Oner, N., Celtik, C., Okutan, O., & Pala, O. (2004). Sensitization to common allergens, especially pollens, among children with respiratory allergy in the Traky Region of Turkey. *Asian Pacific Journal of Allergy and Immunology*, *22*, 183–190.

Zemmer, F. (2014). Update on airborne ragweed pollen monitored in Istanbul. *European Journal of Aerobiology and Environmental Medicine*, *2/2014*(Volume x, 2/2014), 31.

Zemmer, F., Cenk, E., Dahl, A., Galan, C., & Ozkaragoz, F. (2021). A multidisciplinary approach of outdoor aeroallergen selection for skin prick testing in the geographical area of Greater Istanbul. *European Annals of Allergy and Clinical Immunology*, *online first*. https://doi.org/10.23822/EurAnnACI.1764-1489.188

Zemmer, F., Karaca, F., & Ozkaragoz, F. (2012). Ragweed pollen observed in Turkey: Detection of sources using back trajectory models. *Science of The Total Environment*, *430*, 101–108. https://doi.org/10.1016/j.scitotenv.2012.04.067

Zhang, S., Soltis, D. E., Yang, Y., Li, D., & Yi, T. (2011). Multi-gene analysis provides a well-supported phylogeny of Rosales. *Molecular Phylogenetics and Evolution*, *60*(1), 21–28. https://doi.org/10.1016/j.ympev.2011.04.008

Ziello, C., Sparks, T. H., Estrella, N., Belmonte, J., Bergmann, K. C., Bucher, E., Brighetti, M. A., Damialis, A., Detandt, M., Galán, C., Gehrig, R., Grewling, L., Gutiérrez Bustillo, A. M., Hallsdóttir, M., Kockhans-Bieda, M.-C., De Linares, C., Myszkowska, D., Pàldy, A., Sánchez, A., … Menzel, A. (2012). Changes to Airborne Pollen Counts across Europe. *PLoS ONE*, *7*(4), e34076. https://doi.org/10.1371/journal.pone.0034076

ANNEX I

PATIENT QUESTIONNAIRE (ENGLISH) SECTION A

ALLERGY QUESTIONNAIRE

Your information will contribute to a study investigating airborne allergen sensitization rates among the population of Istanbul and help finding the best types of allergens to use in a standard skin prick test panel to use in this region.

I CITY /TOWN ____________________ AGE_____________ WEIGHT ___ GENDER M F

II HISTORY

Allergic since Year ____________

For this study you will be tested for 40 different types of allergens.

Do other members in your family have allergies? Mother Father Sibling Child

IV **SYMPTOMS: Which organs are affected?**

Eyes ☐ **Nose** ☐ **Lungs** ☐

1. **Intermittent** ☐ Less than 4 days a week OR for less than 4 weeks
2. **Persistent** ☐ More than 4 days a week AND for more than 4 weeks
3. **Mild** ☐ **NON of the following are present:**
   Sleep disturbance
   Impairment of daily activities, leisure and/or sport
   Impairment of school or work
   Troublesome symptoms
4. **Moderate-severe** ☐ **ONE OR MORE of the following are present**
   Sleep disturbance
   Impairment of daily activities, leisure and/or sport
   Impairment of school or work
   Troublesome symptoms
5. HAY FEVER DIARY (pollendiary.com): This study uses an online diary for patients connencted to the pollen monitoring station at Fatih University. You can record your daily symptoms and obtain information on pollen airborne during those days. This diary will help you manage your allergy better.

6. **I agree to keep a hay fever diary for the duration of the study.**
7. **If necessary, I will allow to have blood sampled for study purposes (in vitro IgE).**

Date **Name** **Surname** **Mobile phone** **Signature**

SYMPTOM SCORES
Record in www.pollendiary.com within 3 days you had symptoms

Eyes	**Problems**	☐ None ☐ Mild ☐ Moderate ☐ Severe	**Symptoms**	☐ Itching ☐ Foreign body sensation ☐ Redness ☐ Watering
Nose	**Problems**	☐ None ☐ Mild ☐ Moderate ☐ Severe	**Symptoms**	☐ Nose Itching ☐ Sneezing ☐ Nose Running ☐ Nose Blocked
Lungs	**Sorunlar**	☐ None ☐ Mild ☐ Moderate ☐ Severe	**Symptoms**	☐ Wheezing ☐ Shortness of Breath ☐ Cough ☐ Asthma
Medication		☐ None ☐ Eye Drops ☐ Nose Spray ☐ Anti-Allergy Tablets ☐ Homepathic Remedy ☐ Other		

Comments

PATIENT QUESTIONNAIRE (TURKISH) SECTION A

ALERJİ ANKETİ

Verdiğiniz bilgiler İstanbul halkı arasındaki hava kaynaklı alerjen hassasiyeti oranı ile ilgili çalışmalarımıza katkı sağlayacaktır ve bölgede kullanılacak standart bir 'Prik Testi Paneli' için uygun alerjen türlerinin belirlenmesine yardımcı olacaktır.

I İL/İLÇE ____________________ YAŞ _____________ KİLO ___ CİNSİYET E K

II **GEÇMİŞ**

Alerjinizi ne zaman farkettiniz Year ____

Bu çalışma için size 40 farklı alerjen Skin Prick testi yapılacaktır

Ailenizin başka bir üyesinde alerjisi olan var mı? Anne Baba Kardeş Çocuk

IV **SEMPTOMLER: Hangi organlarınız etkleniyor**

Göz ☐ **Burun** ☐ **Akciğer** ☐

1. **Aralıklı** ☐ Senede haftada en az 4 gün veya 4 haftadan daha az sürede
2. **Devamlı** ☐ Haftada en fazla 4 gün ve 4 haftadan daha uzun bir sürede
3. **Hafif** ☐ **Aşağıdakilerden OLMAYAN varsa:**
   Uyku bozukluğu
   Günlük aktivitelerdeki halsizlik eğlence ve/veya spor
   İş veya okulda halsizlik
   Sıkıntılı semptom
4. **Orta-Şiddetli** ☐ **Aşağıdakilerden BİR VE YA FAZLASI varsa:**
   Uyku bozukluğu
   Günlük aktivitelerdeki halsizlik eğlence ve/veya spor
   İş veya okulda halsizlik
   Sıkıntılı semptom
5. **ALERJİ GÜNLÜĞÜ** (**pollendiary.com**): Bu çalışma hastaların online günlüğe bağlanıp Fatih Üniversitesindeki polen izleme istasyonunda kullanılacaktır. O günlerde havada asılı olan polenler ile ilgili bilgilerinizi içeren günlük belirtilerinizi kayıt edebilirsiniz . Bu günlük, size alerjinizi daha iyi kontrol etmenize yardımcı olacaktır. Çalışma kodunuzu IPASS dır. Allergi semptom (belirti) görürseniz hemen günlüğe bilgilerinizi girin.

6. **Çalışma boyunca Alerji Günlüğü tutacağımı onaylıyorum.**
7. **Eğer gerekli olursa çalışma için kar örneği vermeyi onaylıyorum.**

Tarih **Adı** **Soyadı** **Telefon** **Email** **Imza**

PATIENT QUESTIONNAIRE (TURKISH) SECTION B

SEMPTOM SKORU DETAILLERI pollen günlüğünden alınmıştır
www.pollendiary.com 3 gün içersinde semptom kayit edin

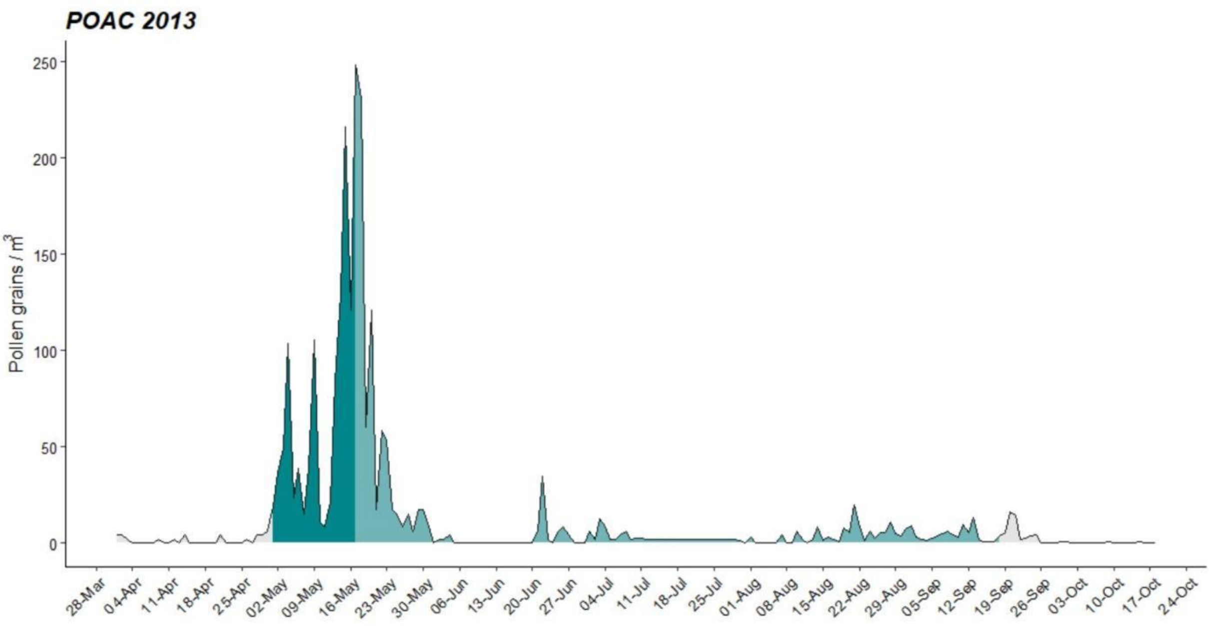
Gözler	**Sorunlar**	☐ Yok	**Semptomlar**	☐ Kaşıntı
		☐ Hafif		☐ Batma
		☐ Orta		☐ Kızarma
		☐ Şiddetli		☐ Sulanma
Burun	**Sorunlar**	☐ Yok	**Semptomlar**	☐ Burun kaşıntı
		☐ Hafif		☐ Hapşırma
		☐ Orta		☐ Burun akıntısı
		☐ Şiddetli		☐ Tıkanma
Akciğerler	**Sorunlar**	☐ Yok	**Semptomlar**	☐ Hırıltı
		☐ Hafif		☐ Nefes tıkanıklığı
		☐ Orta		☐ Öksürük
		☐ Şiddetli		☐ Astım
İlaçlar		☐ Hiçbiri		
		☐ Göz damlası		
		☐ **Burun damlası (ya da spreyi)**		
		☐ Anti histaminik tabletler		
		☐ Homeopatik tedavi		
		☐ Diğer		

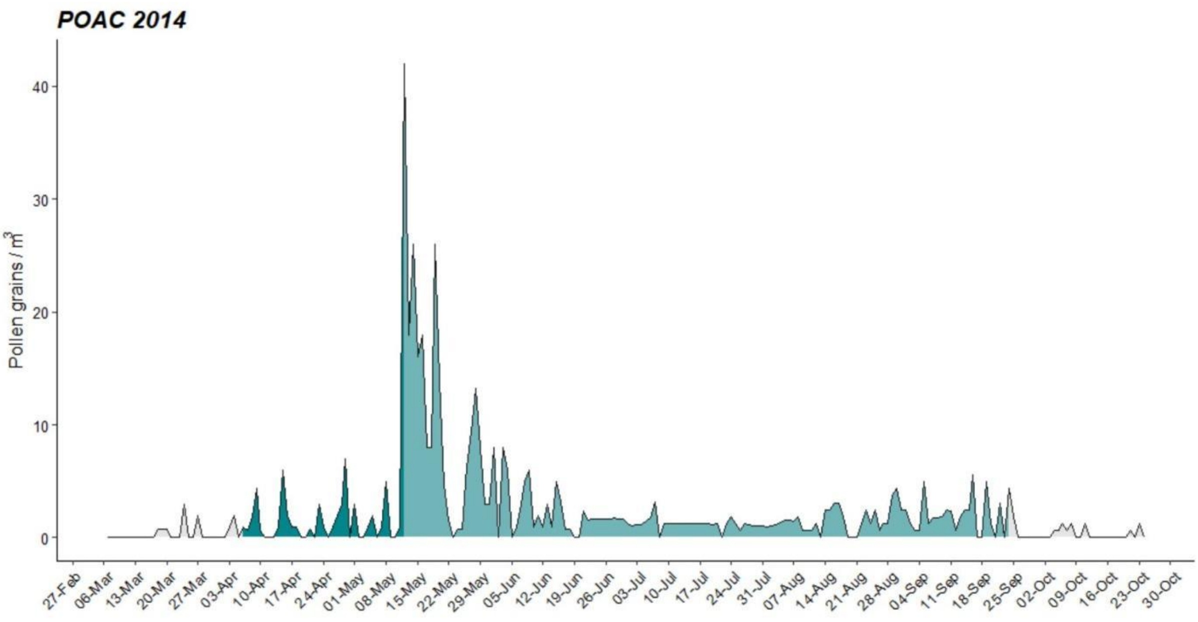
Yorumlar

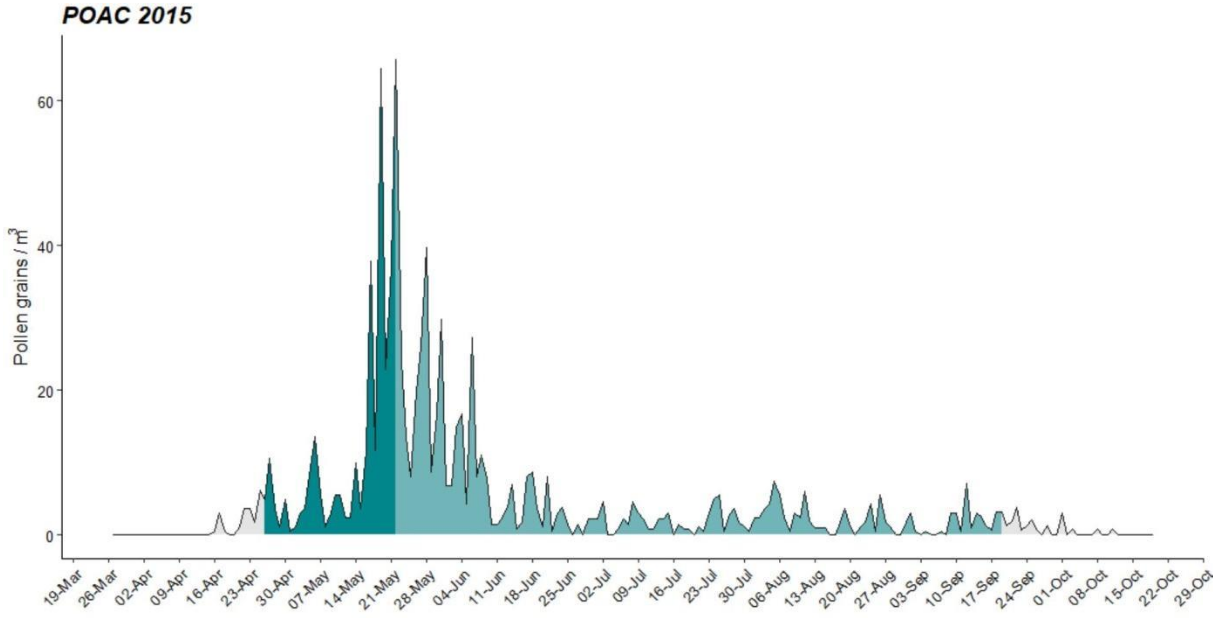
ANNEX II

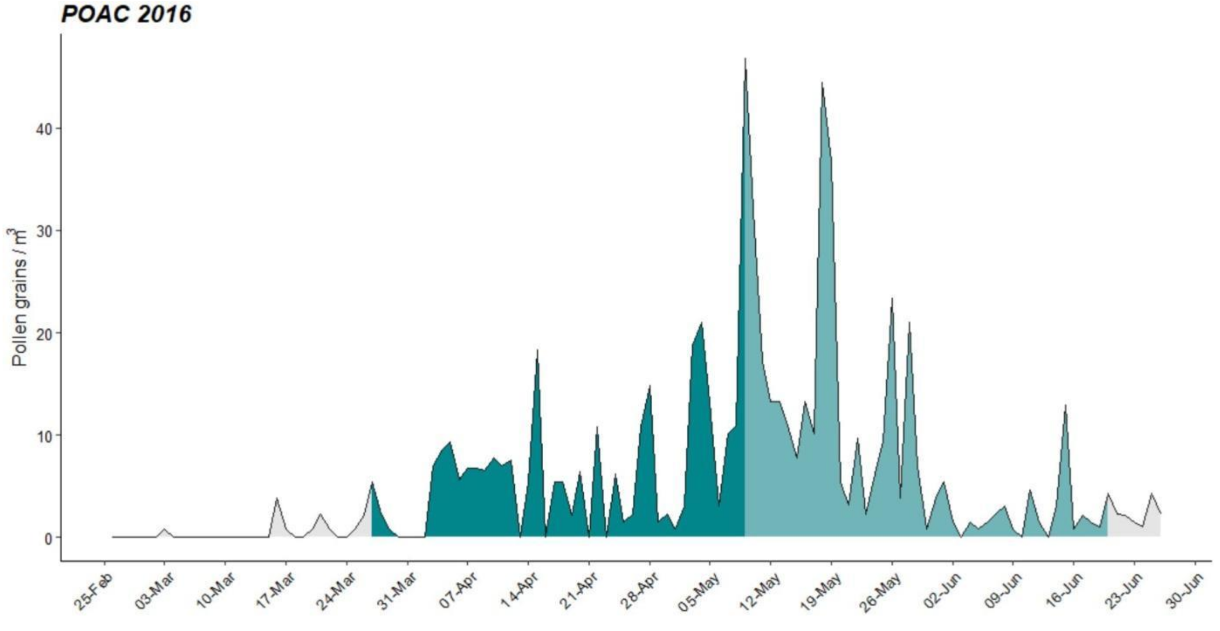
POLLEN CURVES OF POACEAE

The darker area of the curve marks the pre-peak period. No coloured areas are out of the main pollen season.



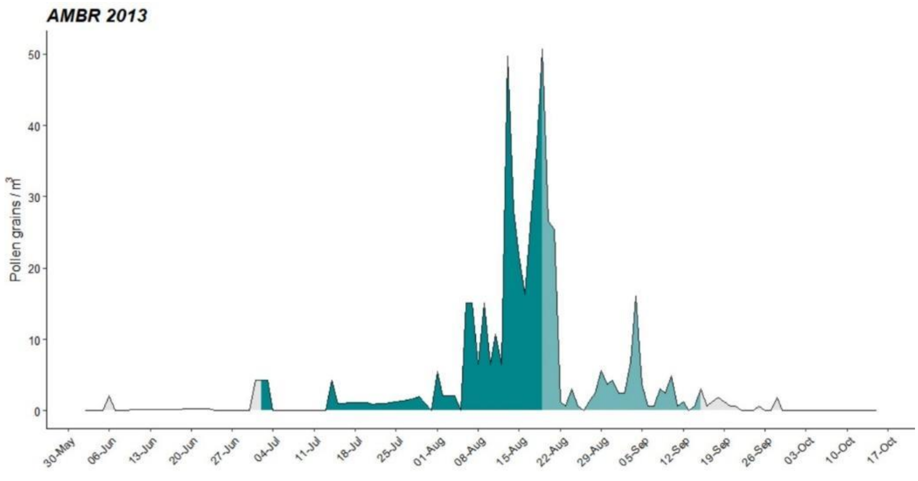


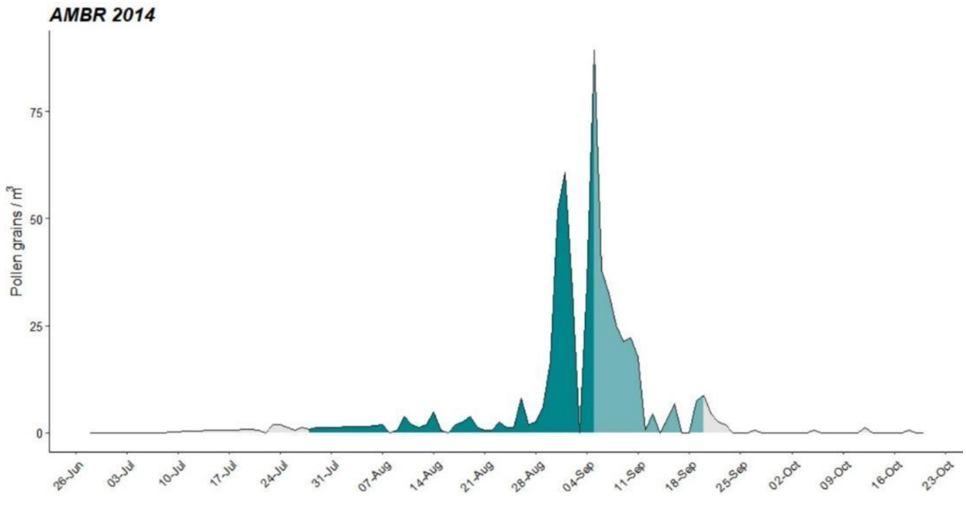

POAC 2015
Pollen grains / m3
0
20
40
60
19-Mar
26-Mar
02-Apr
09-Apr
16-Apr
23-Apr
30-Apr
07-May
14-May
21-May
28-May
04-Jun
11-Jun
18-Jun
25-Jun
02-Jul
09-Jul
16-Jul
23-Jul
30-Jul
06-Aug
13-Aug
20-Aug
27-Aug
03-Sep
10-Sep
17-Sep
24-Sep
01-Oct
08-Oct
15-Oct
22-Oct
29-Oct

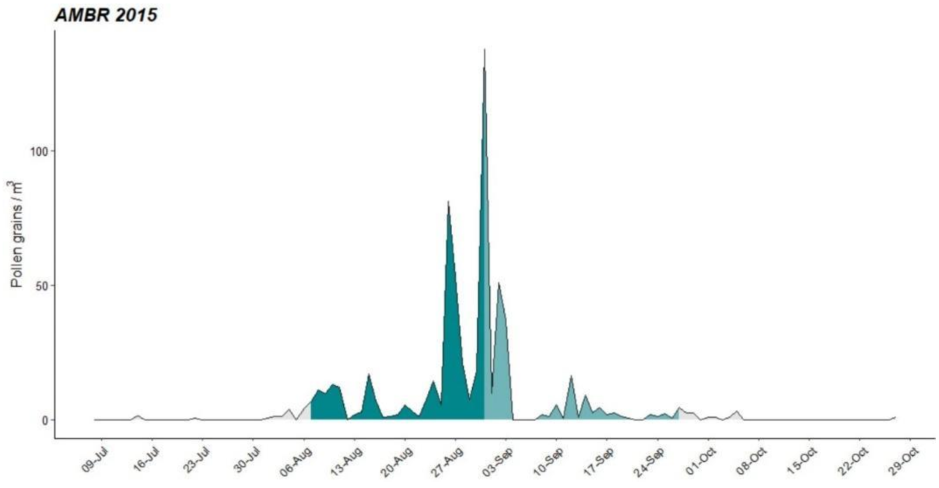

POAC 2016
Pollen grains / m3
0
10
20
30
40
25-Feb
03-Mar
10-Mar
17-Mar
24-Mar
31-Mar
07-Apr
14-Apr
21-Apr
28-Apr
05-May
12-May
19-May
26-May
02-Jun
09-Jun
16-Jun
23-Jun
30-Jun

POLLEN CURVES OF AMBROSIA

The darker area of the curve marks the pre-peak period. No coloured areas are out of the main pollen season.






AMBR 2015
Pollen grains / m3
100
50
0
09-Jul
16-Jul
23-Jul
30-Jul
06-Aug
13-Aug
20-Aug
27-Aug
03-Sep
10-Sep
17-Sep
24-Sep
01-Oct
08-Oct
15-Oct
22-Oct
29-Oct

ANNEX III

EXAMPLES OF GAMS

Total Medicine Score and Total pollen Istanbul

Highlighted in green in Model 48a are the estimate for the calculation of RR with standard error and significance level. Cupressaceae, the main pollen contributor in Istanbul, was added as confounding factor together with PM10, Time, and maximum average daily temperature (MaxT). When the spline function (s) was set on the predictor, the effect curve can be plotted (Model 48aa).

```
Model48a<-
gam(TotalMedicineScore~(TotalPollen)+s(Cupressaceae,k=10,fx=F)+s(PM10,k=10,fx=F)+s(Time,
k=6,fx=F)+s(MaxT,k=10,fx=F),data=IstanbulGAM3,family=quasipoisson(log))
> summary(Model48a)

Family: quasipoisson
Link function: log

Formula:
TotalMedicineScore ~ (TotalPollen) + s(Cupressaceae, k = 10,
   fx = F) + s(PM10, k = 10, fx = F) + s(Time, k = 6, fx = F) +
   s(MaxT, k = 10, fx = F)

Parametric coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) -1.228979   0.085945 -14.300   <2e-16 ***
TotalPollen  0.003689   0.001536   2.402   0.0166 *
---
Signif. codes:
0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Approximate significance of smooth terms:
                  edf Ref.df     F  p-value
s(Cupressaceae) 1.000  1.001 5.505   0.0192 *
s(PM10)         1.971  2.495 0.606   0.4561
s(Time)         2.720  3.301 7.372 4.30e-05 ***
s(MaxT)         3.861  4.828 8.994 5.57e-08 ***
---
Signif. codes:
0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

R-sq.(adj) =  0.103   Deviance explained = 11.4%
GCV = 0.83444  Scale est. = 0.88935    n = 725
```

```
Model48aa<-gam(TotalMedicineScore~s(TotalPollen)+s(Cupressaceae,k=10,fx=F)+
s(PM10,k=10,fx=F)+s(Time,k=6,fx=F)+s(MaxT,k=10,fx=F),
data=IstanbulGAM3,family=quasipoisson(log))
> summary(Model48aa)

Family: quasipoisson
Link function: log

Formula:
TotalMedicineScore ~ s(TotalPollen) + s(Cupressaceae, k = 10,
    fx = F) + s(PM10, k = 10, fx = F) + s(Time, k = 6, fx = F) +
    s(MaxT, k = 10, fx = F)

Parametric coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) -1.10701    0.06405  -17.29   <2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Approximate significance of smooth terms:
                 edf Ref.df     F  p-value
s(TotalPollen)  1.000  1.000 5.767   0.0166 *
s(Cupressaceae) 1.000  1.000 5.507   0.0192 *
s(PM10)         1.971  2.495 0.606   0.4561
s(Time)         2.720  3.301 7.372 4.30e-05 ***
s(MaxT)         3.861  4.828 8.994 5.57e-08 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

R-sq.(adj) =  0.103   Deviance explained = 11.4%
GCV = 0.83444  Scale est. = 0.88935   n = 725
```

Total pollen TMS with Poaceae added

In Model 48b Poaceae was added changing significances of the independent variables Total pollen, Cupressaceae, and PM10.

```
Model48b<-gam(TotalMedicineScore~(TotalPollen)+s(Cupressaceae,k=10,fx=F)+s(Poaceae,k=4,
fx=F)+s(PM10,k=10,fx=F)+s(Time,k=6,fx=F)+s(MaxT,k=10,fx=F),data=IstanbulGAM3,family=qua
sipoisson(log))
> summary(Model48b)

Family: quasipoisson
Link function: log

Formula:
TotalMedicineScore ~ (TotalPollen) + s(Cupressaceae, k = 10,
    fx = F) + s(Poaceae, k = 4, fx = F) + s(PM10, k = 10, fx = F) +
    s(Time, k = 6, fx = F) + s(MaxT, k = 10, fx = F)

Parametric coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept) -1.156e+00  9.168e-02 -12.611   <2e-16 ***
TotalPollen -5.124e-06  1.918e-03  -0.003    0.998
---
Signif. codes:
0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Approximate significance of smooth terms:
                 edf Ref.df     F  p-value
s(Cupressaceae) 1.000  1.001 1.630 0.202140
s(Poaceae)      2.829  2.976 9.016 8.23e-06 ***
s(PM10)         2.326  2.943 0.841 0.400525
s(Time)         4.403  4.837 7.994 5.50e-07 ***
s(MaxT)         3.427  4.318 5.331 0.000251 ***
---
Signif. codes:
0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

R-sq.(adj) =  0.155   Deviance explained = 15.2%
GCV = 0.80836  Scale est. = 0.87358   n = 725
```

RR estimates in Yuvojvod

	YUVOJVOD 2014-2016	
Allergen with additive taxa and significance codes for symptom estimate/medicine use	**RR symptom estimate and standard error**	**RR medicine use estimate and standard error**
Poaceae	NS (negative estimate)	0.020282+-0.009543*
Poaceae with added PM10 (*), Urticaceae (*), Carpinus (NS), Betula (NS), Quercus (NS), Platanus (NS), Morus (NS)	NS	0.030 +-0.010**
Fagales		
Betulaceae		
Betula with PM10		
Carpinus with PM10	0.051+-0.004***	0.016+-0.005886**
Carpinus with Betulaceae	0.037+-0.005***	0.018 +-0.007*
Carpinus with PM10, Fraxinus, Betula, Poaceae	0.040+-0.005***	0.019+-0.007**
Betula with additives	0.011 +-0.003***	NS
Betula with PM10	0.022+-0.002***	NS
Betula with PM10, Acer, Carpinus**		
Betula with PM10, Fraxinus, Carpinus, Poaceae		
Ambrosia	0.048+-0.002***	0.011+-0.004**
Ambrosia with added PM10 (NS)/(*), Urticaceae (*)/(*), Artemisia (.)/(NS), Poaceae (NS)/(**)	0.051+-0.003***	0.011+-0.003***
Quercus with additives	0.046+-0.007***	NS
Urticaceae	NS (negative estimate)	NS (negative estimate)
Urticaceae with added PM10 (NS)/(NS) Corylus (***)/(**), Alnus (**)/, Carpinus (*)/(NS), Betula (NS)/(NS), Poaceae (*)/(***), Fraxinus (**)/(NS), Olea (.)/(NS)		
PM10	0.004+-0.001***	0.004+-0.001***
PM10 with additives	0.002+-0.001*	0.003+-0.001***
Olea	NS	NS

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1