Geographic and temporal variations in pollen exposure across Europe

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

2 **Geographic and temporal variations in pollen exposure across Europe**

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34 **ABSTRACT (248 words)**

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

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

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

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

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

58 **WORD COUNT: 2457**

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

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86 from 13 pollen-monitoring sites that were located as close as possible to the survey centres
87 in EuroPrevall (Fig.1). Centres were chosen to represent different geographic and climatic
88 regions in Europe, including Alpine, Mediterranean, Central, Nordic, and Maritime areas. The
89 focus was on pollen of the Betulaceae and Oleaceae (tree pollen), Poaceae (grass pollen)
90 and Asteraceae (weed pollen).

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92 **MATERIALS AND METHODS**

93 *Collection and analysis of pollen data*

94 Criteria for selecting the pollen-monitoring sites were: (a) where possible, they were located
95 in the same city as the clinical centres involved in EuroPrevall; (b) if this was not possible
96 then selected sites representative of the region with regard to climate and plant species
97 distribution were identified; (c) the station contained the longest available pollen dataset for
98 the region. Pollen data were accessed via the European Aeroallergen Network database
99 (EAN - <https://ean.polleninfo.eu/Ean/>). A total of 1888 datasets (years of pollen data), from
100 24 different pollen types were downloaded from EAN and included in the analysis.

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

110 The following genera are included in the Betulaceae family: *Alnus* (alder), *Betula*
111 (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 “Cereal 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).

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132 *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

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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 Łódź, 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% $\pm$ 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, Łódź, 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

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

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

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198 *Asteraceae pollen exposure*

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

209 Start dates in Asteraceae pollen seasons were shown to be getting significantly
210 earlier at Derby (Table 4). There were no significant trends in season duration, but
211 Asteraceae pollen seasons were becoming significantly less intense at Derby, Leiden and
212 Strasbourg. Asteraceae pollen counts at Legnano are increasing and there is a

213 corresponding increase in *Ambrosia* pollen at the site (slope=105.082; $p=0.147$), but the
214 trends are not significant.

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216 DISCUSSION

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

225 On a European scale, the greatest exposure was to allergenic pollen from the
226 Betulaceae family. This is largely due to the fact that Betulaceae pollen was the most
227 important at 9 out of the 13 sites included in this study, and mean annual Betulaceae pollen
228 counts exceeded 5000 grains at all 9 of these sites. The second most important pollen type
229 was Poaceae, which was the dominant at 3 out of the 13 sites (Reykjavik, Madrid and
230 Derby). However, in comparison to Betulaceae, mean values of > 5000 Poaceae pollen
231 grains were only recorded at two sites (Derby and Leiden). Mean annual Oleaceae pollen
232 counts only exceeded 5000 grains at Zürich. It is interesting to note that the results of the
233 GA2LEN skin test study showed that the highest sensitization rate to olive was in Zürich
234 (45.5%), where *Fraxinus* is the main Oleaceae pollen in the air (mean contribution 98.6%) (4,
235 24). In general, Asteraceae accounted for < 10% of the pollen types examined (11 out of 13
236 centres). The exceptions were Siauliai (16% - mainly *Artemisia*) and Legnano (30% - mainly
237 *Ambrosia*). Legnano was also the only site where mean annual Asteraceae pollen counts
238 were > 5000 grains. It is important to note that, because of their relatively large and heavy
239 pollen grains, numbers of some pollen types like *Ligustrum* (Oleaceae) (25) and *Artemisia*

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(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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276 Authors Contribution to the work:

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

284 Conflict of Interest:

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

287 FIGURE LEGENDS

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

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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.

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

2 **Geographic and temporal variations in pollen exposure across Europe**

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

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34 **ABSTRACT (248 words)**

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

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

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

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

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

58 **WORD COUNT: 2457**

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

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3 86 from 13 pollen-monitoring sites that were located as close as possible to the survey centres
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5 87 in EuroPrevall (Fig.1). Centres were chosen to represent different geographic and climatic
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7 88 regions in Europe, including Alpine, Mediterranean, Central, Nordic, and Maritime areas. The
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9 89 focus was on pollen of the Betulaceae and Oleaceae (tree pollen), Poaceae (grass pollen)
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11 90 and Asteraceae (weed pollen).
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17 92 **MATERIALS AND METHODS**

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20 93 *Collection and analysis of pollen data*

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22 94 Criteria for selecting the pollen-monitoring sites were: (a) where possible, they were located
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24 95 in the same city as the clinical centres involved in EuroPrevall; (b) if this was not possible
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26 96 then selected sites representative of the region with regard to climate and plant species
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28 97 distribution were identified; (c) the station contained the longest available pollen dataset for
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30 98 the region. Pollen data were accessed via the European Aeroallergen Network database
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32 99 (EAN - <https://ean.polleninfo.eu/Ean/>). A total of 1888 datasets (years of pollen data), from
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34 100 24 different pollen types were downloaded from EAN and included in the analysis.
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37 101 Daily average pollen data for the Betulaceae, Oleaceae, Poaceae and Asteraceae
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39 102 families recorded during the period 1990-2009 were collected at 13 pollen-monitoring sites in
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41 103 Europe (Fig. 1), using volumetric spore traps of the Hirst design (11) situated at roof level.
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43 104 Samples were examined by light microscopy using internationally recognised and
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45 105 comparable methods (12). To assess the duration of the pollen season, the start and end of
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47 106 the pollen season were defined using the 95% method, whereby the season starts on the
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49 107 day when 2.5% of the season's catch had been recorded and the end occurs when 97.5% of
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51 108 the total catch had been reached (13). The intensity of the pollen season was defined as the
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53 109 sum of pollen recorded in a season.
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56 110 The following genera are included in the Betulaceae family: *Alnus* (alder), *Betula*
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58 111 (birch), *Carpinus* (hornbeam), *Corylus* (hazel) and *Ostrya* (hop-hornbeam). Due to changes
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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 “Cereal 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

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3 138 possible to calculate start dates of the season due to missing values (Table 1). However, the
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5 139 same years could still be included in the analysis of season intensity because the dataset still
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7 140 contained the main peaks of the *Alnus* and *Betula* pollen seasons.
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10 141 The following statistics are presented: the mean, Standard Deviation (SD), slope of
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12 142 the simple linear regression over time, Standard Error of the regression slope (SE),
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14 143 probability level (*p*) and dominant taxa (%). Trends were considered significant with
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16 144 probability levels < 0.05. The calculations were carried out using the statistical software
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18 145 packages SPSS version 12.0.
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24 147 **RESULTS**
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26 148 *Overall pollen exposure*
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29 149 Pollen from the Betulaceae family is the most dominant pollen type recorded with total
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31 150 seasonal pollen grains summed up for the 13 centres reaching a mean of 98,180 grains,
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33 151 compared to 42,676 for Poaceae, 19,118 for Oleaceae and only 13,399 for Asteraceae (Fig.
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35 152 2, Tables 1-4). The sum of mean exposure to the four major families of allergenic pollen (Fig.
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37 153 2) differed by more than an order of magnitude between the centres with the highest (Zürich)
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39 154 and the lowest (Thessaloniki) exposure.
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45 156 *Betulaceae pollen exposure*
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48 157 The total Betulaceae pollen count was predominantly made up of *Betula* pollen at most sites,
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50 158 with mean *Betula* contributions ranging from 50% at Strasbourg to 100% at Reykjavik (Table
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52 159 1 and Fig.2). The exceptions were Leiden (49% *Alnus*), Madrid (62% *Alnus*) and
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54 160 Thessaloniki (40% *Carpinus* and 41% *Corylus*). The sites that recorded the most intensive
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56 161 Betulaceae pollen counts were Łódź, Zurich and Prague (Table 1 and Fig.2). The lowest
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58 162 Betulaceae pollen counts were recorded at Reykjavik, Thessaloniki and Madrid. The sites
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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% $\pm$ 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, Łódź, 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

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

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

197
198 *Asteraceae pollen exposure*

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

209 Start dates in Asteraceae pollen seasons were shown to be getting significantly
210 earlier at Derby (Table 4). There were no significant trends in season duration, but
211 Asteraceae pollen seasons were becoming significantly less intense at Derby, Leiden and
212 Strasbourg. Asteraceae pollen counts at Legnano are increasing and there is a

213 corresponding increase in *Ambrosia* pollen at the site (slope=105.082; $p=0.147$), but the
214 trends are not significant.

215

216 DISCUSSION

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

225 On a European scale, the greatest exposure was to allergenic pollen from the
226 Betulaceae family. This is largely due to the fact that Betulaceae pollen was the most
227 important at 9 out of the 13 sites included in this study, and mean annual Betulaceae pollen
228 counts exceeded 5000 grains at all 9 of these sites. The second most important pollen type
229 was Poaceae, which was the dominant at 3 out of the 13 sites (Reykjavik, Madrid and
230 Derby). However, in comparison to Betulaceae, mean values of > 5000 Poaceae pollen
231 grains were only recorded at two sites (Derby and Leiden). Mean annual Oleaceae pollen
232 counts only exceeded 5000 grains at Zürich. It is interesting to note that the results of the
233 GA2LEN skin test study showed that the highest sensitization rate to olive was in Zürich
234 (45.5%), where *Fraxinus* is the main Oleaceae pollen in the air (mean contribution 98.6%) (4,
235 24). In general, Asteraceae accounted for < 10% of the pollen types examined (11 out of 13
236 centres). The exceptions were Siauliai (16% - mainly *Artemisia*) and Legnano (30% - mainly
237 *Ambrosia*). Legnano was also the only site where mean annual Asteraceae pollen counts
238 were > 5000 grains. It is important to note that, because of their relatively large and heavy
239 pollen grains, numbers of some pollen types like *Ligustrum* (Oleaceae) (25) and *Artemisia*

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(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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276 Authors Contribution to the work:

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

284 Conflict of Interest:

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

286

287 **FIGURE LEGENDS**

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

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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.

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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
Łódź	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

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

²Site 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*.

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

⁴Betulaceae 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
Łódź	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, other Oleaceae^m, *Syringa*ⁿ

¹No Oleaceae pollen recorded at Reykjavik

²Note 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
Łódź 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

¹Monitoring 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
Łódź 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).

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
Łódź (PL)	Łódź	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	<i>p</i>	N	Mean	SD	Slope	SE	<i>p</i>	N	Mean	SD	Slope	SE	<i>p</i>	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
Łódź	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

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

²Site 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*.

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

⁴Betulaceae 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
Łódź	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, other Oleaceae^m, *Syringa*ⁿ

¹No Oleaceae pollen recorded at Reykjavik

²Note 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	<i>p</i>	N	Mean	SD	Slope	SE	<i>p</i>	N	Mean	SD	Slope	SE	<i>p</i>	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
Łódź 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

¹Monitoring 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
Łódź 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).