Analysis of pollen counts of *Betulaceae* in Timisoara, 2001–2004

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Abstract This work presents the first aerobiological monitoring results for *Betulaceae* in Timisoara using a volumetric spore-trap which is now the most commonly used aerobiologic measurement instrument in Europe. In order to collect the airborne pollen, we used a Hirst-type volumetric spore-trap, model Lanzoni VPPS 2000, placed on the West University terrace. The aim of this study was to determine characteristics of *Betulaceae* pollen occurrence in Timisoara atmosphere by measuring daily pollen concentrations and by comparing *Betulaceae* pollen season start dates calculated by different methods, as the base for a future forecasting model. Sampling was carried out during 4 years, starting in 2001, when the aerobiological monitoring unit was set up in the town, until 2004. The data showed that during the early spring the precise pollination periods for these allergenic tree species are highly variable. There were considerable variations in season length and cumulative season total (pollen index).

Key words aerobiological monitoring, *Betulaceae* airpollen, aeroallergens

The knowledge of pollination peak and period for each pollen enables a better correlation between skin responses and symptomatology and a more accurate management of pharmacological or hyposensibilisation treatment. Long term monitoring of the composition of aeroplankton in different sites has been undertaken in order to provide the current data to allergologists and to establish tendencies characteristic for a given region [26]. *Betulaceae* species are not a dominant tree species in Timisoara and its vicinity, it is important to evaluate the occurrence of airborne *Betulaceae* pollen in Timisoara, in order to assist clinicians and patients, particularly since there is an increase in the planting of birch trees in public and private gardens. Their pollen concentrations represent important values in the atmospheric measurement of Timisoara, which means they may produce polinosis in its population [18].

The genus *Betula* belongs to the *Betulaceae* family, which also includes *Alnus* (alder), *Corylus* (hazel) and *Carpinus* (hornbeam). Extensive fluctuations in flowering are characteristic of trees belonging to the *Betulaceae* family. Sarvas (1952, 1955) described large annual variations in pollen catch and seed set of two European tree-like *Betula* species, the silver birch (*Betula pendula*) and the downy birch (*Betula pubescens*). Shiabata et al. (1998) observed the strong synchronization of annual fluctuations of different *Carpinus* species in Japan. Fluctuation in flowering is likely to have a major effect on the reproduction of tree populations. It also has implications for the populations of organisms consuming pollen, flowers and seeds as their major food [5; 22].

Two mutually exclusive theories, the predator-satiation hypothesis and the wind-pollination hypothesis, aim at identifying the ultimate causes for the evolution of intermittent large seed crops (reviewed in Kelly, 1994). According to the predator satiation theory, seed survival increases; seed predators starve during low years, while during abundant years they are unable to consume all the seeds. The wind-pollination hypothesis in turn postulates that pollination efficiency increases during the high years. For wind-pollinated *Betulaceae* trees this seems obvious; an exponential positive relationship exists between on the one hand the amount of pollen production, on the other pollination efficiency and seed viability [20; 21]. Likewise Shiabata et al. (1998) found that the percentage of mature seeds of *Carpinus* species was positively correlated with annual staminate ament production.

Birches are monoecious trees. Male catkins, 2–4 together are grouped on ends of long shoots, while 1–2 female catkins are formed on reproductive dwarf shoots [4]. The reproductive output of silver birch and downy birch, two common forest trees with wide distribution covering most of Europe and Asia, fluctuates synchronously over wide geographical areas. This phenomenon, in particular the synchronization of
good years, is likely to have a positive impact on pollination success and seed viability, and to affect gene flow even between different birch species. Since the concentrations of airborne birch pollen are high during years of abundant flowering, and since pollen is frequently transported from remote areas in northern Europe, the effective population size may be vast, considering that the pollen is viable and the flowering times of distant trees overlap. The magnitude of catkin production is connected to weather variables the year before the observed flowering [12;19].

Material and Method

Measurements were performed by the volumetric method. The sampler is calibrated to handle a flow of 10 litres of air per minute, which roughly corresponds to human breathing. Pollen grains are impacted on a cylindrical drum covered by a melinex film coated with a uniform layer of sticky substance, and rotated by a 7-day clockwork at a speed of 2 mm/h. The tapes were changed weekly and cut into one day segments which were mounted onto microscope slides. Slides were stained with glycerin-jelly containing basic fuchsin and examined microscopically. Microscope counts were converted into atmospheric concentrations and expressed as pollen grains per cubic meter of air (PG/m$^3$) [17].

Results and Discussions

In recent decades pollen-related allergic reactions have attracted increased attention in European countries [14]. 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 [24].

Allergic airborne species appear in the area of Timisoara only from the end of January to the middle of October. The beginning of Betulaceae pollination was observed at the same time in all the years: February for Alnus and Corylus, March for Betula and Carpinus. More than 70% of Betulaceae pollen was collected in March and April. The pollination period of Betulaceae trees was relatively long. Its main pollination started with just a few days of difference between the four years under study, at the end of May. Such long pollen season duration enlarges risk of occurrence of cross-reactions between Betulaceae pollen and fruit allergens [15;17].

Pollen grains of Alnus are oblate to oblate-spheroidal and usually 4-5 porate. Their exinous thickened bands that extend in pairs from aperture to aperture distinguish the grains. Pollen grains vary in size from 19-21 $\times$ 23-30 $\mu$m [8]. The male flower forms groups of hanging balls and each ball produces about 4.5 million pollen grains. Flowering precedes the opening of leaf. The highest annual pollen count of Alnus was measured in 2003 (636 PG/m$^3$). More than 90% of the total annual Alnus pollen was collected during February and March. With regard to the annual concentrations, Alnus followed a pattern in which 2 years of low concentrations alternated with another of high concentrations. Different authors have studied the possible presence of this kind of cycle in different tree species, and the results are not homogeneous in the case of Alnus [1]. Andersen (1980) observed this kind of behavior while, in the study carried out in Stockholm by Atkinson and Larsson (1990), there was no such trend towards periodical cycles. 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 [10; 16]. Because Alnus generally flowers before Betula, exposure to Alnus pollen early in the spring can prime sensitised individuals. Although believed to be only mildly allergenic (the minimum threshold for clinical symptoms has been reported as 50 PG/m$^3$ daily average) a severe alder pollen season can elicit stronger reactions to birch pollen and effectively extend the birch pollen season. 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. Alnus incana typically flowers first, followed by Alnus glutinosa about 2 weeks later. However, in years when spring warming occurs rapidly, both Alnus glutinosa and Alnus incana can flower simultaneously, which may affect the length of the Alnus pollen season [24; 25].

The flowering of hazel (Corylus sp.) marks the beginning of the botanical early spring season. The decorative male catkins appear in late winter, and are fully open by spring when the new leaves and the female flowers appear. The pollen grains (20-25 $\times$ 26-28 $\mu$m) are triporate with a profound oncus at each pore. Pollen grains of Corylus are generally isopolar, suboblate to oblate or oblate-spheroidal. Hazelnuts are a common cause of food allergy. Allergy to hazelnut is most frequently observed in patients with birch pollinosis. This can largely be explained by crossreactive IgE against the major birch pollen allergen Bet v 1 and its homologue in hazelnuts Cor a 1. In addition, profilin and carbohydrate structures can be involved [9; 26]. In Timisoara a long pollination period was observed for Corylus in 2003, with high annual total count of sporomorphs (1081 PG/m$^3$).

Betula is considered the most important aeroallergen among tree species [17]. Birch trees are abundant pollen producers, each ball produces about 5.5 million pollen grains. The grains are suboblate to oblate. The grains (18-23 $\times$ 21-30 $\mu$m in size) are triporate with pronounced aspides at the pores [8; 9; 26]. The number of pollen grains per inflorescence was estimated by Erdtman [1954] as $6 \times 10^6$. Betula is important component in the pollen spectrum from Timisoara as shown by the percentages of total annual
pollen they represent 4.87%, 4.89%, 9.86% and 5.85%. The highest annual pollen count of Betula measured in 2003 (2436 PG/m³). The Betula pollen season usually ended in May except for 2003 when finished their season in June. A two year cycle in which a low annual birch pollen sum is followed by a high one has been reported by many authors. The cause of this biennial rhythm and its synchronicity over large areas is not clear. However, some authors suggest that it is physiological factors that are mostly involved: the high pollen production usually results in high fruit production, which takes a lot energy, inhibiting the development of new flowers and pollen for the next year. The high fruit production also inhibits other processes, for example the vegetative growth of the tree [12].

*Carpinus* pollen grains are isopolar, suboblate to oblate or oblate-spheroidal and usually have 3-4 pores. The pores are small and usually circular. The exine is smooth but can be granular around the pores. Pollen grains vary in size from 26-31 x 28-35 µm. Hornbeam flowering occurs generally simultaneously with birch anthesis. In Geneva among symptomatic patients during the March-April period, 73.5% were hypersensitive to *Betulaceae* pollen. Overall, 71.7% of the patients allergic to *Betulaceae* pollen were found to be sensitive to hornbeam, and 12.5% of the same group of patients were sensitive to hornbeam pollen only [7]. In Timisoara the highest annual pollen count of *Carpinus* measured in 2003 (1544 PG/m³).

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**Table 1**

The seasonal *Betulaceae* pollen index

<table>
<thead>
<tr>
<th>Pollen type/ Pollen index %</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corylus</td>
<td>1.04</td>
<td>1.77</td>
<td>4.17</td>
<td>1.65</td>
</tr>
<tr>
<td>Alnus</td>
<td>2.71</td>
<td>3.04</td>
<td>1.6</td>
<td>1.71</td>
</tr>
<tr>
<td>Betula</td>
<td>4.87</td>
<td>4.89</td>
<td>9.86</td>
<td>5.85</td>
</tr>
<tr>
<td>Carpinus</td>
<td>4.02</td>
<td>3.51</td>
<td>6.25</td>
<td>2.15</td>
</tr>
</tbody>
</table>
Figure 5. The pollination season of Betulaceae in 2001

Figure 6. The pollination season of Betulaceae in 2002

Figure 7. The pollination season of Betulaceae in 2003

Figure 8. The pollination season of Betulaceae in 2004
Conclusions

The onset of pollen allergy symptoms has been correlated to atmospheric concentrations of allergenic pollen. During the 4-year period (2001-2004), pollen of the representatives of the family Betulaceae accounted for a significant proportion of total pollen, predominated by Betula pollen and a considerably lower proportion of Alnus sp., Carpinus sp. and Corylus sp. pollen. The flowering period of European hazel (Corylus avellana L.) extends over February and March; that of black alder (Alnus glutinosa) and grey alder (Alnus incana) lasts from February to April, whereas the birch tree (Betula pendula) and hornbeam (Carpinus betulus) have flowering period in April and May. Throughout the years monitored, the Betula pollen season shows great variation with respect to the total pollen sum, the number of days in the season, its dynamics and the maximum daily pollen concentration values. The annual sums of atmospheric Betula pollen for the period 2001–2004 show a clear two-year cyclic rhythm. Exposure to Alnus pollen early in the spring can therefore prime sensitized individuals, eliciting stronger reactions to Betula pollen and extend the birch pollen season. The amount of Betulaceae pollen released into the atmosphere in places such as Timisoara may effectively increase its allergenic capacity and make it one of the more important aeroallergens present.

References