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Short Title - Pollen exposure across Europe: Analysis of 2 decades

Geographic and temporal variations in pollen exposure across Europe

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Key words: Asteraceae, Betulaceae, epidemiology, Oleaceae, Poaceae

ABSTRACT (248 words)

Background: The EC-funded EuroPrevall project examined the prevalence of food allergy across Europe. A well-established factor in the occurrence of food allergy is primary sensitization to pollen.

Objective: To analyse geographic and temporal variations in pollen exposure, allowing investigating how these variations influence the prevalence and incidence of food allergies across Europe.

Methods: Airborne pollen data for two decades (1990-2009) were obtained from 13 monitoring sites located as close as possible to the EuroPrevall survey centres. Start dates, intensity and duration of Betulaceae, Oleaceae, Poaceae and Asteraceae pollen seasons were examined. Mean, slope of the regression, probability level (p) and dominant taxa (%) were calculated. Trends were considered significant at $p < 0.05$.

Results: On a European scale Betulaceae, in particular *Betula*, is the most dominant pollen exposure, 2-fold higher than to Poaceae, and > 5-fold higher than to Oleaceae and Asteraceae. Only in Reykjavik, Madrid and Derby was Poaceae the dominant pollen, as was Oleaceae in Thessaloniki. Weed pollen (Asteraceae) were never dominant, exposure accounted for > 10% of total pollen exposure only in Siauliai (*Artemisia*) and Legnano (*Ambrosia*). Consistent trends towards changing intensity or duration of exposure were not observed, possibly with the exception of (not significant) decreased exposure to *Artemisia* and increased exposure to *Ambrosia*.

Conclusions: This is the first comprehensive study quantifying exposure to the major allergenic pollen families Betulaceae, Oleaceae, Poaceae and Asteraceae across Europe. These data can now be used for studies into patterns of sensitization and allergy to pollen and foods.

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INTRODUCTION

IgE-mediated reactions to foods can occur either as a consequence of primary sensitization to foods or as a cross-reactive phenomenon by primary sensitization to airborne allergen sources, particularly pollen, the oral allergy-syndrome (1, 2). The latter includes reactions to fruits, tree nuts, legumes, vegetables and spices. The main structures responsible for pollen-vegetable food IgE cross-reactivity, listed in decreasing order of clinical relevance for food allergy, are the major birch pollen allergen (Bet v 1) also known as a PR 10 protein, the profilins from grass, tree and weed pollen, and cross-reactive carbohydrate determinants (CCD). Lipid transfer proteins (LTP) are mostly reported as primary sensitizers from food, but have more recently also been described as primary sensitizers in weed pollen, e.g. *Artemisia*, leading to cross-reactive food allergies (2-4).

The EC funded EuroPrevall project set out to examine the prevalence of food allergy across Europe and to determine factors contributing to the onset and persistence of food allergies such as concomitant pollen exposure (5). To this end, cross-sectional (general population and out-patient clinic) and longitudinal (birth cohort) surveys were carried out in up to 13 cities across Europe (6, 7). EuroPrevall focused on twenty-four foods that includes some where the role of cross-reactivity to pollen is well-established, e.g. apple and hazelnut linked to birch pollen Bet v 1 (8, 9) or melon to pollen profilins (10). For many of the other foods, much less is known about the role of cross-reactivity to pollen.

To get better insight into the role of pollen sensitization in the occurrence of cross-reactive allergies to foods across Europe, it is essential to evaluate pollen exposure data on a European scale for a prolonged period of time. This allows qualitative and quantitative differences between countries to be established, which can subsequently be used to search for associations between food sensitization and food allergy found in the EuroPrevall community surveys. In addition, changes over time, possibly linked to climate change or globalization, can be used to explain and predict trends in the incidence of food allergies. In the present study, data of airborne pollen for a 20-year period (1990-2009) were obtained from 13 pollen-monitoring sites that were located as close as possible to the survey centres in EuroPrevall (Fig.1). Centres were chosen to represent different geographic and climatic regions in Europe, including Alpine, Mediterranean, Central, Nordic, and Maritime areas. The focus was on pollen of the Betulaceae and Oleaceae (tree pollen), Poaceae (grass pollen) and Asteraceae (weed pollen).

MATERIALS AND METHODS

Collection and analysis of pollen data

Criteria for selecting the pollen-monitoring sites were: (a) where possible, they were located in the same city as the clinical centres involved in EuroPrevall; (b) if this was not possible then selected sites representative of the region with regard to climate and plant species distribution were identified; (c) the station contained the longest available pollen dataset for the region. Pollen data were accessed via the European Aeroallergen Network database (EAN - https://ean.polleninfo.eu/Ean/). A total of 1888 datasets (years of pollen data), from 24 different pollen types were downloaded from EAN and included in the analysis.

Daily average pollen data for the Betulaceae, Oleaceae, Poaceae and Asteraceae families recorded during the period 1990-2009 were collected at 13 pollen-monitoring sites in Europe (Fig. 1), using volumetric spore traps of the Hirst design (11) situated at roof level. Samples were examined by light microscopy using internationally recognised and comparable methods (12). To assess the duration of the pollen season, the start and end of the pollen season were defined using the 95% method, whereby the season starts on the day when 2.5% of the season's catch had been recorded and the end occurs when 97.5% of the total catch had been reached (13). The intensity of the pollen season was defined as the sum of pollen recorded in a season.

The following genera are included in the Betulaceae family: *Alnus* (alder), *Betula* (birch), *Carpinus* (hornbeam), *Corylus* (hazel) and *Ostrya* (hop-hornbeam). Due to changes in taxonomic classification, the Corylaceae family (containing *Corylus, Carpinus* and *Ostrya*) is now included within an expanded Betulaceae family (14). Betulaceae pollen grains can be identified to genus level, but the pollen-monitoring site at Legnano (near Milano) recorded them as either Betulaceae or Corylaceae (Table 1).

The two genera belonging to the Oleaceae family that are most frequently recorded in the air are *Fraxinus* (ash) and *Olea* (olive). In addition, some sites also recorded *Forsythia*, *Ligustrum* and *Syringa* but these are rarely found in air samples. An alternative classification of "other Oleaceae" pollen was occasionally used for *Forsythia*, *Ligustrum* and *Syringa*. Legnano simply identified Oleaceae pollen to family level until 2009 (Table 2).

Poaceae (grass) pollen grains are morphologically similar, and most can only be identified to family level by light microscopy. However, it is possible to identify some cereals to genus level. Apart from Poaceae, several pollen-monitoring sites included in this study also recorded *Secale cereale* and *Zea mays.* Alternatively, the more generic term "Cerealia pollen type" was used (Table 3).

Pollen grains from the genera *Ambrosia* (ragweed) and *Artemisia* (e.g. mugwort) are identifiable by light microscopy and so are usually recorded separately (note that *Ambrosia* was not present at Reykjavik or Madrid). Some sites record additional taxa such as Cichoriaceae or *Taraxacum*, but more often sites simply use the family name Asteraceae (Compositae) for pollen types not belonging to the *Ambrosia* or *Artemisia* genera (Table 4).

Data preprocessing and statistical analysis

Datasets were examined for missing values and irregularities. Years that contained missing values were removed from the analysis when it was deemed that it had a noticeable effect on the results. For example, the Betulaceae family includes some of the earliest spring flowering trees. Not all pollen-monitoring sites commence monitoring on the 1st January each year and can miss the start of the *Corylus* and *Alnus* pollen seasons. Therefore, it was not always possible to calculate start dates of the season due to missing values (Table 1). However, the same years could still be included in the analysis of season intensity because the dataset still contained the main peaks of the *Alnus* and *Betula* pollen seasons.

The following statistics are presented: the mean, Standard Deviation (SD), slope of the simple linear regression over time, Standard Error of the regression slope (SE), probability level (*p*) and dominant taxa (%). Trends were considered significant with probability levels < 0.05. The calculations were carried out using the statistical software packages SPSS version 12.0.

RESULTS

Overall pollen exposure

Pollen from the Betulaceae family is the most dominant pollen type recorded with total seasonal pollen grains summed up for the 13 centres reaching a mean of 98,180 grains, compared to 42,676 for Poaceae, 19,118 for Oleaceae and only 13,399 for Asteraceae (Fig. 2, Tables 1-4). The sum of mean exposure to the four major families of allergenic pollen (Fig. 2) differed by more than an order of magnitude between the centres with the highest (Zürich) and the lowest (Thessaloniki) exposure.

Betulaceae pollen exposure

The total Betulaceae pollen count was predominantly made up of *Betula* pollen at most sites, with mean *Betula* contributions ranging from 50% at Strasbourg to 100% at Reykjavik (Table 1 and Fig.2). The exceptions were Leiden (49% *Alnus*), Madrid (62% *Alnus*) and Thessaloniki (40% *Carpinus* and 41% *Corylus*). The sites that recorded the most intensive Betulaceae pollen counts were Łodz, Zurich and Prague (Table 1 and Fig.2). The lowest Betulaceae pollen counts were recorded at Reykjavik, Thessaloniki and Madrid. The sites with the longest Betulaceae pollen seasons were Leiden, Legnano and Thessaloniki. The sites with the shortest seasons were Reykjavik, Prague and Strasbourg (Table 1).

Three pollen-monitoring sites had trends towards significantly earlier start dates of the Betulaceae pollen season; these were Reykjavik, Derby and Sofia (Table 1). Of these, only Reykjavik, and Sofia had significantly longer seasons. Betulaceae pollen counts at Reykjavik also showed a significant trend toward more intensive seasons. The only other site where this occurred was Zurich.

Oleaceae pollen exposure

The highest exposure to pollen from the Oleaceae family, almost exclusively to *Fraxinus* pollen, was observed in Zürich, followed by Strasbourg and Sofia (Table 2 and Fig. 2). No Oleaceae pollen was detected in Reykjavik. *Fraxinus* pollen was also found in Madrid, but Oleaceae exposure was dominated by *Olea* (64% ± 24 % (SD)). The sites with the longest Oleaceae pollen seasons were Legnano, Thessaloniki and Madrid where *Olea* pollen is recorded. The shortest Oleaceae pollen seasons were at Siauliai, Łodz, Prague and Zurich.

There were no significant trends in start dates of Oleaceae pollen seasons (Table 2). There was only one significant trend in the duration of Oleaceae pollen season, which was towards shorter seasons in Derby. Similarly, the only significant trend in the amount of Oleaceae recorded annually was at Madrid where seasons were becoming less intense. This did not reach significance when *Olea* pollen exposure was evaluated separately (slope = -73.70, p = 0.117).

Poaceae pollen exposure

Derby, Leiden and Legnano recorded the most Poaceae pollen (Table 3 and Fig.2). Whereas the pollen-monitoring sites included in this study with the lowest Poaceae pollen count were

Reykjavik and Siauliai in the north and Thessaloniki in the south. The longest Poaceae pollen seasons were at Legnano, Thessaloniki and Madrid. The shortest Poaceae pollen seasons were recorded at Reykjavik, Siauliai, and Łodz (Table 3).

Significant trends towards earlier Poaceae pollen seasons were recorded at Reykjavik and Strasbourg. Poaceae pollen seasons at Neustrelitz and Thessaloniki became significantly longer. Conversely, Poaceae pollen seasons at Derby were shown to be becoming significantly shorter. There were also opposite trends observed in the amount of Poaceae pollen recorded annually, with seasons becoming significantly less intense in Leiden and more intense in Legnano (Table 3).

Asteraceae pollen exposure

By far the highest Asteraceae pollen counts were recorded at Legnano, where *Ambrosia* pollen accounts for 90% of the total catch (Table 4 and Fig. 2). The next highest Asteraceae pollen counts were recorded at Siauliai and Prague, where *Artemisia* pollen dominates (mean 97% and 82% at Siauliai and Prague, respectively). The lowest Asteraceae pollen counts were at Reykjavik, Derby and Zurich. The longest Asteraceae pollen seasons were at Derby, Zurich and Madrid. Interestingly, a large proportion of Asteraceae pollen in Zurich (mean 42%) belonged to “other Asteraceae” (not *Ambrosia* or *Artemisia*). These other Asteraceae pollen grains were airborne from April onward and extended the season. The shortest Asteraceae seasons were at Siauliai, Prague and Legnano, where either *Ambrosia* or *Artemisia* predominated.

Start dates in Asteraceae pollen seasons were shown to be getting significantly earlier at Derby (Table 4). There were no significant trends in season duration, but Asteraceae pollen seasons were becoming significantly less intense at Derby, Leiden and Strasbourg. Asteraceae pollen counts at Legnano are increasing and there is a corresponding increase in *Ambrosia* pollen at the site (slope=105.082; *p*=0.147), but the trends are not significant.

DISCUSSION

Pollen count data are considered to be a proxy for aeroallergen exposure (15). A number of studies have examined regional differences in the timing and magnitude of pollen seasons of different allergenic taxa, such as *Alnus* (16), *Betula* (17, 18), *Olea* (19), *Artemisia* (20) and the Poaceae family (21, 22). However, this is the first comprehensive study that quantifies exposure, in terms of the magnitude and duration of pollen seasons across Europe over 20 years, for the Betulaceae, Oleaceae, Poaceae and Asteraceae families. As a consequence of the cross-reactive nature of some of their major and/or minor allergens, these allergenic taxa in Europe (23) have major impact on the prevalence of pollen-associated food allergies.

On a European scale, the greatest exposure was to allergenic pollen from the Betulaceae family. This is largely due to the fact that Betulaceae pollen was the most important at 9 out of the 13 sites included in this study, and mean annual Betulaceae pollen counts exceeded 5000 grains at all 9 of these sites. The second most important pollen type was Poaceae, which was the dominant at 3 out of the 13 sites (Reykjavik, Madrid and Derby). However, in comparison to Betulaceae, mean values of > 5000 Poaceae pollen grains were only recorded at two sites (Derby and Leiden). Mean annual Oleaceae pollen counts only exceeded 5000 grains at Zürich. It is interesting to note that the results of the GA2LEN skin test study showed that the highest sensitization rate to olive was in Zürich (45.5%), where *Fraxinus* is the main Oleaceae pollen in the air (mean contribution 98.6%) (4, 24). In general, Asteraceae accounted for < 10% of the pollen types examined (11 out of 13 centres). The exceptions were Siauliai (16% - mainly *Artemisia*) and Legnano (30% - mainly *Ambrosia*). Legnano was also the only site where mean annual Asteraceae pollen counts were > 5000 grains. It is important to note that, because of their relatively large and heavy pollen grains, numbers of some pollen types like *Ligustrum* (Oleaceae) (25) and *Artemisia*

(Asteraceae) (26) can be underestimated at roof level where monitoring stations are usually situated. However, this is unlikely to change the overall picture of pollen exposure being dominated by Betulaceae and, to a lesser extent, by Poaceae.

In the present study, we have searched for significant changes in exposure to four major families of allergenic pollen. Developments like climate change and urbanization are expected to impact on pollen exposure (27). However, very few consistent trends were actually observed, apart from a trend towards decreased exposure to Asteraceae pollen at 8 out of 13 sites (including 3 significant trends). No consistent changes were observed in the start or duration of pollen seasons. Previous studies have shown changes in the timing of *Betula* pollen seasons in Europe (17, 28). Similar changes were also seen at Reykjavik, Derby and Sofia but these results were not consistent at all sites, possibly because such changes may be slowing or approaching a limit in some places (29). With respect to the duration of pollen seasons, it should be noted that due to the method used for defining season start and end (retrospective 95% method), low amounts of pollen can extend the season at sites with low annual sums, therefore a direct comparison between stations with differing annual sums should be done with caution.

Overall, notable differences in exposure to the four major families of pollen have been observed. These analyses now offer the possibility to investigate the impact of these differences on the patterns of food sensitization and allergy observed in the EuroPrevall project. In a first analysis of sensitization to pollen and foods in EuroPrevall, it has clearly been demonstrated that pollen sensitization is the most dominant factor in determining sensitization to foods (30). The current study now provides the opportunity to carry out a detailed analysis of the associations between pollen exposure, food sensitization and food allergy.

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Authors Contribution to the work:

The project outline was drafted by RvR, MS, SJ, UB and KHS. The data acquisition and coordination of pollen sets was performed by BS, MS SJ, and UB. Finally all the authors MS, SJ, UB, BS, MH, IS, KB, CHP, LW, BMW, OR, MT, RG, MB, RY, AD, DV, AMGB, KHS and RvR provided substantial contributions to conception and design of the study, acquired data or analysed and interpreted data. Subsequently they all provided input to drafting the article and revising it critically for important intellectual content and gave final approval of the version to be published.

Conflict of Interest:

None of the authors declared a conflict of interest in relation to the current manuscript.

FIGURE LEGENDS

Fig. 1. Location of EuroPrevall allergy centres and pollen-monitoring sites

Fig. 2 Pollen exposure across Europe. The size of each circle represents the mean exposure level to all four pollen families (Betulaceae, Oleaceae, Poaceae and Asteraceae) together (mean yearly sum of daily pollen concentrations shown in parenthesis) in the thirteen centres across Europe, ranging from 2,565 in Thessaloniki to 28,177 in Zurich. The pie charts illustrate the relative importance (% indicated) of each of the four main families of allergenic pollen per centre.

REFERENCES

1. Burks AW, Tang M, Sicherer S, Muraro A, Eigenmann PA, Ebisawa M, et al. ICON: Food allergy. *J Allergy Clin Immunol* 2012;**129**(4):906-920.

2. Chapman JA, Bernstein I, Lee RE, Oppenheimer J, Nicklas RA, Portnoy JM, et al. Food allergy: a practice parameter. *Ann Allergy Asthma Immunol* 2006;**96**(3, Suppl. 2).

3. Lombardero M, García-Sellés FJ, Polo F, Jimeno L, Chamorro MJ, García-Casado G, et al. Prevalence of sensitization to *Artemisia* allergens Art v 1, Art v 3 and Art v 60 kDa. Cross-reactivity among Art v 3 and other relevant lipid-transfer protein allergens. *Clin Exp Allergy* 2004;**34**(9):1415-1421.

4. Heinzerling LM, Burbach GJ, Edenharter G, Bachert C, Bindslev-Jensen C, Bonini S, et al. GA2LEN skin test study I: GA²LEN harmonization of skin prick testing: novel sensitization patterns for inhalant allergens in Europe. *Allergy* 2009;**64**(10):1498-1506.

5. Mills ENC, Mackie AR, Burney P, Beyer K, Frewer L, Madsen C, et al. The prevalence, cost and basis of food allergy across Europe. *Allergy* 2007;**62**(7):717-722.

6. McBride D, Keil T, Grabenhenrich L, Dubakiene R, Drasutiene G, Fiocchi A, et al. The EuroPrevall birth cohort study on food allergy: baseline characteristics of 12,000 newborns and their families from nine European countries. *Ped Allergy Immunol* 2012;**23**(3):230-239.

7. Kummeling I, Mills ENC, Clausen M, Dubakiene R, Pérez CF, Fernández-Rivas M, et al. The EuroPrevall surveys on the prevalence of food allergies in children and adults: background and study methodology. *Allergy* 2009;**64**(10):1493-1497.

8. Fernández-Rivas M, Bolhaar S, González-Mancebo E, Asero R, van Leeuwen A, Bohle B, et al. Apple allergy across Europe: How allergen sensitization profiles determine the clinical expression of allergies to plant foods. *J Allergy Clin Immunol* 2006;**118**(2):481-488.

9. Skamstrup Hansen K, Ballmer-Weber BK, Sastre J, Lidholm J, Andersson K, Oberhofer H, et al. Component-resolved in vitro diagnosis of hazelnut allergy in Europe. *J Allergy Clin Immunol* 2009;**123**(5):1134-1141.e1133.

10. Pastor C, Cuesta-Herranz J, Cases B, Pérez-Gordo M, Figueredo E, de las Heras M, et al. Identification of Major Allergens in Watermelon. *Int Arch Allergy Immunol* 2009;**149**(4):291-298.

11. Hirst JM. An automatic volumetric spore trap. *Ann Appl Biol* 1952;**39**(2):257-265.

12. Cariñanos P, Emberlin J, Galán C, Dominguez Vilches E. Comparison of two pollen counting methods of slides from a Hirst type volumetric trap. *Aerobiologia* 2000;**16**(3/4):339-346.

13. Goldberg C, Buch H, Moseholm L, Weeke EV. Airborne pollen records in Denmark, 1977-1986. *Grana* 1988;**27**:209-217.

14. APGIII. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Bot J Linn Soc* 2009;**161**:105-121.

15. Buters JTM, Thibaudon M, Smith M, Kennedy R, Rantio-Lehtimäki A, Albertini R, et al. Release of Bet v 1 from birch pollen from 5 European countries. Results from the HIALINE study. *Atmos Environ* 2012;**55**:496-505.

16. Smith M, Emberlin J, Stach A, Czarnecka-Operacz M, Jenerowicz D, Silny W. The regional importance of *Alnus* pollen as an aeroallergen: A comparative study of Alnus pollen counts from Worcester (UK) and Poznań (Poland). *Ann Agric Environ Med* 2007(14):123-128.

17. Emberlin J, Detandt M, Gehrig R, Jaeger S, Nolard N, Rantio-Lehtimäki A. Responses in the start of *Betula* (birch) pollen seasons to recent changes in spring temperatures across Europe. *Int J Biometeorol* 2002;**46**:159-170.

18. Stach A, Emberlin J, Smith M, Adams-Groom B, Myszkowska D. Factors that determine the severity of *Betula* spp. pollen seasons in Poland (Poznań and Krakow) and the United Kingdom (Worcester and London). *Int J Biometeorol* 2008;**52**(4):311-321.

19. Díaz de la Guardia C, Alba F, Del Mar Trigo M, Galán C, Ruíz L, Sabariego S. Aerobiological analysis of *Olea europaea* L. pollen in different localities of southern Spain. *Grana* 2003;**42**(4):234-243.

20. Grewling Ł, Šikoparija B, Skjøth CA, Radišić P, Apatini D, Magyar D, et al. Variation in Artemisia pollen seasons in Central and Eastern Europe. *Agric For Meteorol* 2012;**160**(0):48-59.

21. Emberlin J, Jaeger S, Dominguez Vilches E, Galan Soldevilla C, Hodal L, Mandrioli P, et al. Temporal and geographical variations in grass pollen seasons in areas of western Europe: and analysis of season dates at sites of the European pollen information system. *Aerobiologia* 2000;**16**:373-379.

22. Smith M, Emberlin J, Stach A, Rantio-Lehtimäki A, Caulton E, Thibaudon M, et al. Influence of the North Atlantic Oscillation on grass pollen counts in Europe. *Aerobiologia* 2009;**25**(4):321-332.

23. Skjøth CA, Šikoparija B, Jäger S, EAN. Pollen Sources. In: Sofiev M, Bergmann K-C, editors. Allergenic Pollen: Springer Netherlands; 2013. p. 9-27.

24. Burbach GJ, Heinzerling LM, Edenharter G, Bachert C, Bindslev-Jensen C, Bonini S, et al. GA2LEN skin test study II: clinical relevance of inhalant allergen sensitizations in Europe. *Allergy* 2009;**64**(10):1507-1515.

25. Cariñanos P, Alcázar P, Galán C, Domínguez E. Privet pollen (Ligustrum sp.) as potential cause of pollinosis in the city of Cordoba, south-west Spain. *Allergy* 2002;**57**(2):92-97.

26. Spieksma FT, van Noort P, Nikkels H. Influence of nearby stands of Artemisia on street-level versus roof-top-level ratio's of airborne pollen quantities. *Aerobiologia* 2000;**16**:21-24.

27. Ziello C, Sparks TH, Estrella N, Belmonte J, Bergmann KC, Bucher E, et al. Changes to airborne pollen counts across europe. *PLoS ONE* 2012;**7**(4): e34076. doi:34010.31371/journal.pone.0034076.

28. Emberlin J, Mullins J, Cordon J, Millington W, Brooke M, Savage M, et al. The trend to earlier birch pollen seasons in the UK: a biotic response to changes in weather conditions? *Grana* 1997;**36**: 29-33.

29. Newnham RM, Sparks TH, Skjøth CA, Head K, Adams-Groom B, Smith M. Pollen season and climate: Is the timing of birch pollen release in the UK approaching its limit? *Int J Biometeorol* 2012;**57**(3):391-400.

30. Burney PGJ, Potts J, Kummeling I, Mills ENC, Clausen M, Dubakiene R, et al. The prevalence and distribution of food sensitization in European adults. *Allergy* 2013:doi: 10.1111/all.12341.

Short Title - Pollen exposure across Europe: Analysis of 2 decades

Geographic and temporal variations in pollen exposure across Europe

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ABSTRACT (248 words)

Background: The EC-funded EuroPrevall project examined the prevalence of food allergy across Europe. A well-established factor in the occurrence of food allergy is primary sensitization to pollen.

Objective: To analyse geographic and temporal variations in pollen exposure, allowing investigating how these variations influence the prevalence and incidence of food allergies across Europe.

Methods: Airborne pollen data for two decades (1990-2009) were obtained from 13 monitoring sites located as close as possible to the EuroPrevall survey centres. Start dates, intensity and duration of Betulaceae, Oleaceae, Poaceae and Asteraceae pollen seasons were examined. Mean, slope of the regression, probability level (p) and dominant taxa (%) were calculated. Trends were considered significant at $p < 0.05$.

Results: On a European scale Betulaceae, in particular *Betula*, is the most dominant pollen exposure, 2-fold higher than to Poaceae, and > 5-fold higher than to Oleaceae and Asteraceae. Only in Reykjavik, Madrid and Derby was Poaceae the dominant pollen, as was Oleaceae in Thessaloniki. Weed pollen (Asteraceae) were never dominant, exposure accounted for > 10% of total pollen exposure only in Siauliai (*Artemisia*) and Legnano (*Ambrosia*). Consistent trends towards changing intensity or duration of exposure were not observed, possibly with the exception of (not significant) decreased exposure to *Artemisia* and increased exposure to *Ambrosia*.

Conclusions: This is the first comprehensive study quantifying exposure to the major allergenic pollen families Betulaceae, Oleaceae, Poaceae and Asteraceae across Europe. These data can now be used for studies into patterns of sensitization and allergy to pollen and foods.

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INTRODUCTION

IgE-mediated reactions to foods can occur either as a consequence of primary sensitization to foods or as a cross-reactive phenomenon by primary sensitization to airborne allergen sources, particularly pollen, the oral allergy-syndrome (1, 2). The latter includes reactions to fruits, tree nuts, legumes, vegetables and spices. The main structures responsible for pollen-vegetable food IgE cross-reactivity, listed in decreasing order of clinical relevance for food allergy, are the major birch pollen allergen (Bet v 1) also known as a PR 10 protein, the profilins from grass, tree and weed pollen, and cross-reactive carbohydrate determinants (CCD). Lipid transfer proteins (LTP) are mostly reported as primary sensitizers from food, but have more recently also been described as primary sensitizers in weed pollen, e.g. *Artemisia*, leading to cross-reactive food allergies (2-4).

The EC funded EuroPrevall project set out to examine the prevalence of food allergy across Europe and to determine factors contributing to the onset and persistence of food allergies such as concomitant pollen exposure (5). To this end, cross-sectional (general population and out-patient clinic) and longitudinal (birth cohort) surveys were carried out in up to 13 cities across Europe (6, 7). EuroPrevall focused on twenty-four foods that includes some where the role of cross-reactivity to pollen is well-established, e.g. apple and hazelnut linked to birch pollen Bet v 1 (8, 9) or melon to pollen profilins (10). For many of the other foods, much less is known about the role of cross-reactivity to pollen.

To get better insight into the role of pollen sensitization in the occurrence of cross-reactive allergies to foods across Europe, it is essential to evaluate pollen exposure data on a European scale for a prolonged period of time. This allows qualitative and quantitative differences between countries to be established, which can subsequently be used to search for associations between food sensitization and food allergy found in the EuroPrevall community surveys. In addition, changes over time, possibly linked to climate change or globalization, can be used to explain and predict trends in the incidence of food allergies. In the present study, data of airborne pollen for a 20-year period (1990-2009) were obtained from 13 pollen-monitoring sites that were located as close as possible to the survey centres in EuroPrevall (Fig.1). Centres were chosen to represent different geographic and climatic regions in Europe, including Alpine, Mediterranean, Central, Nordic, and Maritime areas. The focus was on pollen of the Betulaceae and Oleaceae (tree pollen), Poaceae (grass pollen) and Asteraceae (weed pollen).

MATERIALS AND METHODS

Collection and analysis of pollen data

Criteria for selecting the pollen-monitoring sites were: (a) where possible, they were located in the same city as the clinical centres involved in EuroPrevall; (b) if this was not possible then selected sites representative of the region with regard to climate and plant species distribution were identified; (c) the station contained the longest available pollen dataset for the region. Pollen data were accessed via the European Aeroallergen Network database (EAN - https://ean.polleninfo.eu/Ean/). A total of 1888 datasets (years of pollen data), from 24 different pollen types were downloaded from EAN and included in the analysis.

Daily average pollen data for the Betulaceae, Oleaceae, Poaceae and Asteraceae families recorded during the period 1990-2009 were collected at 13 pollen-monitoring sites in Europe (Fig. 1), using volumetric spore traps of the Hirst design (11) situated at roof level. Samples were examined by light microscopy using internationally recognised and comparable methods (12). To assess the duration of the pollen season, the start and end of the pollen season were defined using the 95% method, whereby the season starts on the day when 2.5% of the season's catch had been recorded and the end occurs when 97.5% of the total catch had been reached (13). The intensity of the pollen season was defined as the sum of pollen recorded in a season.

The following genera are included in the Betulaceae family: *Alnus* (alder), *Betula* (birch), *Carpinus* (hornbeam), *Corylus* (hazel) and *Ostrya* (hop-hornbeam). Due to changes in taxonomic classification, the Corylaceae family (containing *Corylus, Carpinus* and *Ostrya*) is now included within an expanded Betulaceae family (14). Betulaceae pollen grains can be identified to genus level, but the pollen-monitoring site at Legnano (near Milano) recorded them as either Betulaceae or Corylaceae (Table 1).

The two genera belonging to the Oleaceae family that are most frequently recorded in the air are *Fraxinus* (ash) and *Olea* (olive). In addition, some sites also recorded *Forsythia*, *Ligustrum* and *Syringa* but these are rarely found in air samples. An alternative classification of “other Oleaceae” pollen was occasionally used for *Forsythia*, *Ligustrum* and *Syringa*. Legnano simply identified Oleaceae pollen to family level until 2009 (Table 2).

Poaceae (grass) pollen grains are morphologically similar, and most can only be identified to family level by light microscopy. However, it is possible to identify some cereals to genus level. Apart from Poaceae, several pollen-monitoring sites included in this study also recorded *Secale cereale* and *Zea mays.* Alternatively, the more generic term “Cerealia pollen type” was used (Table 3).

Pollen grains from the genera *Ambrosia* (ragweed) and *Artemisia* (e.g. mugwort) are identifiable by light microscopy and so are usually recorded separately (note that *Ambrosia* was not present at Reykjavik or Madrid). Some sites record additional taxa such as Cichoriaceae or *Taraxacum*, but more often sites simply use the family name Asteraceae (Compositae) for pollen types not belonging to the *Ambrosia* or *Artemisia* genera (Table 4).

Data preprocessing and statistical analysis

Datasets were examined for missing values and irregularities. Years that contained missing values were removed from the analysis when it was deemed that it had a noticeable effect on the results. For example, the Betulaceae family includes some of the earliest spring flowering trees. Not all pollen-monitoring sites commence monitoring on the 1st January each year and can miss the start of the *Corylus* and *Alnus* pollen seasons. Therefore, it was not always possible to calculate start dates of the season due to missing values (Table 1). However, the same years could still be included in the analysis of season intensity because the dataset still contained the main peaks of the *Alnus* and *Betula* pollen seasons.

The following statistics are presented: the mean, Standard Deviation (SD), slope of the simple linear regression over time, Standard Error of the regression slope (SE), probability level (p) and dominant taxa (%). Trends were considered significant with probability levels < 0.05. The calculations were carried out using the statistical software packages SPSS version 12.0.

RESULTS

Overall pollen exposure

Pollen from the Betulaceae family is the most dominant pollen type recorded with total seasonal pollen grains summed up for the 13 centres reaching a mean of 98,180 grains, compared to 42,676 for Poaceae, 19,118 for Oleaceae and only 13,399 for Asteraceae (Fig. 2, Tables 1-4). The sum of mean exposure to the four major families of allergenic pollen (Fig. 2) differed by more than an order of magnitude between the centres with the highest (Zürich) and the lowest (Thessaloniki) exposure.

Betulaceae pollen exposure

The total Betulaceae pollen count was predominantly made up of *Betula* pollen at most sites, with mean *Betula* contributions ranging from 50% at Strasbourg to 100% at Reykjavik (Table 1 and Fig.2). The exceptions were Leiden (49% *Alnus*), Madrid (62% *Alnus*) and Thessaloniki (40% *Carpinus* and 41% *Corylus*). The sites that recorded the most intensive Betulaceae pollen counts were Łodz, Zurich and Prague (Table 1 and Fig.2). The lowest Betulaceae pollen counts were recorded at Reykjavik, Thessaloniki and Madrid. The sites with the longest Betulaceae pollen seasons were Leiden, Legnano and Thessaloniki. The sites with the shortest seasons were Reykjavik, Prague and Strasbourg (Table 1).

Three pollen-monitoring sites had trends towards significantly earlier start dates of the Betulaceae pollen season; these were Reykjavik, Derby and Sofia (Table 1). Of these, only Reykjavik, and Sofia had significantly longer seasons. Betulaceae pollen counts at Reykjavik also showed a significant trend toward more intensive seasons. The only other site where this occurred was Zurich.

Oleaceae pollen exposure

The highest exposure to pollen from the Oleaceae family, almost exclusively to *Fraxinus* pollen, was observed in Zürich, followed by Strasbourg and Sofia (Table 2 and Fig. 2). No Oleaceae pollen was detected in Reykjavik. *Fraxinus* pollen was also found in Madrid, but Oleaceae exposure was dominated by *Olea* (64% ± 24 % (SD)). The sites with the longest Oleaceae pollen seasons were Legnano, Thessaloniki and Madrid where *Olea* pollen is recorded. The shortest Oleaceae pollen seasons were at Siauliai, Łodz, Prague and Zurich.

There were no significant trends in start dates of Oleaceae pollen seasons (Table 2). There was only one significant trend in the duration of Oleaceae pollen season, which was towards shorter seasons in Derby. Similarly, the only significant trend in the amount of Oleaceae recorded annually was at Madrid where seasons were becoming less intense. This did not reach significance when *Olea* pollen exposure was evaluated separately (slope = -73.70, p = 0.117).

Poaceae pollen exposure

Derby, Leiden and Legnano recorded the most Poaceae pollen (Table 3 and Fig.2). Whereas the pollen-monitoring sites included in this study with the lowest Poaceae pollen count were

Reykjavik and Siauliai in the north and Thessaloniki in the south. The longest Poaceae pollen seasons were at Legnano, Thessaloniki and Madrid. The shortest Poaceae pollen seasons were recorded at Reykjavik, Siauliai, and Łodz (Table 3).

Significant trends towards earlier Poaceae pollen seasons were recorded at Reykjavik and Strasbourg. Poaceae pollen seasons at Neustrelitz and Thessaloniki became significantly longer. Conversely, Poaceae pollen seasons at Derby were shown to be becoming significantly shorter. There were also opposite trends observed in the amount of Poaceae pollen recorded annually, with seasons becoming significantly less intense in Leiden and more intense in Legnano (Table 3).

Asteraceae pollen exposure

By far the highest Asteraceae pollen counts were recorded at Legnano, where *Ambrosia* pollen accounts for 90% of the total catch (Table 4 and Fig. 2). The next highest Asteraceae pollen counts were recorded at Siauliai and Prague, where *Artemisia* pollen dominates (mean 97% and 82% at Siauliai and Prague, respectively). The lowest Asteraceae pollen counts were at Reykjavik, Derby and Zurich. The longest Asteraceae pollen seasons were at Derby, Zurich and Madrid. Interestingly, a large proportion of Asteraceae pollen in Zurich (mean 42%) belonged to "other Asteraceae" (not *Ambrosia* or *Artemisia*). These other Asteraceae pollen grains were airborne from April onward and extended the season. The shortest Asteraceae seasons were at Siauliai, Prague and Legnano, where either *Ambrosia* or *Artemisia* predominated.

Start dates in Asteraceae pollen seasons were shown to be getting significantly earlier at Derby (Table 4). There were no significant trends in season duration, but Asteraceae pollen seasons were becoming significantly less intense at Derby, Leiden and Strasbourg. Asteraceae pollen counts at Legnano are increasing and there is a corresponding increase in *Ambrosia* pollen at the site (slope=105.082; *p*=0.147), but the trends are not significant.

DISCUSSION

Pollen count data are considered to be a proxy for aeroallergen exposure (15). A number of studies have examined regional differences in the timing and magnitude of pollen seasons of different allergenic taxa, such as *Alnus* (16), *Betula* (17, 18), *Olea* (19), *Artemisia* (20) and the Poaceae family (21, 22). However, this is the first comprehensive study that quantifies exposure, in terms of the magnitude and duration of pollen seasons across Europe over 20 years, for the Betulaceae, Oleaceae, Poaceae and Asteraceae families. As a consequence of the cross-reactive nature of some of their major and/or minor allergens, these allergenic taxa in Europe (23) have major impact on the prevalence of pollen-associated food allergies.

On a European scale, the greatest exposure was to allergenic pollen from the Betulaceae family. This is largely due to the fact that Betulaceae pollen was the most important at 9 out of the 13 sites included in this study, and mean annual Betulaceae pollen counts exceeded 5000 grains at all 9 of these sites. The second most important pollen type was Poaceae, which was the dominant at 3 out of the 13 sites (Reykjavik, Madrid and Derby). However, in comparison to Betulaceae, mean values of > 5000 Poaceae pollen grains were only recorded at two sites (Derby and Leiden). Mean annual Oleaceae pollen counts only exceeded 5000 grains at Zürich. It is interesting to note that the results of the GA2LEN skin test study showed that the highest sensitization rate to olive was in Zürich (45.5%), where *Fraxinus* is the main Oleaceae pollen in the air (mean contribution 98.6%) (4, 24). In general, Asteraceae accounted for < 10% of the pollen types examined (11 out of 13 centres). The exceptions were Siauliai (16% - mainly *Artemisia*) and Legnano (30% - mainly *Ambrosia*). Legnano was also the only site where mean annual Asteraceae pollen counts were > 5000 grains. It is important to note that, because of their relatively large and heavy pollen grains, numbers of some pollen types like *Ligustrum* (Oleaceae) (25) and *Artemisia*

(Asteraceae) (26) can be underestimated at roof level where monitoring stations are usually situated. However, this is unlikely to change the overall picture of pollen exposure being dominated by Betulaceae and, to a lesser extent, by Poaceae.

In the present study, we have searched for significant changes in exposure to four major families of allergenic pollen. Developments like climate change and urbanization are expected to impact on pollen exposure (27). However, very few consistent trends were actually observed, apart from a trend towards decreased exposure to Asteraceae pollen at 8 out of 13 sites (including 3 significant trends). No consistent changes were observed in the start or duration of pollen seasons. Previous studies have shown changes in the timing of *Betula* pollen seasons in Europe (17, 28). Similar changes were also seen at Reykjavik, Derby and Sofia but these results were not consistent at all sites, possibly because such changes may be slowing or approaching a limit in some places (29). With respect to the duration of pollen seasons, it should be noted that due to the method used for defining season start and end (retrospective 95% method), low amounts of pollen can extend the season at sites with low annual sums, therefore a direct comparison between stations with differing annual sums should be done with caution.

Overall, notable differences in exposure to the four major families of pollen have been observed. These analyses now offer the possibility to investigate the impact of these differences on the patterns of food sensitization and allergy observed in the EuroPrevall project. In a first analysis of sensitization to pollen and foods in EuroPrevall, it has clearly been demonstrated that pollen sensitization is the most dominant factor in determining sensitization to foods (30). The current study now provides the opportunity to carry out a detailed analysis of the associations between pollen exposure, food sensitization and food allergy.

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Authors Contribution to the work:

The project outline was drafted by RvR, MS, SJ, UB and KHS. The data acquisition and coordination of pollen sets was performed by BS, MS SJ, and UB. Finally all the authors MS, SJ, UB, BS, MH, IS, KB, CHP, LW, BMW, OR, MT, RG, MB, RY, AD, DV, AMGB, KHS and RvR provided substantial contributions to conception and design of the study, acquired data or analysed and interpreted data. Subsequently they all provided input to drafting the article and revising it critically for important intellectual content and gave final approval of the version to be published.

Conflict of Interest:

None of the authors declared a conflict of interest in relation to the current manuscript.

FIGURE LEGENDS

Fig. 1. Location of EuroPrevall allergy centres and pollen-monitoring sites

Fig. 2 Pollen exposure across Europe. The size of each circle represents the mean exposure level to all four pollen families (Betulaceae, Oleaceae, Poaceae and Asteraceae) together (mean yearly sum of daily pollen concentrations shown in parenthesis) in the thirteen centres across Europe, ranging from 2,565 in Thessaloniki to 28,177 in Zurich. The pie charts illustrate the relative importance (% indicated) of each of the four main families of allergenic pollen per centre.

REFERENCES

1. Burks AW, Tang M, Sicherer S, Muraro A, Eigenmann PA, Ebisawa M, et al. ICON: Food allergy. *J Allergy Clin Immunol* 2012;**129**(4):906-920.

2. Chapman JA, Bernstein I, Lee RE, Oppenheimer J, Nicklas RA, Portnoy JM, et al. Food allergy: a practice parameter. *Ann Allergy Asthma Immunol* 2006;**96**(3, Suppl. 2).

3. Lombardero M, García-Sellés FJ, Polo F, Jimeno L, Chamorro MJ, García-Casado G, et al. Prevalence of sensitization to *Artemisia* allergens Art v 1, Art v 3 and Art v 60 kDa. Cross-reactivity among Art v 3 and other relevant lipid-transfer protein allergens. *Clin Exp Allergy* 2004;**34**(9):1415-1421.

4. Heinzerling LM, Burbach GJ, Edenharter G, Bachert C, Bindslev-Jensen C, Bonini S, et al. GA2LEN skin test study I: GA²LEN harmonization of skin prick testing: novel sensitization patterns for inhalant allergens in Europe. *Allergy* 2009;**64**(10):1498-1506.

5. Mills ENC, Mackie AR, Burney P, Beyer K, Frewer L, Madsen C, et al. The prevalence, cost and basis of food allergy across Europe. *Allergy* 2007;**62**(7):717-722.

6. McBride D, Keil T, Grabenhenrich L, Dubakiene R, Drasutiene G, Fiocchi A, et al. The EuroPrevall birth cohort study on food allergy: baseline characteristics of 12,000 newborns and their families from nine European countries. *Ped Allergy Immunol* 2012;**23**(3):230-239.

7. Kummeling I, Mills ENC, Clausen M, Dubakiene R, Pérez CF, Fernández-Rivas M, et al. The EuroPrevall surveys on the prevalence of food allergies in children and adults: background and study methodology. *Allergy* 2009;**64**(10):1493-1497.

8. Fernández-Rivas M, Bolhaar S, González-Mancebo E, Asero R, van Leeuwen A, Bohle B, et al. Apple allergy across Europe: How allergen sensitization profiles determine the clinical expression of allergies to plant foods. *J Allergy Clin Immunol* 2006;**118**(2):481-488.

9. Skamstrup Hansen K, Ballmer-Weber BK, Sastre J, Lidholm J, Andersson K, Oberhofer H, et al. Component-resolved in vitro diagnosis of hazelnut allergy in Europe. *J Allergy Clin Immunol* 2009;**123**(5):1134-1141.e1133.

10. Pastor C, Cuesta-Herranz J, Cases B, Pérez-Gordo M, Figueredo E, de las Heras M, et al. Identification of Major Allergens in Watermelon. *Int Arch Allergy Immunol* 2009;**149**(4):291-298.

11. Hirst JM. An automatic volumetric spore trap. *Ann Appl Biol* 1952;**39**(2):257-265.

12. Cariñanos P, Emberlin J, Galán C, Dominguez Vilches E. Comparison of two pollen counting methods of slides from a Hirst type volumetric trap. *Aerobiologia* 2000;**16**(3/4):339-346.

13. Goldberg C, Buch H, Moseholm L, Weeke EV. Airborne pollen records in Denmark, 1977-1986. *Grana* 1988;**27**:209-217.

14. APGIII. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Bot J Linn Soc* 2009;**161**:105-121.

15. Buters JTM, Thibaudon M, Smith M, Kennedy R, Rantio-Lehtimäki A, Albertini R, et al. Release of Bet v 1 from birch pollen from 5 European countries. Results from the HIALINE study. *Atmos Environ* 2012;**55**:496-505.

16. Smith M, Emberlin J, Stach A, Czarnecka-Operacz M, Jenerowicz D, Silny W. The regional importance of *Alnus* pollen as an aeroallergen: A comparative study of Alnus pollen counts from Worcester (UK) and Poznań (Poland). *Ann Agric Environ Med* 2007(14):123-128.

17. Emberlin J, Detandt M, Gehrig R, Jaeger S, Nolard N, Rantio-Lehtimäki A. Responses in the start of *Betula* (birch) pollen seasons to recent changes in spring temperatures across Europe. *Int J Biometeorol* 2002;**46**:159-170.

18. Stach A, Emberlin J, Smith M, Adams-Groom B, Myszkowska D. Factors that determine the severity of *Betula* spp. pollen seasons in Poland (Poznań and Krakow) and the United Kingdom (Worcester and London). *Int J Biometeorol* 2008;**52**(4):311-321.

19. Díaz de la Guardia C, Alba F, Del Mar Trigo M, Galán C, Ruíz L, Sabariego S. Aerobiological analysis of *Olea europaea* L. pollen in different localities of southern Spain. *Grana* 2003;**42**(4):234-243.

20. Grewling Ł, Šikoparija B, Skjøth CA, Radišić P, Apatini D, Magyar D, et al. Variation in Artemisia pollen seasons in Central and Eastern Europe. *Agric For Meteorol* 2012;**160**(0):48-59.

21. Emberlin J, Jaeger S, Dominguez Vilches E, Galan Soldevilla C, Hodal L, Mandrioli P, et al. Temporal and geographical variations in grass pollen seasons in areas of western Europe: and analysis of season dates at sites of the European pollen information system. *Aerobiologia* 2000;**16**:373-379.

22. Smith M, Emberlin J, Stach A, Rantio-Lehtimäki A, Caulton E, Thibaudon M, et al. Influence of the North Atlantic Oscillation on grass pollen counts in Europe. *Aerobiologia* 2009;**25**(4):321-332.

23. Skjøth CA, Šikoparija B, Jäger S, EAN. Pollen Sources. In: Sofiev M, Bergmann K-C, editors. Allergenic Pollen: Springer Netherlands; 2013. p. 9-27.

24. Burbach GJ, Heinzerling LM, Edenharter G, Bachert C, Bindslev-Jensen C, Bonini S, et al. GA2LEN skin test study II: clinical relevance of inhalant allergen sensitizations in Europe. *Allergy* 2009;**64**(10):1507-1515.

25. Cariñanos P, Alcázar P, Galán C, Domínguez E. Privet pollen (Ligustrum sp.) as potential cause of pollinosis in the city of Cordoba, south-west Spain. *Allergy* 2002;**57**(2):92-97.

26. Spieksma FT, van Noort P, Nikkels H. Influence of nearby stands of Artemisia on street-level versus roof-top-level ratio's of airborne pollen quantities. *Aerobiologia* 2000;**16**:21-24.

27. Ziello C, Sparks TH, Estrella N, Belmonte J, Bergmann KC, Bucher E, et al. Changes to airborne pollen counts across europe. *PLoS ONE* 2012;**7**(4): e34076. doi:34010.31371/journal.pone.0034076.

28. Emberlin J, Mullins J, Cordon J, Millington W, Brooke M, Savage M, et al. The trend to earlier birch pollen seasons in the UK: a biotic response to changes in weather conditions? *Grana* 1997;**36**: 29-33.

29. Newnham RM, Sparks TH, Skjøth CA, Head K, Adams-Groom B, Smith M. Pollen season and climate: Is the timing of birch pollen release in the UK approaching its limit? *Int J Biometeorol* 2012;**57**(3):391-400.

30. Burney PGJ, Potts J, Kummeling I, Mills ENC, Clausen M, Dubakiene R, et al. The prevalence and distribution of food sensitization in European adults. *Allergy* 2013:doi: 10.1111/all.12341.

Table 1. Start dates, intensity (sum of pollen recorded in the season) and duration of Betulaceae pollen seasons recorded at 13 pollen-monitoring stations included in this study. The following statistics are included: the mean, Standard Deviation (SD), slope of the regression over time, Standard Error of the regression slope (SE), probability level (*p*) and number of years in the analysis (N).

Pollen-monitoring station	Pollen types	Start (DOY)						Sum Year (grains)						Duration (days)						Most dominant taxa	
		Mean	SD	Slope	SE	*p*	N	Mean	SD	Slope	SE	*p*	N	Mean	SD	Slope	SE	*p*	N	Taxa	Mean %
Reykjavik[1]	a, b	142	8.2	-1.0	**0.2**	**0.000**	20	512	545	46.6	**18.7**	**0.023**	20	38	13.8	1.1	**0.5**	**0.034**	20	b	100
Siauliai	a, b, c, d	78	-	-	-	-	6	8973	-	-	-	-	6	63	-	-	-	-	6	b	73
Neustrelitz	a, b, c, d	53	20.6	-0.2	1.1	0.878	16	9870	5846	-240.8	292.4	0.423	17	75	19.7	-0.1	1.0	0.925	16	b	58
Derby	a, b, c, d	49	18.0	-1.8	**0.6**	**0.005**	19	3622	2313	116.0	89.5	0.212	19	83	12.5	-0.4	0.5	0.393	19	b	68
Leiden	a, b, c, d	35	19.5	-1.0	0.8	0.235	19	7865	3464	-187.5	130.7	0.169	20	102	41.7	0.3	1.8	0.846	19	a	49
Łodz	a, b, c, d	67	-	-	-	-	4	17325	-	-	-	-	4	59	-	-	-	-	4	b	79
Prague[2]	a, b, c, d, e	72	-	-	-	-	7	10821	5241	44.7	283.7	0.877	15	52	-	-	-	-	7	b	84
Strasbourg[2]	a, b, c, d	56	-	-	-	-	6	9281	5222	141.5	217.2	0.525	17	79	-	-	-	-	6	b	50
Zürich	a, b, c, d, e	39	20.3	0.1	0.8	0.921	20	14921	4948	454.1	**165.6**	**0.013**	20	87	18.4	0.1	0.7	0.852	20	b	66
Legnano[3]	a, b, c, d, e, f, g	41	9.3	-0.1	0.6	0.824	14	8311	4498	470.6	246.6	0.079	15	107	19.6	-0.8	1.2	0.502	14	f	67
Sofia	a, b, c, d	71	24.8	-5.7	**1.2**	**0.001**	11	5904	2021	165.5	174.0	0.366	11	56	25.6	6.0	**1.2**	**0.001**	11	b	74
Thessaloniki	a, b, c, d	48	8.2	-0.0	0.3	0.989	20	533	357	26.5	12.8	0.053	20	97	12.8	-0.6	0.5	0.237	20	c d	40 41
Madrid[4]	a, b, d	-	-	-	-	-	-	169	99	-1.16	5.6	0.839	15	-	-	-	-	-	-	a	62

Pollen types – *Alnus*[a], *Betula*[b], *Carpinus*[c], *Corylus*[d], *Ostrya*[e], Betulaceae (*Alnus* + *Betula*)[f], Corylaceae (*Corylus* + *Carpinus* + *Ostrya*)[g]

[1]The majority of Betulaceae pollen recorded at Reykjavik was from *Betula* (99.8%) and only a small amount of *Alnus* pollen was registered.

[2]Site regularly commenced pollen-monitoring after the start of *Corylus* flowering and so no trend can be shown. Sum of pollen reflects mainly *Alnus* and *Betula*.

[3]Legnano changed from simply recording Betulaceae and Corylaceae pollen to recording *Alnus*, *Betula*, *Carpinus*, *Corylus* and *Ostrya* pollen in 2009.

[4]Betulaceae pollen counts at Madrid are intermittent and highly variable (from 33 to 356 grains in a year), it was therefore not possible to calculate start and end of the season.

Table 2. Start dates, intensity (sum of pollen recorded in the season) and duration of Oleaceae pollen seasons recorded at 13 pollen-monitoring stations included in this study. The following statistics are included: the mean, Standard Deviation (SD), slope of the regression over time, Standard Error of the regression slope (SE), probability level (*p*) and number of years in the analysis (N).

Pollen-monitoring station	Pollen types	Start (DOY)						Sum Year (grains)						Duration (days)						Most dominant taxa	
		Mean	SD	Slope	SE	*p*	N	Mean	SD	Slope	SE	*p*	N	Mean	SD	Slope	SE	*p*	N	Taxa	Mean %
Reykjavik[1]	NA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Siauliai	i	113	-	-	-	-	5	200	-	-	-	-	5	32	-	-	-	-	5	i	100
Neustrelitz	h, i, j,	95	9.9	-0.3	0.5	0.604	16	348	233	5.8	11.8	0.631	16	40	26.4	-1.2	1.3	0.374	16	i	99
Derby	h, i, j, n	83	16.0	0.4	0.6	0.525	20	913	681	31.7	26.1	0.240	20	57	33.1	-3.4	1.0	**0.004**	20	i	94
Leiden	h, i, j	81	15.6	0.7	0.6	0.296	19	1300	922	-7.6	36.9	0.840	19	67	41.4	-2.1	1.6	0.201	19	i	96
Łodz	i	103	-	-	-	-	4	990	-	-	-	-	4	34	-	-	-	-	4	i	100
Prague	i, j,	89	11.5	0.2	0.6	0.737	15	1543	926	-12.9	50.0	0.801	15	35	20.7	-1.6	1.0	0.142	15	i	99
Strasbourg	h, i, j,	75	14.4	0.3	0.7	0.669	15	2250	1268	24.7	54.3	0.656	17	48	25.1	-0.5	1.3	0.696	15	i	99
Zurich[2]	i, m	86	10.7	0.2	0.4	0.659	19	5750	3261	42.5	135.9	0.759	19	35	13.6	-0.1	0.6	0.875	19	i	99
Legnano	i, j, k, l, m	76	12.9	-0.7	0.8	0.413	15	1065	894	82.4	50.5	0.127	15	94	17.4	0.1	1.1	0.953	15	l	100
Sofia	i, j,	77	15.3	-1.1	1.0	0.275	12	2036	1408	65.5	86.8	0.466	13	41	14.1	0.6	0.9	0.530	12	i	99
Thessaloniki	k	94	12.7	-0.2	0.5	0.752	20	853	407	20.0	15.5	0.214	20	78	15.3	0.0	0.6	0.951	20	k	100
Madrid	i, j, k	20	9.5	0.3	0.7	0.668	13	1870	815	-113.2	39.9	**0.015**	14	158	19.2	-0.3	1.3	0.826	13	k	64

Pollen types – *Forsythia*[h], *Fraxinus*[i], *Ligustrum*[j], *Olea*[k], Oleaceae[l], other Oleaceae[m], *Syringa*[n]

[1]No Oleaceae pollen recorded at Reykjavik

[2]Note that only *Fraxinus excelsior* pollen grains are recorded as "*Fraxinus*" pollen in Zurich. Low amounts of *Fraxinus ornus* pollen are also seen in the samples, but these are recorded as "other Oleaceae".

Table 3. Start dates, intensity (sum of pollen recorded in the season) and duration of Poaceae pollen seasons recorded at 13 pollen-monitoring stations included in this study. The following statistics are included: the mean, Standard Deviation (SD), slope of the regression over time, Standard Error of the regression slope (SE), probability level (p) and number of years in the analysis (N).

Pollen-monitoring station	Pollen types	Start (DOY)						Sum Year (grains)						Duration (days)						Most dominant taxa	
		Mean	SD	Slope	SE	*p*	N	Mean	SD	Slope	SE	*p*	N	Mean	SD	Slope	SE	*p*	N	Taxa	Mean %
Reykjavik	p	174	6.0	-0.5	0.2	**0.020**	20	1747	707	35.6	26.9	0.202	20	69	9.4	-0.4	0.4	0.254	20	p	100
Siauliai	p, q	158	-	-	-	-	6	2007	-	-	-	-	6	83	-	-	-	-	6	p	99
Neustrelitz	p, q, r	142	9.6	-0.7	0.4	0.141	14	3100	1059	-52.2	47.8	0.291	18	93	20.6	2.7	0.7	**0.001**	14	p	97
Derby	p	145	10.6	0.1	0.4	0.841	20	5467	1995	85.3	76.9	0.282	20	91	19.5	-2.2	0.6	**0.002**	20	p	100
Leiden	p	133	9.7	-0.5	0.4	0.220	20	5122	1898	-231.8	52.3	**0.000**	20	118	10.5	0.4	0.4	0.305	20	p	100
Łodz[1]	o, p, q	142	-	-	-	-	2	3874	-	-	-	0.681	5	82	-	-	-	-	2	p	95
Prague	p, q, r	136	6.7	-0.7	0.4	0.138	13	2946	1758	-211.6	101.3	0.061	13	92	17.2	0.3	1.2	0.822	13	p	98
Strasbourg	p	130	7.7	-0.9	0.3	**0.021**	11	2942	1145	-40.8	48.7	0.414	18	94	19.5	-1.3	1.0	0.221	11	p	100
Zürich	p, q	133	5.7	-0.2	0.2	0.290	20	3753	788	51.6	28.9	0.091	20	96	10.6	-0.1	0.4	0.793	20	p	100
Legnano	p	105	7.6	-0.2	0.5	0.650	15	4250	1551	182.1	81.9	**0.044**	15	148	13.7	0.3	0.8	0.736	15	p	100
Sofia	p	126	11.6	0.1	0.6	0.874	16	3736	1601	-101.8	64.9	0.136	18	114	16.1	1.0	0.7	0.183	16	p	100
Thessaloniki	p	98	16.1	0.0	0.6	0.998	20	591	339	24.3	12.2	0.063	20	152	38.7	3.0	1.4	**0.045**	20	p	100
Madrid[1]	p	58	-	-	-	-	4	3141	2123	-95.9	126.1	0.464	12	211	-	-	-	-	4	p	100

Pollen types – Cerealia[o], Poaceae[p], *Secale*[q], *Zea mays*[r]

[1]Monitoring station did not operate continuously and so it was not always possible to calculate start and end of the season. However, it was still possible to calculate intensity.

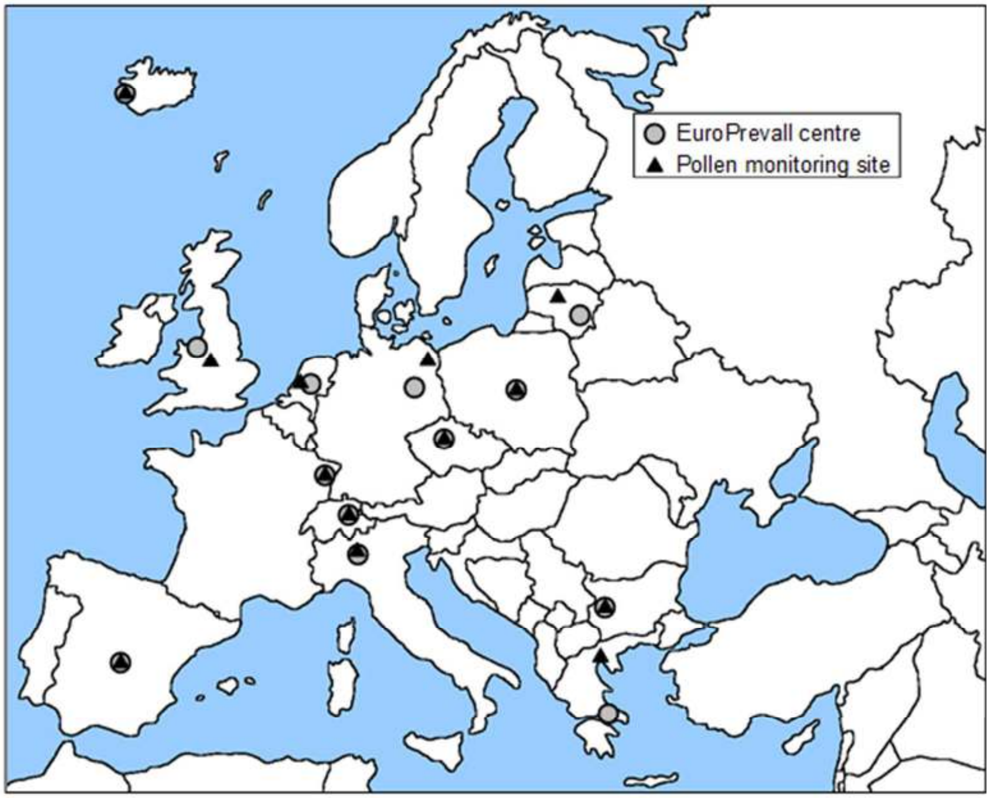
Table 4. Start dates, intensity (sum of pollen recorded in the season) and duration of Asteraceae pollen seasons recorded at 13 pollen-monitoring stations included in this study. The following statistics are included: the mean, Standard Deviation (SD), slope of the regression over time, Standard Error of the regression slope (SE), probability level (p) and number of years in the analysis (N).

Pollen-monitoring station	Pollen types	Start (DOY)						Sum Year (grains)						Duration (days)						Most dominant taxa	
		Mean	SD	Slope	SE	*p*	N	Mean	SD	Slope	SE	*p*	N	Mean	SD	Slope	SE	*p*	N	Taxa	Mean %
Reykjavik[1]	t, u, v, x	161	-	-	-	-	5	54	-	-	-	-	5	90	-	-	-	-	5	v + x	92
Siauliai	s, t, u	202	-	-	-	-	7	2168	-	-	-	-	7	40	-	-	-	-	7	t	97
Neustrelitz	s, t, u, v	181	22.0	0.2	1.1	0.830	15	777	261	-17.2	12.0	0.174	15	67	27.1	1.6	1.3	0.222	15	t	88
Derby	s, t, x	153	33.9	-3.4	1.1	**0.005**	20	80	42	-3.6	1.5	**0.022**	20	115	43.0	1.3	1.7	0.440	20	t	97
Leiden	s, t, u	167	28.8	-0.2	1.1	0.850	20	491	320	-46.6	6.1	**0.000**	19	84	29.3	0.7	1.2	0.545	20	t	87
Łodz[1]	s, t	191	-	-	-	-	3	994	-	-	-	-	3	64	-	-	-	-	3	t	85
Prague	s, t, u, v	197	13.1	0.9	0.7	0.229	15	1534	1126	-107.6	54.7	0.069	16	60	17.0	-0.9	1.0	0.387	15	t	82
Strasbourg	s, t, u, v	147	29.2	-0.7	1.2	0.568	19	237	112	-12.8	3.3	**0.001**	19	105	32.5	0.5	1.3	0.685	19	t	74
Zürich	s, t, u	122	17.4	-0.5	0.7	0.452	20	82	35	1.1	1.4	0.418	20	138	18.4	0.9	0.7	0.199	20	u	42
																				t	33
Legnano	s, t, u	219	3.6	-0.2	0.2	0.441	15	5393	1224	71.7	73.3	0.346	15	53	7.4	0.1	0.5	0.783	15	s	90
Sofia	s, t, u, v	188	13.4	0.3	1.0	0.744	13	1281	416	-29.3	31.0	0.365	13	78	15.7	0.5	1.2	0.713	13	s	42
																				t	54
Thessaloniki	s, t, u, v	124	35.5	0.2	1.5	0.919	19	148	63	-1.3	2.7	0.636	19	152	39.3	0.3	1.7	0.881	19	s	45
																				t	36
Madrid[1]	t, w	92	50.3	-3.5	2.8	0.227	15	160	130	-13.1	6.7	0.070	15	196	67.3	0.7	3.9	0.868	15	w	70

Pollen types – *Ambrosia*[s], *Artemisia*[t], Asteraceae[u], Cichoriaceae[v], Compositae (Asteraceae)[w], *Taraxacum*[x]

[1] Reykjavik – Asteraceae pollen were only recorded from 2005 to 2009. Cichoriaceae and *Taraxacum* were counted separately in 2005 and 2009. However, in other years only *Taraxacum* was counted. Therefore Cichoriaceae and *Taraxacum* have been combined to produce the most dominant taxa (2005-2009).

Fig. 1. Location of EuroPrevall allergy centres and pollen-monitoring sites



EuroPrevall allergy centre	Pollen-monitoring station	Location of pollen-monitoring site			Available years
		Latitude	Longitude	Height above sea level (m)	
Reykjavik (IS)	Reykjavik	64° 07' N	21° 54' W	52	1990-2009
Vilnius (LT)	Siauliai	55° 55' N	23° 18' E	128	2003-2009
Berlin (DE)	Neustrelitz	53° 22' N	13° 05' E	70	1992-2009
Manchester (GB)	Derby	52° 55' N	01° 30' W	78	1990-2009
Leiden (NL)	Leiden	52° 09' N	04° 28' E	-1	1990-2009
Łodz (PL)	Łodz	51° 45' N	19° 27' E	187	2005-2009
Prague (CZ)	Prague	50° 05' N	14° 25' E	245	1993-2009
Strasbourg (FR)	Strasbourg	48° 34' N	07° 44' E	142	1990-2009
Zurich (CH)	Zurich	47° 23' N	08° 33' E	556	1990-2009
Milan (IT)	Legnano	45° 35' N	08° 55' E	219	1995-2009
Sofia (BG)	Sofia	42° 42' N	23° 17' E	586	1991-2009
Athens (GR)	Thessaloniki	40° 38' N	22° 57' E	24	1990-2009
Madrid (ES)	Madrid	40° 25' N	03° 43' W	615	1994-2009

IS – Iceland; LT – Lithuania; DE – Germany; NL – The Netherlands; PL – Poland; CZ – Czech Republic; FR – France; CH – Switzerland; IT – Italy; BG – Bulgaria; GR – Greece; ES – Spain.

Fig. 2 Pollen exposure across Europe. The size of each circle represents the mean exposure level to all four pollen families (Betulaceae, Oleaceae, Poaceae and Asteraceae) together (mean yearly sum of daily pollen concentrations shown in parenthesis) in the thirteen centres across Europe, ranging from 2,565 in Thessaloniki to 28,177 in Zurich. The pie charts illustrate the relative importance (% indicated) of each of the four main families of allergenic pollen per centre.

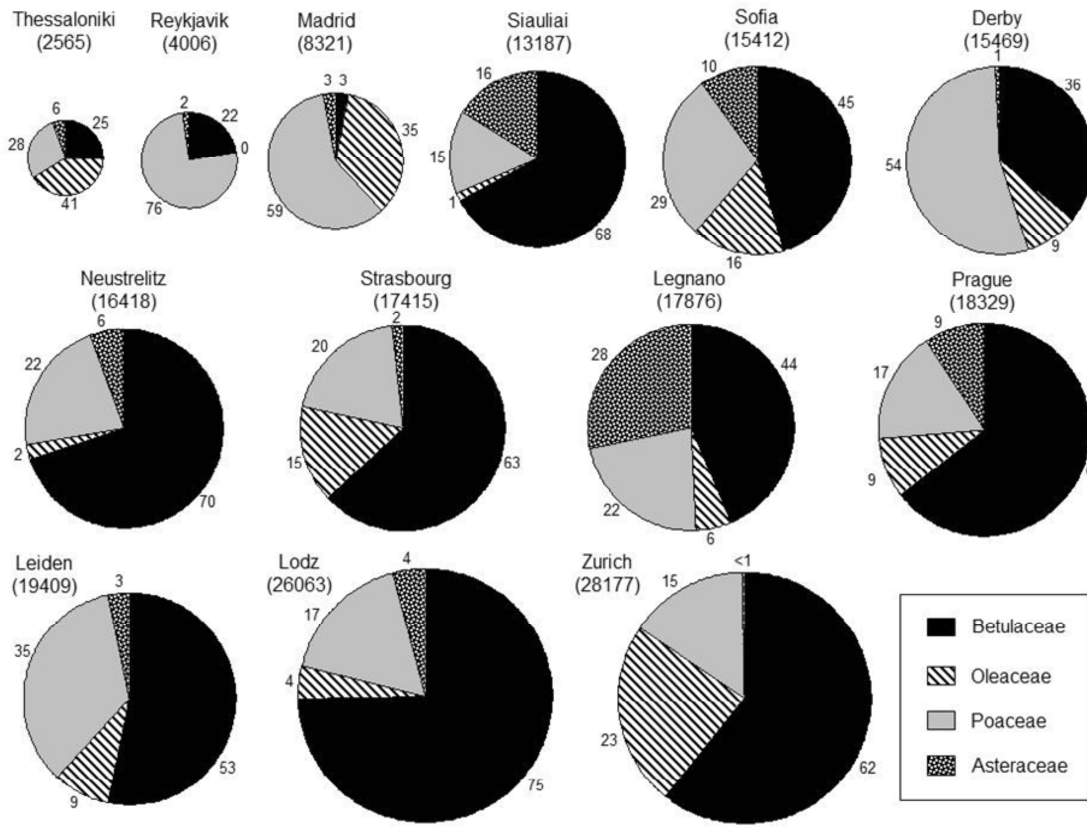


Table 1. Start dates, intensity (sum of pollen recorded in the season) and duration of Betulaceae pollen seasons recorded at 13 pollen-monitoring stations included in this study. The following statistics are included: the mean, Standard Deviation (SD), slope of the regression over time, Standard Error of the regression slope (SE), probability level (*p*) and number of years in the analysis (N).

Pollen-monitoring station	Pollen types	Start (DOY)						Sum Year (grains)						Duration (days)						Most dominant taxa	
		Mean	SD	Slope	SE	*p*	N	Mean	SD	Slope	SE	*p*	N	Mean	SD	Slope	SE	*p*	N	Taxa	Mean %
Reykjavik[1]	a, b	142	8.2	-1.0	**0.2**	**0.000**	20	512	545	46.6	**18.7**	**0.023**	20	38	13.8	1.1	**0.5**	**0.034**	20	b	100
Siauliai	a, b, c, d	78	-	-	-	-	6	8973	-	-	-	-	6	63	-	-	-	-	6	b	73
Neustrelitz	a, b, c, d	53	20.6	-0.2	1.1	0.878	16	9870	5846	-240.8	292.4	0.423	17	75	19.7	-0.1	1.0	0.925	16	b	58
Derby	a, b, c, d	49	18.0	-1.8	**0.6**	**0.005**	19	3622	2313	116.0	89.5	0.212	19	83	12.5	-0.4	0.5	0.393	19	b	68
Leiden	a, b, c, d	35	19.5	-1.0	0.8	0.235	19	7865	3464	-187.5	130.7	0.169	20	102	41.7	0.3	1.8	0.846	19	a	49
Łodz	a, b, c, d	67	-	-	-	-	4	17325	-	-	-	-	4	59	-	-	-	-	4	b	79
Prague[2]	a, b, c, d, e	72	-	-	-	-	7	10821	5241	44.7	283.7	0.877	15	52	-	-	-	-	7	b	84
Strasbourg[2]	a, b, c, d	56	-	-	-	-	6	9281	5222	141.5	217.2	0.525	17	79	-	-	-	-	6	b	50
Zürich	a, b, c, d, e	39	20.3	0.1	0.8	0.921	20	14921	4948	454.1	**165.6**	**0.013**	20	87	18.4	0.1	0.7	0.852	20	b	66
Legnano[3]	a, b, c, d, e, f, g	41	9.3	-0.1	0.6	0.824	14	8311	4498	470.6	246.6	0.079	15	107	19.6	-0.8	1.2	0.502	14	f	67
Sofia	a, b, c, d	71	24.8	-5.7	**1.2**	**0.001**	11	5904	2021	165.5	174.0	0.366	11	56	25.6	6.0	**1.2**	**0.001**	11	b	74
Thessaloniki	a, b, c, d	48	8.2	-0.0	0.3	0.989	20	533	357	26.5	12.8	0.053	20	97	12.8	-0.6	0.5	0.237	20	c d	40 41
Madrid[4]	a, b, d	-	-	-	-	-	-	169	99	-1.16	5.6	0.839	15	-	-	-	-	-	-	a	62

Pollen types – *Alnus*[a], *Betula*[b], *Carpinus*[c], *Corylus*[d], *Ostrya*[e], Betulaceae (*Alnus* + *Betula*)[f], Corylaceae (*Corylus* + *Carpinus* + *Ostrya*)[g]

[1]The majority of Betulaceae pollen recorded at Reykjavik was from *Betula* (99.8%) and only a small amount of *Alnus* pollen was registered.

[2]Site regularly commenced pollen-monitoring after the start of *Corylus* flowering and so no trend can be shown. Sum of pollen reflects mainly *Alnus* and *Betula*.

[3]Legnano changed from simply recording Betulaceae and Corylaceae pollen to recording *Alnus*, *Betula*, *Carpinus*, *Corylus* and *Ostrya* pollen in 2009.

[4]Betulaceae pollen counts at Madrid are intermittent and highly variable (from 33 to 356 grains in a year), it was therefore not possible to calculate start and end of the season.

Table 2. Start dates, intensity (sum of pollen recorded in the season) and duration of Oleaceae pollen seasons recorded at 13 pollen-monitoring stations included in this study. The following statistics are included: the mean, Standard Deviation (SD), slope of the regression over time, Standard Error of the regression slope (SE), probability level (*p*) and number of years in the analysis (N).

Pollen-monitoring station	Pollen types	Start (DOY)						Sum Year (grains)						Duration (days)						Most dominant taxa	
		Mean	SD	Slope	SE	*p*	N	Mean	SD	Slope	SE	*p*	N	Mean	SD	Slope	SE	*p*	N	Taxa	Mean %
Reykjavik[1]	NA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Siauliai	i	113	-	-	-	-	5	200	-	-	-	-	5	32	-	-	-	-	5	i	100
Neustrelitz	h, i, j,	95	9.9	-0.3	0.5	0.604	16	348	233	5.8	11.8	0.631	16	40	26.4	-1.2	1.3	0.374	16	i	99
Derby	h, i, j, n	83	16.0	0.4	0.6	0.525	20	913	681	31.7	26.1	0.240	20	57	33.1	-3.4	1.0	**0.004**	20	i	94
Leiden	h, i, j	81	15.6	0.7	0.6	0.296	19	1300	922	-7.6	36.9	0.840	19	67	41.4	-2.1	1.6	0.201	19	i	96
Łodz	i	103	-	-	-	-	4	990	-	-	-	-	4	34	-	-	-	-	4	i	100
Prague	i, j,	89	11.5	0.2	0.6	0.737	15	1543	926	-12.9	50.0	0.801	15	35	20.7	-1.6	1.0	0.142	15	i	99
Strasbourg	h, i, j,	75	14.4	0.3	0.7	0.669	15	2250	1268	24.7	54.3	0.656	17	48	25.1	-0.5	1.3	0.696	15	i	99
Zurich[2]	i, m	86	10.7	0.2	0.4	0.659	19	5750	3261	42.5	135.9	0.759	19	35	13.6	-0.1	0.6	0.875	19	i	99
Legnano	i, j, k, l, m	76	12.9	-0.7	0.8	0.413	15	1065	894	82.4	50.5	0.127	15	94	17.4	0.1	1.1	0.953	15	l	100
Sofia	i, j,	77	15.3	-1.1	1.0	0.275	12	2036	1408	65.5	86.8	0.466	13	41	14.1	0.6	0.9	0.530	12	i	99
Thessaloniki	k	94	12.7	-0.2	0.5	0.752	20	853	407	20.0	15.5	0.214	20	78	15.3	0.0	0.6	0.951	20	k	100
Madrid	i, j, k	20	9.5	0.3	0.7	0.668	13	1870	815	-113.2	39.9	**0.015**	14	158	19.2	-0.3	1.3	0.826	13	k	64

Pollen types – *Forsythia*[h], *Fraxinus*[i], *Ligustrum*[j], *Olea*[k], Oleaceae[l], other Oleaceae[m], *Syringa*[n]

[1]No Oleaceae pollen recorded at Reykjavik

[2]Note that only *Fraxinus excelsior* pollen grains are recorded as "*Fraxinus*" pollen in Zurich. Low amounts of *Fraxinus ornus* pollen are also seen in the samples, but these are recorded as "other Oleaceae".

Table 3. Start dates, intensity (sum of pollen recorded in the season) and duration of Poaceae pollen seasons recorded at 13 pollen-monitoring stations included in this study. The following statistics are included: the mean, Standard Deviation (SD), slope of the regression over time, Standard Error of the regression slope (SE), probability level (p) and number of years in the analysis (N).

Pollen-monitoring station	Pollen types	Start (DOY)						Sum Year (grains)						Duration (days)						Most dominant taxa	
		Mean	SD	Slope	SE	*p*	N	Mean	SD	Slope	SE	*p*	N	Mean	SD	Slope	SE	*p*	N	Taxa	Mean %
Reykjavik	p	174	6.0	-0.5	0.2	**0.020**	20	1747	707	35.6	26.9	0.202	20	69	9.4	-0.4	0.4	0.254	20	p	100
Siauliai	p, q	158	-	-	-	-	6	2007	-	-	-	-	6	83	-	-	-	-	6	p	99
Neustrelitz	p, q, r	142	9.6	-0.7	0.4	0.141	14	3100	1059	-52.2	47.8	0.291	18	93	20.6	2.7	0.7	**0.001**	14	p	97
Derby	p	145	10.6	0.1	0.4	0.841	20	5467	1995	85.3	76.9	0.282	20	91	19.5	-2.2	0.6	**0.002**	20	p	100
Leiden	p	133	9.7	-0.5	0.4	0.220	20	5122	1898	-231.8	52.3	**0.000**	20	118	10.5	0.4	0.4	0.305	20	p	100
Łodz[1]	o, p, q	142	-	-	-	-	2	3874	-	-	-	0.681	5	82	-	-	-	-	2	p	95
Prague	p, q, r	136	6.7	-0.7	0.4	0.138	13	2946	1758	-211.6	101.3	0.061	13	92	17.2	0.3	1.2	0.822	13	p	98
Strasbourg	p	130	7.7	-0.9	0.3	**0.021**	11	2942	1145	-40.8	48.7	0.414	18	94	19.5	-1.3	1.0	0.221	11	p	100
Zürich	p, q	133	5.7	-0.2	0.2	0.290	20	3753	788	51.6	28.9	0.091	20	96	10.6	-0.1	0.4	0.793	20	p	100
Legnano	p	105	7.6	-0.2	0.5	0.650	15	4250	1551	182.1	81.9	**0.044**	15	148	13.7	0.3	0.8	0.736	15	p	100
Sofia	p	126	11.6	0.1	0.6	0.874	16	3736	1601	-101.8	64.9	0.136	18	114	16.1	1.0	0.7	0.183	16	p	100
Thessaloniki	p	98	16.1	0.0	0.6	0.998	20	591	339	24.3	12.2	0.063	20	152	38.7	3.0	1.4	**0.045**	20	p	100
Madrid[1]	p	58	-	-	-	-	4	3141	2123	-95.9	126.1	0.464	12	211	-	-	-	-	4	p	100

Pollen types – Cerealia[o], Poaceae[p], *Secale*[q], *Zea mays*[r]

[1]Monitoring station did not operate continuously and so it was not always possible to calculate start and end of the season. However, it was still possible to calculate intensity.

Table 4. Start dates, intensity (sum of pollen recorded in the season) and duration of Asteraceae pollen seasons recorded at 13 pollen-monitoring stations included in this study. The following statistics are included: the mean, Standard Deviation (SD), slope of the regression over time, Standard Error of the regression slope (SE), probability level (p) and number of years in the analysis (N).

Pollen-monitoring station	Pollen types	Start (DOY)						Sum Year (grains)						Duration (days)						Most dominant taxa	
		Mean	SD	Slope	SE	*p*	N	Mean	SD	Slope	SE	*p*	N	Mean	SD	Slope	SE	*p*	N	Taxa	Mean %
Reykjavik[1]	t, u, v, x	161	-	-	-	-	5	54	-	-	-	-	5	90	-	-	-	-	5	v + x	92
Siauliai	s, t, u	202	-	-	-	-	7	2168	-	-	-	-	7	40	-	-	-	-	7	t	97
Neustrelitz	s, t, u, v	181	22.0	0.2	1.1	0.830	15	777	261	-17.2	12.0	0.174	15	67	27.1	1.6	1.3	0.222	15	t	88
Derby	s, t, x	153	33.9	-3.4	1.1	**0.005**	20	80	42	-3.6	1.5	**0.022**	20	115	43.0	1.3	1.7	0.440	20	t	97
Leiden	s, t, u	167	28.8	-0.2	1.1	0.850	20	491	320	-46.6	6.1	**0.000**	19	84	29.3	0.7	1.2	0.545	20	t	87
Łodz[1]	s, t	191	-	-	-	-	3	994	-	-	-	-	3	64	-	-	-	-	3	t	85
Prague	s, t, u, v	197	13.1	0.9	0.7	0.229	15	1534	1126	-107.6	54.7	0.069	16	60	17.0	-0.9	1.0	0.387	15	t	82
Strasbourg	s, t, u, v	147	29.2	-0.7	1.2	0.568	19	237	112	-12.8	3.3	**0.001**	19	105	32.5	0.5	1.3	0.685	19	t	74
Zürich	s, t, u	122	17.4	-0.5	0.7	0.452	20	82	35	1.1	1.4	0.418	20	138	18.4	0.9	0.7	0.199	20	u t	42 33
Legnano	s, t, u	219	3.6	-0.2	0.2	0.441	15	5393	1224	71.7	73.3	0.346	15	53	7.4	0.1	0.5	0.783	15	s	90
Sofia	s, t, u, v	188	13.4	0.3	1.0	0.744	13	1281	416	-29.3	31.0	0.365	13	78	15.7	0.5	1.2	0.713	13	s t	42 54
Thessaloniki	s, t, u, v	124	35.5	0.2	1.5	0.919	19	148	63	-1.3	2.7	0.636	19	152	39.3	0.3	1.7	0.881	19	s t	45 36
Madrid[1]	t, w	92	50.3	-3.5	2.8	0.227	15	160	130	-13.1	6.7	0.070	15	196	67.3	0.7	3.9	0.868	15	w	70

Pollen types – *Ambrosia*[s], *Artemisia*[t], Asteraceae[u], Cichoriaceae[v], Compositae (Asteraceae)[w], *Taraxacum*[x]

[1] Reykjavik – Asteraceae pollen were only recorded from 2005 to 2009. Cichoriaceae and *Taraxacum* were counted separately in 2005 and 2009. However, in other years only *Taraxacum* was counted. Therefore Cichoriaceae and *Taraxacum* have been combined to produce the most dominant taxa (2005-2009).