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ORIGINAL ARTICLE

Clinical relevance of *Corylus* pollen in Poznań, western Poland

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Abstract

Background. In Central Europe, hazel (*Corylus* sp.*)* pollen is considered to be an important aeroallergen in early spring.
Objective. This study examines hazel pollen levels in Poznań, western Poland, and the clinical relevance of this aeroallergen in the city.
Methods. *Corylus* pollen data (1996–2010) were obtained by volumetric spore trap located near the centre of Poznań. Clinical data (2006–2010), i.e. skin prick test (SPT) and allergen-specific IgE measurements (asIgE), were supplied by the Allergy Diseases Diagnostic Centre in Poznań.
Results. Mean diurnal hazel pollen concentrations peaked around 14:00–16:00 when mean bi-hourly pollen concentrations were ~60 P m^{-3}. Onset of the hazel pollen season varied up to 87 days annually, and was significantly ($r=-0.647$; $p<0.01$) related to mean maximum temperature during late December. SPT data revealed that ~11% of allergy patients had positive skin reactions to *Corylus* pollen allergens, and most of these (94.4%) reacted to pollen allergens from other members of the Betulaceae family – alder or birch. Of those sensitized, 53% suffered from atopic dermatitis. Of patients examined for serum asIgE, 26.0% had asIgE measurements in classes 5 and 6.
Conclusions. Hazel pollen has a detrimental effect on the allergic population of Poznań, with more than half of those sensitised to hazel pollen allergens showing symptoms of atopic dermatitis. Hazel pollen concentrations reach levels recognized as being able to induce allergy symptoms, especially in the afternoon and early evening when many people are returning home from work. The cross-reactivity with other members of the Fagales order also increases the allergenic potential of hazel pollen.

Key words

asIgE, hazel, atopic dermatitis, sensitization rates, skin-prick test, RAST, cross-reactivity, Betulaceae

INTRODUCTION

Pollen allergy has a marked clinical impact in Europe [1]. Exposure to aeroallergens is associated with allergic rhinitis (hay fever), asthma and atopic dermatitis (AD), which are closely related diseases [2, 3, 4] that significantly reduce quality of life and have a significant economic impact on society [5, 6, 7, 8, 9]. In northern, central, and eastern Europe, pollen from trees of the Fagales order (including members of the Betulaceae and Fagaceae families) are important sources of allergens. The major allergens in the pollen from these plants are highly homologous and there is therefore a degree of cross-reactivity between them [1, 10].

Hazel (*Corylus* sp.) is a member of the Betulaceae family. Other members of the family include alder (*Alnus* sp.), birch (*Betula* sp.) and hornbeam (*Carpinus* sp.) [11]. Hazel is one of the first trees in Poland to flower and its pollen season typically lasts from the end of January until late March [12]. The major hazel allergen is Cor a 1, a 17.4 kDa protein that shows homology with the major birch antigen- Bet v 1 (with the similarity assessed at 80.5–83%) [13]. Homologous pollen molecules can be found among other evolutionary related members of *Fagales* order. Allergens of alder (Aln g 1), birch (Bet v 1) and hornbeam (Car b 1) may provoke symptoms in persons sensitized to hazel due to the similar epitopes [10, 14]. Cor a 2 is a profilin, and together with Cor a 1 is found in hazelnut; therefore, food-induced allergic symptoms known as oral allergy syndrome can also be observed [15, 16]. The aim of the presented study is to provide information about the prevalence of IgE-associated allergic diseases in relation to *Corylus* pollen in Poznań, and to determine the most important characteristics of the hazel pollen season in relation to weather conditions using long-term data.

MATERIALS AND METHODS

Study area and climate. Poznań is the largest city in western Poland with a population of ~550,000 people [13]. It is the capital of Wielkopolska, an agricultural region located in mid-western Poland. *Corylus avellana* is the only wild species of hazel in Poland, although introduced species are ornamentally planted in cities. *Corylus avellana* is increasingly grown for

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its nuts, with cultivation rising in Poland by about 300% between 1998–2007 [18]. In Wielkopolska, about 90 hectares of land was dedicated to hazel cultivation in 2009 [19].

Poznań has a temperate continental climate with cold winters and warm summers. Mean January and July temperatures in the city are -1.0 °C and +18.0 °C, respectively, and mean annual precipitation approximately 550 mm (1971–2000 average). Westerly winds predominate, particularly from the SW [20].

Aerobiological and meteorological data. *Corylus* sp. pollen data were collected in Poznań by volumetric spore trap of the Hirst design [21]. The trap was located on the roof of a 13-story university students' dormitory (Esculap), approximately 1 km SW of the city centre (52˚24′N 16˚53′E), and operated continuously from 1996 – 2010 [22]. In the neighbourhood around the pollen-monitoring site in Esculap there are 4–8 storey buildings, 2 small parks with ornamental and non-ornamental trees, gardens, patches of grass and ruderal vegetation [23].

The pollen counting methods used in Poznań during the years studied have been comprehensively described in Grewling et al. [22]. The limits of the hazel pollen season were calculated by using the 90% method [24] whereby the season starts when 5% of the total catch was achieved and ends when 95% is reached. Daily average and bi-hourly concentrations of atmospheric particles (pollen grains) are expressed as P m^{-3}. The daily values are averages taken over a 24-hour period, while bi-hourly concentrations are used to show the diurnal variation in *Corylus* pollen. Meteorological data were obtained from the station located at Ławica Airport (52˚25′N 16˚49′E) ~7 km from the city centre and 4.25 km west of the pollen-monitoring site.

Clinical study. Clinical analysis was performed at the Allergic Disease Diagnostic Centre of the University of Medical Sciences in Poznań, which draws on patients from the Wielkopolska region. Skin prick testes (SPT) were carried out from 2006–2010 on 625 patients observed with various manifestations of atopy (AD, allergic rhinitis and conjunctivitis). A set of airborne allergens, including *Corylus* sp. allergen (Allergopharma-Nexter) were used. As a positive control, histamine hydrochloride was used (1:1,000) and as a negative control – 0.9% saline solution. SPT were carried out on the inner surface of the forearm, and a drop of each extract solution was placed on the skin. The skin was then pricked through the drop using the tip of a Morrow-Brown lancet. A positive SPT result was defined as an allergen wheal with mean diameter at least equal to the mean diameter of histamine wheal [25].

Measurements of allergen-specific IgE (asIgE) were carried out using the fluoroenzyme immunoassay (FEIA) CAP system with regard to following tree pollen: hazel (Phadia, t4), common silver birch (Phadia, t3) and grey alder (Phadia, t2). CAP (Phadia) tests are an updated version of the Radio Allergo Sorbent Test (RAST) which has been the standard technique for measuring asIgE antibodies in human serum or plasma. ImmunoCAP asIgE measures IgE antibodies to specific allergens. The test is designed as a sandwich immunoassay – an allergen is covalently coupled to the solid phase and reacts with the specific IgE in the patient serum sample. The CAP tests were carried out on samples from 23 sensitised patients from 2006–2010. Venous blood was collected and the serum separated by centrifugation. Concentrations of asIgE in patient's serum (kU/ml) were classified as follows: 0 (<0.35); 1 (0.35–0.7); 2 (0.7–3.5); 3 (3.5–17.5); 4 (17.5–50); 5 (50–100); 6 (>100). Specimens that needed to be kept for later assay were stored at -70 °C. Repeated freezing and thawing was avoided.

Statistical analysis. The following characteristics of the hazel pollen season were examined: start, end, duration, intensity (sum of pollen grains recorded during the pollen season – Seasonal Pollen Index [SPI]), daily maximum pollen concentration, and the peak day (day on which the maximum value was recorded). Data related to start, end and peak day were converted to the day of the year from 1 January (DOY). In addition to season intensity, the number of days with concentrations above certain thresholds (35 and 80 P m^{-3}) was also examined. These threshold values were based on atmospheric concentrations of hazel pollen reported to evoke allergic symptoms in Poland [26]. Trends in all the above-mentioned seasonal characteristics were estimated by simple linear regression analysis. The following parameters were calculated: slope of regression line (β), goodness-of-fit (R^2) and probability level (p). The intra-diurnal variation in pollen concentration was examined by taking into account the mean bi-hourly pollen concentrations during days with a daily average pollen level > 20 P m^{-3}. Days with rain episodes were excluded from the analysis.

The following variables were entered into correlation analysis with start, peak day and end of the hazel pollen season in order to determine the influence of weather conditions on seasonal characteristics: fortnightly and monthly averages of maximum, minimum and mean daily temperature and the sum of rain recorded in the same periods from October – February. The relationship between daily average hazel pollen concentrations and concurrent daily meteorological data (approximately from the end of January – late March, depending on year and pollen season duration) were also examined using correlation analysis. The meteorological variables were maximum, minimum and mean temperature, rainfall, average wind speed, depth of snow, dew point and relative humidity. Parametric (Pearson) or non-parametric (Spearman) analysis was applied, depending on data distribution (checked by Shapiro–Wilk test with α level= 0.05, result not shown).

RESULTS

Aerobiological data. The beginning of the hazel pollen seasons in Poznań varied greatly, with almost 3 months difference between the earliest and latest start dates during the study period. The hazel pollen season usually lasted for about one-and-a-half months in Poznań (Tab. 1), but high concentrations of *Corylus* pollen were only recorded for short periods (i.e. a few days). During seven pollen seasons, the daily average hazel pollen counts did not reach 35 P m^{-3}, whereas in 1998, the threshold value of 35 P m^{-3} was exceeded for 5 days. The most severe seasons (with a SPI > 350 grains) occurred in 1998, 1999, 2005 and 2006. No significant trends in pollen season characteristics were observed during the 15 year study period. However, there was a slight (but not significant) increase in SPI and maximum daily pollen concentrations (Tab. 1). The start of the hazel pollen season was significantly

Table 1. Variations and trends in pollen season characteristics in Poznan 1996-2010 (DOY- day of the year)

		Start of pollen season (DOY)	Peak date (DOY)	End of pollen season (DOY)	Duration of pollen season	Seasonal pollen index	Max. daily pollen concentrations	No. of days with pollen level >35 P m^{-3}	No. of days with pollen level >80 P m^{-3}
Variations	MEAN	46	64	92	47	244	48	2	0
	MIN	10	35	61	14	112	15	0	0
	MAX	97	99	124	82	473	138	5	1
Trends	slope	-0.629	0.225	-0.618	0.011	5.925	1.696	0.050	-0.007
	p	0.687	0.868	0.569	0.993	0.395	0.414	0.637	0.748
	R^2	0.013	0.002	0.026	0.000	0.056	0.052	0.018	0.008

related to mean daily minimum temperatures recorded during the 2nd fortnight of December (r=-0.647; p<0.01). The average minimum temperature during February revealed the strongest relationship (r=-0.811; p<0.001) with the end of the hazel pollen season (Tab. 2).

Daily average hazel pollen concentrations recorded during the season were generally related to concurrent weather conditions. Almost all the meteorological parameters examined had a significant influence on hazel pollen levels (p<0.05). It was observed that daily average maximum temperatures had a positive affect (r=0.377; p<0.001), whereas rainfall (r=–0.172; p<0.001) and the depth of snow (r=–0.259; p<0.001) negatively affected mean daily hazel pollen concentrations. No influence of wind speed was noticed during studied period (Tab. 2).

Analysis of diurnal (bi-hourly) variations in hazel pollen concentrations revealed that most of the pollen grains (>80%) were recorded between 10:00–20:00 local time. On average, hazel pollen concentrations started increasing before midday and reached their maximum between 14:00–16:00 (Fig. 1). During peak hours, the mean bi-hourly pollen concentration was ~60 P m^{-3}; however, during certain seasons this value can exceed 300 P m^{-3} (data not shown). Hazel pollen counts generally remained low at night, but there were 2 episodes when high night-time concentrations were recorded (between 22:00–02:00), these were 164 and 403 P m^{-3} during 1999 and 2006, respectively (data not shown).

Clinical data. Analysis of SPTs conducted on 625 individuals due to suspected airborne allergy revealed that 90 patients (14.4%) were sensitive to tree pollen allergen, while 71 (11.36%) were allergic to *Corylus* sp. (Tab. 3). The percentage of patients sensitized to *Corylus* sp. pollen allergens recorded in the city of Poznań was the highest in 2008 (14.15%), while the lowest (8.6%) was during 2007. Out of 71 patients who reacted to hazel extract, 67 (94.4%) were also sensitive to other members of Fagales (alder and/or birch). In the period 2006–2010, only 4 patients were allergic solely to hazel (Tab. 3). In the group of patients with airborne allergy symptoms associated with hazel allergens (n=71), 38 (53%) suffered from previously diagnosed AD, 26 (37%) from disseminated eczematous lesions (observed towards AD) and 7 (10%) from rhinitis and conjunctivitis (Fig. 2A). Out of 64 patients, presenting predominantly eczematous skin lesions, 37 individuals (58%) complained of concomitant symptoms of seasonal rhinitis, conjunctivitis and/or wheezing (data not shown).

The results of serum allergen specific IgE serum measurements in relation to hazel pollen allergens are expressed as antibodies class ranging from 0–6 (Fig. 2B). The analysis was performed on 23 patients with positive SPT

Table 2. Correlations between *Corylus* pollen season characteristics/daily pollen concentrations and meteorological parameters

Variables (dependent/*independent*)	Correlation coefficient
Start of pollen season	**Pearson coeff.**
daily average mean temperature during 2nd fortnight of December	–0.621*
daily average maximum temperature during 2nd fortnight of December	–0.554*
daily average minimum temperature during 2nd fortnight of December	–0.647**
daily average mean temperature during December	–0.627*
daily average maximum temperature during December	–0.636*
daily average minimum temperature during December	–0.619*
Peak day during pollen season	
daily average mean temperature during 2nd fortnight of January	–0.707**
daily average maximum temperature during 2nd fortnight of January	–0.745**
daily average minimum temperature during 2nd fortnight of January	–0.676**
daily sum of rain during 2nd fortnight of January	–0.534*
daily average mean temperature during January	–0.648**
daily average maximum temperature during January	–0.649**
daily average minimum temperature during January	–0.653**
End of pollen season	
daily average mean temperature during 1st fortnight of February	–0.641*
daily average maximum temperature during 1st fortnight of February	–0.588*
daily average minimum temperature during 1st fortnight of February	–0.690**
daily average mean temperature during 2nd fortnight of February	–0.726**
daily average maximum temperature during 2nd fortnight of February	–0.677**
daily average minimum temperature during 2nd fortnight of February	–0.708**
daily average mean temperature during February	–0.790***
daily average maximum temperature during February	–0.736**
daily average minimum temperature during February	–0.811***
Daily pollen concentrations	**Spearman coeff.**
daily average temperature	0.377***
daily average maximum temperature	0.409***
daily average minimum temperature	0.314***
daily sum of rain	–0.172***
daily average dew point	0.298***
daily average wind speed	*ns*
daily average depth of snow	–0.259***

ns – not significant
*p<0.05, **p<0.01
***p<0.001

Table 3. Patients with positive SPT to *Corylus* in relation to sensitization to other members of Betulaceae family.

YEAR	Total No. of examined patients	Total No. of patients with positive SPT for *Corylus* sp. (%)	Patients with positive SPT *Corylus* +*Alnus* + *Betula* sp. (%)	Patients with positive SPT *Corylus* + *Alnus* sp. (%)	Patients with positive SPT *Corylus* + *Betula* sp. (%)	Patients with positive SPT Only *Corylus* sp. (%)
2006	38	4 (10.5%)	4 (100%)	0	0	0
2007	93	8 (8.6%)	5 (62.5%)	0	1 (12.5%)	2 (25%)
2008	113	16 (14.2%)	15 (93.8%)	0	1 (6.2%)	0
2009	196	21 (10.7%)	18 (85.7%)	1 (4.8%)	1 (4.8%)	1 (4.8%)
2010	185	22 (11.9%)	19 (86.4%)	0	2 (9.1%)	1 (4.5%)
Total	625	71 (11.4%)	61 (85.9%)	1 (1.4%)	5 (7.1%)	4 (5.6%)

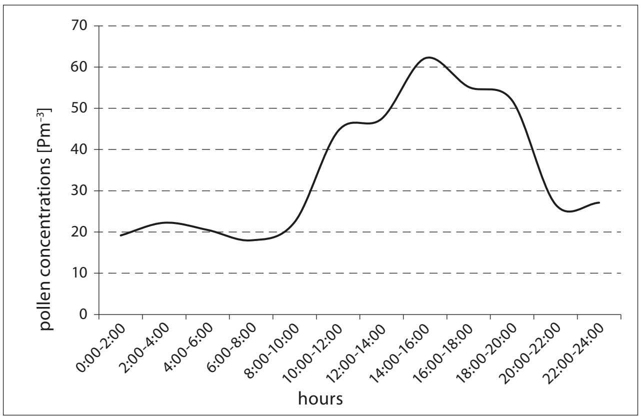

Figure 1. Mean bi-hourly *Corylus* pollen concentrations recorded in Poznań (1996–2010)

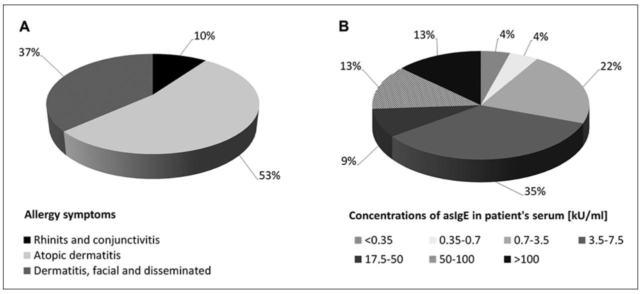

Figure 2. Allergy symptoms in patients with positive SPT to *Corylus* pollen (A) and results of serum allergen-specific IgE (asIgE) measurements in relation to hazel pollen allergens (B)

to hazel pollen and revealed that 21 of them (92.0%) were sensitized to *Corylus* sp. (classes from 2–6). A total of 26.0% of examined patients had asIgE measurements to hazel pollen allergens in classes 5 and 6.

DISCUSSION

Avoiding exposure to aeroallergens is an important part of managing IgE-associated allergic diseases [27]; therefore, monitoring aeroallergen concentrations and disseminating information to the public is crucial in helping individuals plan their activities and avoid high pollen counts. However, only a few recent studies have made detailed analyses of atmospheric concentrations of hazel pollen [12, 28, 29, 30]. This dearth of scientific investigation is probably due to the fact that hazel pollen is not considered to be an important aeroallergen and, depending on region, airborne concentrations generally remain low.

The aerobiological investigation in Poznań have also shown that during most of the studied period the daily average concentrations of hazel pollen did not reach high values. The period exceeding the threshold of 35 P m^{-3} did not usually last more than a few days, and maximum daily pollen concentrations did not exceed 150 P m^{-3}. For comparison, the average daily concentration of alder pollen in Poznań can reach 1,000 P m^{-3} [31]. However, it is worth noting that during certain periods of the day (midday-early evening) the pollen level can often exceeded values that could potentially induce allergy symptoms in people sensitized to hazel pollen, even though daily average hazel pollen concentrations generally remained low. Interestingly, several night-time peaks in hazel pollen concentrations were also observed. Similar episodes of elevated night-time concentrations were previously reported for birch pollen in Poznań by Corden et al. [32]. The authors postulated that the night peaks were caused by the increase in daily mean maximum temperature that extended the time of *Betula* pollen release. Alternatively, night-time peaks in birch pollen have been related to the transport of pollen from distant sources [33, 34]. Unfortunately, a detailed analysis of these night-time hazel pollen episodes was beyond the scope of this study.

Variations in the onset of hazel pollen seasons are one of the highest among early-flowering trees [12, 29]. The presented study supports these earlier findings and shows that the occurrence of hazel pollen in the air of Poznań can vary notably from year-to-year (up to 87 days). It is therefore important to be able to accurately predict the start of hazel pollen seasons so that individuals and health care professionals can plan medication and avoidance strategies. Early flowering trees, such as hazel, are greatly dependent on temperature for timing pollination [28]. In Poznań, the first hazel pollen grains can appear in the air during the first half of January if mean temperatures during the 2nd fortnight of December are high enough (>3.0 °C). Conversely, long cold winters can delay the hazel pollen season until the beginning of April. Elsewhere in Poland, Myszkowska et al. [12] proved that the spatio-temporal variations in the start of hazel pollen seasons was associated with the start of the thermal spring.

The seasonal sum of hazel pollen recorded at Poznań varied by as much as 4-times annually and had a trend towards more intense seasons (not significant), but it is not known whether this is a product of natural variations or the sign of external forcing. External factors could include changes in climate or land use. Climate change, either through rising temperatures or increased atmospheric concentrations of CO_2, can increase pollen productivity [35, 36]. Changes in land use, such as planting ornamental trees in urban areas [37] or changing agricultural practices, like the increased hazel nut cultivation in Poland [18], are known to alter the pollen spectrum at a site [38].

Over 11% of positive skin reactions recorded among patients in Poznań show that hazel pollen can potentially be an important causative or exacerbating factor of allergic diseases. However, the mean sensitization rate for hazel in Europe estimated during the Global Asthma and Allergy European Network (GA^2LEN) was more than twice as much

(22.8%) than in Poznań, but varied distinctly between cities (7.4 % in Coimbra, Portugal, to 50.7% in Zurich, Switzerland) [39]. The percentages of positive SPT reactions in Łódź (the only Polish city included in the GA²LEN study) was similar to the European mean and exceeded 22.3%. It is worth noting that the number of examined patients in Łódź was about a third (n=198) of those in Poznań (n=625). The percentage of positive SPT to hazel allergen in Poznań varied between years from 8.6% (2007) to 14.15% (2008), and this variation cannot be linked with the changes in the seasonal sum of *Corylus* pollen. The percentages of positive SPT reactions and the amount of hazel pollen varied independently. Similarly, no significant relation was observed between positive SPT (%) and seasonal sum of alder pollen in Poznań [31].

This study has shown that more than half (53%) of patients who had a positive response to hazel pollen allergens also showed symptoms of AD. The causal mechanism of this observation is not clear; however, in the pathogenesis of AD, pollen allergens play an undeniable role in IgE-mediated hypersensitivity and there are 2 possible routes for such influence. One route is by inhalation and the other, which currently seems to be considered as far more important, is by direct skin contact. Numerous studies [40, 41, 42] have shown that aeroallergens, such as house dust mite, fungal spores or pollen grains, might trigger AD. Particularly among children suffering from AD, there is an observed correlation between the severity of the disease and the degree of sensitization to aeroallergens, probably enhanced by damage of the skin barrier function. On the other hand, aeroallergen avoidance (e.g., house dust mites and pollen), can result in marked improvement of skin lesions [41]. In the case of patients presenting with AD and IgE-mediated allergy to pollen, exacerbation of skin lesions may be clearly seasonal and involve the so called 'airborne' areas of the skin such as face, neck or hands [40, 41, 42]. The patients investigated for the presented study suffered predominantly from AD, and some of them complained of exacerbations of eczema during springtime. The majority, however, also showed polyvalent allergy (allergic response manifested simultaneously for several or numerous specific allergens) towards perennial allergens (house dust mite, pet dander), and in such cases clear exacerbations as being pollen-related are much more difficult to distinguish.

The presented research has also revealed that most of the patients allergic to hazel (94.4%) reacted to pollen from other members of the Fagales order – alder and/or birch. Hazel pollen sensitization as the only cause of allergic symptoms was only detected in the minority of patients (5.6%). Simultaneous sensitization to all 3 pollen allergens (hazel, alder and birch) was observed in 85.9% of examined patients, which coincidences with the sequence identities (%) between Bet v1- homologous proteins from pollen allergens of these species (similarity >81%) [10]. Members of the different families within the Fagales order are closely related with homology of antigen protein structure. According to recent research conducted by Hauser et al. [43] using ELISA and ImmunoSolid-phase Allergen Chip (ISAC) IgE inhibition assays, Bet v 1-like allergens of the Betuloideae and Coryloideae subfamily might have the potential to induce IgE antibodies with different specificities, while allergic reactions towards Fagaceae allergens are the result of IgE cross-reactivity. The results presented in the current study thereby help to confirm this cross-reactivity between analysed species.

One more phenomenon must also be considered: in northern and central Europe, and also in northern America, *Alnus* spp. and *Corylus* spp. pollen allergens may be the cause of the so-called 'priming effect', which makes allergic patients much more sensitive to other pollen allergens later in the season [1]. Furthermore, pollen allergens from trees that flower in early spring may cause stronger reactions during the birch pollen season by giving a longer period of priming in susceptible individuals, so that their immediate hypersensitivity reactions may then occur at a lower birch threshold concentration [28].

CONCLUSIONS

The presnted study has provided information about the prevalence of IgE-associated allergic diseases in relation to *Corylus* pollen in Poznań. Hazel pollen has a detrimental effect on the allergic population of Poznań, with more than half of those sensitised to hazel pollen allergens showing symptoms of AD. In comparison to other allergenic taxa – birch and alder – hazel pollen counts in Poznań remain relatively low. The cross-reactivity with other members of the Fagales order, and potential as a primer for allergic sensitization to Betulaceae pollen allergens, increases the allergenic potential of hazel pollen. Inter-annual variations in pollen season start dates, which in Poznań can be almost 3 months from one year to the next, was significantly related to mean maximum temperature during late December. Daily hazel pollen concentrations were positively related to daily average maximum temperatures, whereas rainfall and the depth of snow had a negative effect. During sunny dry weather, hazel pollen concentrations can reach levels recognized as being able to induce allergy symptoms, especially in the afternoon and early evening, the time when many people are outdoors, returning home from work.

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