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REGIONAL IMPORTANCE OF *ALNUS* POLLEN AS AN AEROALLERGEN: A COMPARATIVE STUDY OF *ALNUS* POLLEN COUNTS FROM WORCESTER (UK) AND POZNAŃ (POLAND)

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Abstract: Daily average *Alnus* pollen counts (1996-2005) from Worcester (UK) and Poznań (Poland) were examined with the aim of assessing the regional importance of *Alnus* pollen as an aeroallergen. The average number of *Alnus* pollen grains recorded annually at Poznań was more than 2.5 times that of Worcester. Furthermore, daily average *Alnus* pollen counts exceeded the thresholds of 100, 500 and 1,000 grains/m^3 more times at Poznań than Worcester. Skin prick test results (1996-2005) and allergen-specific IgE (asIgE) measurements using the CAP (Pharmacia) system (2002-2005), were supplied by the Allergic Diseases Diagnostic Centre in Poznań. The annual number of positive skin prick tests to *Alnus* pollen allergens was significantly related ($p<0.05$) to seasonal variations in the magnitude of the *Alnus* pollen catch recorded at Poznań ($r=0.70$). The symptoms of patients with positive skin prick tests to *Alnus* pollen allergens were: 51% pollinosis, 43% atopic dermatitis, 4% asthma, 1% chronic urticaria and 1% eczema. On a scale of 0-6, 20.5% of patients examined for serum asIgE in relation to *Alnus* pollen allergens had asIgE measurements in classes 5 and 6. *Alnus* pollen is generally considered to be mildly allergenic. However, the amount of *Alnus* pollen released into the atmosphere in places such as Poznań may increase its impact on the population and make it one of the more important aeroallergens present.

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Key words: *Alnus* pollen, skin prick tests, allergen-specific IgE measurements, CAP (Pharmacia) system, Worcester, Poznań.

INTRODUCTION

This study focuses on pollen grains from *Alnus* (alder), a genus that is common and widespread in central and northern Europe, and is considered to be a significant contributor to the incidence of pollinosis (seasonal allergic rhinitis or hay fever) [9, 23, 40]. The major allergens of *Alnus* (Aln gI) are known to cross react with other members of the Fagales order (Betulaceae and Fagaceae), although the cross reactivity appears to be strongest within botanically established families rather than between them [13, 30, 37].

The genus *Alnus* belongs to the Betulaceae family, which also includes *Betula* (birch), *Corylus* (hazel) and *Carpinus* (hornbeam) [3]. In central and northern Europe, where *Betula* pollen is rated one of the most important allergenic pollen types, an estimated 10-20% of the population are allergic to birch pollen [1, 13, 14]. Because *Alnus* generally flowers before *Betula*, exposure to *Alnus* pollen early in the

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spring can prime sensitised individuals. Although believed to be only mildly allergenic (the minimum threshold for clinical symptoms has been reported as 50 *Alnus* pollen grains/m^3 daily average) a severe alder pollen season can elicit stronger reactions to birch pollen and effectively extend the birch pollen season [12, 13, 37, 41].

Aerobiologists have often measured and reported quantities of airborne pollen (pollen counts) as a service to allergy sufferers [6, 28, 35]. A range of factors needs to be considered when deciding which airborne pollen types are the most important, these include allergenic capacity, but also the amount of pollen released into the atmosphere [16, 30]. It is therefore important to examine spatial variations in the magnitude of the airborne catch and attempt to quantify the relative importance of different allergenic pollen types for different regions. The aim of this paper is to assess the contrasting regional importance of *Alnus* pollen as an aeroallergen by examining alder pollen counts from two sites, Worcester (UK) and Poznań (Poland), which have different biogeographical and climatic regimes.

METHODS

Pollen monitoring sites. The two cities, Worcester and Poznań, featured in this study are situated in regions where agriculture is a major activity [7, 38]. The City of Worcester (population 93,358) is the administrative seat of the county of Worcestershire (population 542,107) [34]. *Alnus* pollen data for Worcester were obtained from the National Pollen and Aerobiology Research Unit. The trap is situated on the roof of the main building of the University of Worcester (52° 11´ N, 002° 14´ W) at a height of 10 m. The pollen-monitoring site is approximately 1.4 km from the centre of Worcester.

Poznań (population 578,235) is the capital of Wielkopolska (population 3,350,437), a region situated in midwestern Poland [20]. *Alnus* data for Poznań were collected by the Laboratory of Aeropalynology at Adam Mickiewicz University. In 1996, the trap in Poznań was situated in an old district of the city at a height of 36 m (52˚24´N, 16˚55´E). However, from 1997-2005 the trap was sited on the roof of a 13 story university students' dormitory (52˚24´N, 16˚53´E) at a height of about 33 m, approximately 1 km southwest of the city centre [7]. The two *Alnus* pollen data sets from Poznań were spliced together to make a single dataset running from 1996-2005.

Climate. The United Kingdom has a temperate climate but is considerably warmer than some locations at similar latitudes, such as central Poland, because of the maritime influence. The weather in the UK has a tendency to fluctuate rapidly due to the influence throughout the year of low-pressure zones moving in from the Atlantic [18]. In Worcester, the mean temperature for January and July is 3.5ºC and 16ºC respectively, and mean annual rainfall is approximately 600 mm (1971-2000 average) [32]. Poland has a temperate continental climate and as a result has colder winters and warmer summers than the UK. Mean January and July temperatures in Poznań are -1.4ºC and 19.2ºC respectively, and mean annual precipitation is approximately 500 mm [7, 40].

***Alnus* pollen data.** Daily average *Alnus* pollen counts from 1996-2005 were collected at both sites by Burkard volumetric spore traps of the Hirst design [21]. The methodology used for collecting the *Alnus* pollen data at Worcester followed the standard method of the UK National Pollen Monitoring Network, described in the British Aerobiology Federation (BAF) guide for trapping and counting airborne pollen and spores [4]. In Poznań, 2 different counting methods have been employed. From 1995-1999 pollen data were collected following the methods outlined by Stach [40] where pollen grains were counted along 12 latitudinal transects. From 2000-2005 this method was changed and pollen grains were counted along 4 longitudinal transects, which were divided into 2 mm (1 hourly) intervals.

In the UK the genus *Alnus* is naturally represented by *Alnus glutinosa*, although *A. cordata* and *A. incana* are frequently introduced as ornamental species [36]. *Alnus* typically flowers in the UK, during February, March and April [19]. In Poland, there are 3 species of alder: *Alnus glutinosa*, *A. incana* and *A. viridis* (rare). Flowering usually begins at the end of February or the beginning of March, and lasts until April [26, 41]. *Alnus* incana typically flowers first, followed by *A. glutinosa* about 2 weeks later. However, in years when spring warming occurs rapidly, both *Alnus glutinosa* and *A. incana* can flower simultaneously, which may affect the length of the *Alnus* pollen season [25, 27].

Defining the limits of the *Alnus* pollen season. A number of methods have been used over the years for defining the start and end dates of pollen seasons. The methods considered in this study included the Σ75 and Σ100 methods, whereby the start of the pollen season is calculated as the day when the cumulative daily count reaches a certain figure (75 or 100 pollen grains) [1, 10, 11]. These methods are mostly used for defining the start of the season and not the end, and were therefore not used. Also considered, were the 90% [33], 95% [17] and 98% [14] methods. These techniques are retrospective, and define the pollen season as the period in which a certain percentage of the total seasons catch occurred.

Start dates calculated using the 90% method produced the lowest standard deviation, which is the technique described by Jato *et al.* [24] for selecting the method to be used for defining the start dates of *Alnus* pollen seasons. Furthermore, it is known that the 90% method is suitable for use with taxa that flower early in the spring, like *Alnus* [27]. Unfortunately, the use of this method omitted a number of noteworthy *Alnus* pollen counts from the Poznań dataset (counts in excess of 100 *Alnus* pollen grains/m^3). As the aim of this study is to assess the regional importance of

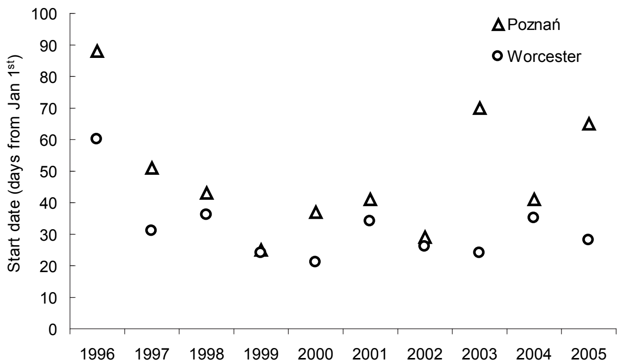

Figure 1. Start dates of *Alnus* pollen seasons in Worcester and Poznań from 1996-2005. Season defined using 98% method.

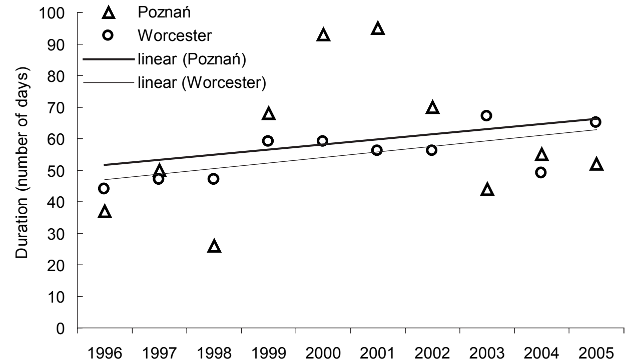

Figure 2. Duration of *Alnus* pollen seasons in Worcester and Poznań from 1996-2005. Season defined using 98% method.

Alnus pollen as an aeroallergen, it was decided to employ a method that would leave the maximum number of data points in the analysis. It was, therefore, decided to use the 98% method, whereby the start is defined as the day when 1% of the season's catch had been recorded and the end occurs when 99% of the total catch had been reached.

Clinical data. The Allergic Diseases Diagnostic Centre at the Department of Dermatology, University of Medical Sciences in Poznań, which draws on patients from the Wielkopolska region, supplied the clinical data. Skin prick tests (SPTs) were carried out on patients with allergy symptoms who attended the Allergic Diseases Diagnostic Centre from 1996-2005. SPTs were performed using the airborne set of allergens, including *Alnus* sp. allergen, from Nexter/Allergopharma, Reinbek, Germany. As a positive control, histamine hydrochloride was used (1:1,000) and as a negative control – 0.9% saline solution. SPTs were carried out on the inner 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 [8]. Pearson correlation analysis was used to determine whether a relationship existed between SPTs carried out at the Allergic Diseases Diagnostic Centre and the amount of *Alnus* pollen recorded in a season at Poznań.

Measurements of allergen-specific IgE (asIgE) were carried out using the fluoroenzyme immunoassay (FEIA) CAP system by Pharmacia. CAP (Pharmacia) 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 [42]. ImmunoCAP asIgE measures IgE antibodies to specific allergens. Specific IgE appears in human serum or plasma as a result of sensitisation to an allergen and can be detected following exposure. The CAP tests were carried out on samples from patients from 2001-2005. Venous blood was collected and the serum separated by centrifugation. Specimens that needed to be kept for later assay were stored at -70°C. Repeated freezing and thawing was avoided.

RESULTS

Analysis of temporal variations in *Alnus* pollen seasons at Worcester and Poznań (Fig. 1) shows that the *Alnus* pollen season generally starts earlier in Worcester than in Poznań. There is also a trend toward earlier *Alnus* pollen seasons at the 2 sites, but this trend is no longer evident when data from 1996 (the year that recorded the latest season start in the 2 cities) are removed from the analysis. In actual fact, *Alnus* pollen seasons in Poznań show a trend toward later starts when 1996 is omitted from the study. Further investigation shows that there is also a trend toward longer *Alnus* pollen seasons in both Worcester and Poznań (Fig. 2). Interestingly, this trend toward longer *Alnus* pollen seasons is still present at the 2 sites when data from 1996 are removed from the investigation.

Examination of cumulative totals of pollen grains recorded during the season is a measure of season severity. In Worcester, the lowest amount of *Alnus* pollen recorded in a season was 686 grains in 2001, and the highest was 3,407 grains in 2004. The 1996-2005 mean at Worcester was 1,363 *Alnus* pollen grains. The lowest number of *Alnus* pollen grains recorded in a season in Poznań was similar to that of Worcester (727 grains in 2002), but the highest amount recorded in Poznań was far in excess of the highest catch at Worcester (15,011 grains in 1998). The average (1996-2005 mean) number of *Alnus* pollen grains recorded during a season in Poznań was 3,599, but without data from 1998 this figure was lowered to 2,331 *Alnus* pollen grains which was still higher than the Worcester average (Fig. 3).

The magnitude of daily catches of *Alnus* pollen in Worcester and Poznań were also compared. The number of times that the daily average *Alnus* pollen counts exceeded a certain threshold were noted and evaluated (Tables 1 and 2). In Poznań, the *Alnus* pollen count was ≥100 grains/m^3 on 57 occasions. Furthermore, 17 of the Poznań counts were ≥500 *Alnus* pollen grains/m^3 and 5 of these were ≥1,000 *Alnus* pollen grains/m^3. In Worcester, the daily average *Alnus* pollen count was ≥100 grains/m^3 on only 27 occasions and never exceeded the other 2 thresholds.

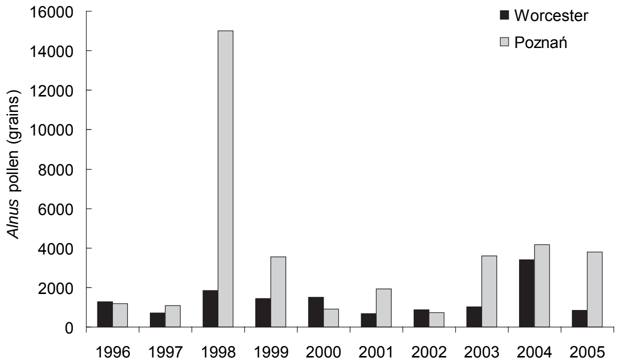

Figure 3. Total season counts of airborne *Alnus* pollen in Worcester and in Poznań from 1996-2005. Season defined using 98% method.

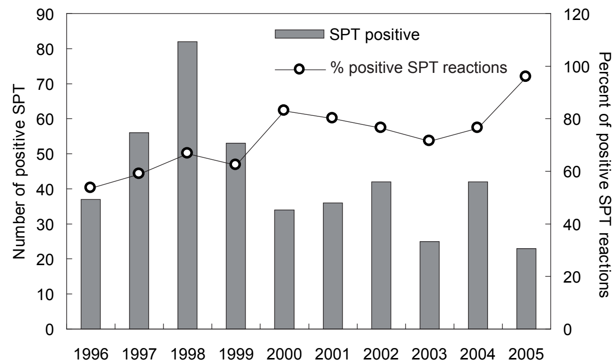

Figure 4. Positive SPT and percentage of positive SPT reactions to alder pollen allergens and in patients attending Allergic Diseases Diagnostic Centre at the Department of Dermatology, University of Medical Sciences in Poznań.

The results of SPTs carried out at the Allergic Diseases Diagnostic Centre between 1996-2005 show that the number of patients to have positive SPT results each year to alder pollen allergens is related to the amount of *Alnus* pollen recorded annually in Poznań ($r = 0.70$, $p<0.05$). Approximately 11% of people given SPTs for pollen allergens (n = 5,032) tested positive to tree pollen, and 430 of these were SPT positive to *Alnus* pollen. The percentages of positive SPT reactions varied annually but were not significantly related to the amount of *Alnus* pollen recorded in a season. The symptoms of patients with positive SPTs to alder pollen allergens can be broken down as follows: 51% pollinosis, 43% atopic dermatitis, 4% asthma, 1% chronic urticaria and 1% eczema (Fig. 4).

The results of serum allergen specific IgE measurements in relation to alder pollen allergens taken from patients attending the Allergic Diseases Diagnostic Centre in Poznań between 2001-2005 are expressed as antibodies class ranging from 0-6 (Tab. 3).

The results show that 73.5% of patients examined for serum allergen-specific IgE in relation to *Alnus* pollen allergens had asIgE measurements in classes 2, 3 and 4, and that 20.5% had asIgE measurements to alder pollen allergens in classes 5 and 6.

Table 1. Frequency of *Alnus* pollen counts above certain thresholds recorded at Worcester.

Daily *Alnus* pollen counts	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	Sum
>100 grains/m^3	3	0	6	3	0	2	0	2	11	0	27
>500 grains/m^3	0	0	0	0	0	0	0	0	0	0	0
>1000 grains/m^3	0	0	0	0	0	0	0	0	0	0	0

Table 2. Frequency of *Alnus* pollen counts above certain thresholds recorded at Poznań.

Daily *Alnus* pollen counts	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	Sum
>100 grains/m^3	3	1	15	8	0	5	1	8	7	9	57
>500 grains/m^3	0	0	8	2	0	0	0	3	2	2	17
>1000 grains/m^3	0	0	4	0	0	0	0	0	1	0	5

Table 3. Results of serum allergen-specific IgE (asIgE) measurements in relation to alder pollen allergens in patients attending Allergic Diseases Diagnostic Center, Department of Dermatology, University of Medical Sciences in Poznań between 2001-2005.

Year	Number of examined patients	Antibodies class/concentration (kU/ml)						
		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)
2001	12	0	1	0	5	3	1	1
2002	36	0	4	9	5	10	7	1
2003	13	0	0	1	5	1	0	4
2004	11	0	0	2	3	2	1	2
2005	16	0	0	5	9	1	0	0

Classes 0-1 – result considered negative; classes 2-6 – result considered positive.

DISCUSSION

A study into *Betula* pollen counts at Poznań and Derby (UK) by Corden *et al.* [7] showed that the birch pollen season usually started earlier in Derby than Poznań. This is similar to the results of this study which show that the *Alnus* pollen season usually starts earlier in Worcester than Poznań, a phenomenon that is probably related to the higher winter temperatures experienced in Worcester caused by the maritime influence on the UK's climate [7].

This paper does not examine trends in the timing or severity of *Alnus* pollen seasons per se, but it is important to consider temporal variations in pollen seasons when taking into account allergy sufferers. Jäger *et al.* [23] showed that *Alnus* exhibits a wide variation with regard to flowering time and pollen production, and that start days of *Alnus* pollen seasons are getting earlier. This study has not shown whether *Alnus* pollen seasons at Worcester and Poznań are getting earlier, a trend does exist, but it is removed when data from 1996 are omitted from the analysis.

This investigation has shown, however, that there is a trend toward longer *Alnus* pollen seasons in both Worcester and Poznań, which could have a detrimental affect on allergy sufferers. This may be related to increases in global average surface temperatures over the last century. In Europe, warmer temperatures produced the warmest decade (1990-1999) on instrumental record, annually and for winter [2, 22]. Such increases in temperature have caused the length of the growing season to increase, on average, by about 10-days since the early 1960s [22, 31].

A number of tree species including *Betula* exhibit cyclic patterns of alternating high followed by low pollen production years [13, 14, 29]. These rhythmic patterns are sometimes interrupted or broken by asynchronous years [13, 39], which may explain why there was not such a distinctive pattern for *Alnus* at either Worcester or Poznań.

The results show that Poznań has more severe *Alnus* pollen seasons than Worcester. The average number of *Alnus* pollen grains recorded annually at Poznań is more than two and a half times that of Worcester. Furthermore, analysis of daily data shows that Poznań had more than twice the number of daily average *Alnus* pollen counts above 100 grains/m^3 than Worcester, and that *Alnus* pollen counts at Poznań exceeded 500 grains/m^3 on 17 occasions and 1,000 grains/m^3 on 5 occasions (counts at Worcester did not exceed either of these higher thresholds). Variations in the magnitude of *Alnus* pollen counts should therefore be reflected in the amount of importance given to this pollen type in this region.

This study has also examined clinical data supplied by the Allergic Diseases Diagnostic Centre in Poznań. The results show that the number of positive SPTs to alder pollen allergens recorded annually is related to seasonal variations in the magnitude of *Alnus* pollen counts recorded at Poznań. The symptoms of allergic diseases such as pollinosis are irritating and can be debilitating. Such disorders can impact significantly on a person's quality of life and can have high socio-economic costs; either directly through treatment or indirectly through decreased productivity caused by absence from education or work or by impaired performance [5, 15]. More than half (51%) of the patients who had a positive response to alder pollen allergens showed symptoms of pollinosis; other symptoms included atopic dermatitis (43%), asthma (4%), chronic urticaria (1%) and eczema (1%).

Measuring circulating IgE antibodies to specific allergens provides an objective measurement of the sensitisation to the suspected allergen, including airborne allergens. The method allows quantitative measurements of a wide range of specificities representing all or individual allergenic components of an allergen source. Measuring specific IgE levels helps identify provoking allergens and predict the risk of developing allergy in the future, and guides clinical decisions. The results of this study show that the majority (73.5%) of patients in Poznań examined for serum allergen-specific IgE in relation to *Alnus* pollen allergens had asIgE measurements in classes 2, 3 and 4, and that a significant number (>20%) had asIgE measurements in the highest 2 classes (classes 5 and 6). It should be noted, however, that the diagnostic performance of CAP varies in an allergen specific manner, and CAP scores do not always correlate with clinical severity [42].

Alnus pollen is considered mildly allergenic, especially in areas such as the United Kingdom where the magnitude of daily and annual counts is relatively low. However, the major allergens of *Alnus* pollen are known to cross react with the major allergens of *Betula*. Exposure to *Alnus* pollen early in the spring can therefore prime sensitised individuals, eliciting stronger reactions to birch pollen and extend the birch pollen season [12, 13]. Furthermore, the amount of alder pollen released into the atmosphere in places such as Poznań may effectively increase its allergenic capacity and make it one of the more important aeroallergens present.

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