



Pollen allergen reports help to understand preseason symptoms

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Abstract

Early symptoms of pollen allergies are frequently reported before pollination period of allergenic plant starts. At northern latitudes the main concern is Betulaceae-trees (alders, hazel, birches). Pollen allergen information, based on the analysis of actual airborne allergen concentrations, beside the traditional pollen reports, based on pollen counts, has been given in SW Finland since 1998. 1–2 weeks before the onset of flowering pollen allergens are detected airborne in quantities high enough to provoke symptoms. Information is given on radio and in local newspapers. During the first season of the new service (1998) we carried out a questionnaire study. The results showed that new information service, though not as frequently used as we had hoped for, was useful to allergic persons for starting their medication in time. They also received explanation for their earlier inexplicable symptoms. We recommend both information services to be run concurrently to give the best information to the public.

Introduction

Pollen reports are published in the mass media in 28 European countries (EPI (European Pollen Information) – website <http://www.cat.at/pollen/>). This information is based on microscopical counts of intact pollen grains. By means of immunochemical methods, however, it has been shown that especially small-sized allergenic particles first appear several weeks before the actual pollen release period (Pehkonen and Rantio-Lehtimäki, 1994). The most sensitive pollen-allergic individuals may experience symptoms when the allergen level reaches about 500 SQ-units per cubic metre of air (i.e. about 0.005 ng of Bet v 1, unpublished).

In SW Finland, the main cause of pollen allergic symptoms is the pollen of Betulaceae trees (*Alnus*, *Corylus*, *Betula*). The most sensitive subjects experience symptoms as early as two weeks before either *Corylus avellana* L.(hazel) or *Alnus incana* (L.) Moench (grey alder) start flowering. Concurrently with the onset of the first symptoms, the allergen concentration has been shown to increase in outdoor air samples (Rantio-Lehtimäki, Viander and Koivikko, 1994). This is due in particular to small-sized particles

(less than 7.4 μm in aerodynamic diameter), assumed to be emitted by the plants (El-Ghazaly et al., 1995; Fountain et al., 1992; Spieksma and Nikkels, 1999; Staff, Schäppi and Taylor, 1999; Schäppi et al., 1997; Takahashi et al., 1995).

Allergen reports give relevant information about the factor which elicits the symptoms, i.e. the allergen concentration in outdoor inhalation air. Pollen reports based entirely on airborne particle counts may be misleading, since the visual inspection of airborne materials by microscope cannot distinguish between viable pollen grains and empty shells, which no longer contain any allergenic material.

In principle, the only proper way to run an information service would be to use human immunoresponse as a measure, for instance using immuno-chemical analyses.

The purpose of this study was to evaluate: 1. the level of airborne allergen concentration feasible to start reporting, and the idea of replacing pollen reports with allergen reports (in the future), 2. how much allergen information is lost when only intact pollen grains are trapped and counted, 3. the disturbance in allergen analyses caused by overlapping of pollination periods of Betulaceae-trees, and 4. the utilization of

the new information system, best channels for allergen information service and further education the clients might need.

Material and methods

Particle size selection was carried out with a continuously operating size-selective bioaerosol sampler (Kauppinen et al. 1989), SSBAS, which divides airborne particles into four size-classes simulating the human respiratory tract; stage 1 $>7.2 \mu\text{m}$, stage 2 $7.2\text{--}2.4 \mu\text{m}$, stage 3 $<2.4 \mu\text{m}$ and stage 4 submicronic antigen-bearing particles in a condenser Unit (Pehkonen and Rantio-Lehtimäki, 1994). Particle samples in the outdoor air were collected between 10 February and 26 May 1998, before and during the flowering of Betulaceae-trees.

The SSBAS was operated continuously round the clock, 20–23 hours for each daily sample. The sampling height was 15 m above ground level. The air intake volume was 18.5 l/min.

The analysis of the air samples was performed with an ELISA procedure (Rantio-Lehtimäki et al., 1994). Bound birch antigen was detected with polyclonal antibodies against *Betula* pollen produced in rabbit (ALK A/S, Copenhagen, Denmark, dilution of 1:1 000, 125 μl /well). The antigenic activities of each stage were converted to correspond to SQ units m^{-3} of air. The analysis takes 6–8 hours/day.

For comparison, airborne pollen counts of alder and birch (hazel pollen counts are near zero in this area; daily averages of pollen grains per cubic metre of air) were obtained from a continuously operating Burkard trap (Burkard Ltd., Rickmansworth, UK) situated at 5 metres distance from the SSBAS (The Finnish Pollen Bulletin, 1990–1998).

Daily reports on allergen concentrations were combined with the traditional pollen report based on particle counts. Both pollen and allergen information was given using expressions: 'low', 'moderate' or 'high' for the concentration and 'increasing' or 'decreasing' for changes.

A questionnaire (see Appendix) concerning the use of pollen information services was sent to 800 households with at least one family member included in the local Asthma and Allergy Society database.

Statistical analyses were carried out with SYSTAT (1992) procedures.

Table 1. Antigenic activity (polyclonal antibodies against *Betula* pollen; SQ-units) expressed as percentages of the total activity, maximum values and standard deviations in IgG ELISA analyses. Stages 1–4 of the SSBAS (stage 1 $<7.2 \mu\text{m}$, 2 $2.4\text{--}7.2 \mu\text{m}$, 3 $<2.4 \mu\text{m}$, 4 molecular size class) in the SSBAS; n = number of samples; Aln Burk = pollen counts of alder, and Bet Burk = pollen counts of birch in Burkard air samples.

	s1	s2	s3	s4	Aln Burk	Bet Burk
Activity, SQ	28%	18%	22%	32%		
Max	10184	26190	16217	26225	566	5737
Std dev	2234	3163	3097	5034	86	804
n	100	97	98	85	106	106

Results

Variations in concentrations and daily service

Allergen reports in the media started on 19 February, when the birch antigen concentration reached 500 SQ-units. This was estimated to be about 0.005 ng allergen/ m^3 (ALK-Abelló, Denmark, personal communication). The first alder pollen grains (probably long-range transported) were caught on the Burkard tape three days later, 22 February. The pollen concentration stayed at a low level during the following two weeks while the allergen concentration was rather high.

The continuous flowering of alder started as late as 27 March. Pollen maximum was reached on 2 April. Allergen activity on stage 1, where all intact pollen grains are caught did not correlate with alder pollen counts. (Table 2). High antigen activity was detected in stages 2 and 3 about two weeks before the alder pollination period.

Immediately following the alder peak pollen period, 25 April, the flowering of birch began. Pollen counts were high (maximum 5737 p.g. per cubic metre of air, Table 1) and the pollen period lasted for an unusually long time, for almost two months.

The pollen peaks of both alder and birch were preceded by high allergen activities in stages 2 and 3 (Figures 1 and 2). Activities in stages 2 and 3 correlated with each other but not significantly with alder pollen counts (Table 1). However, antigen activity in stage 1 correlated with birch pollen counts as expected.

The daily information service on allergen concentrations started first alone, and at the stage when pollen reports began (23 February), the two informa-

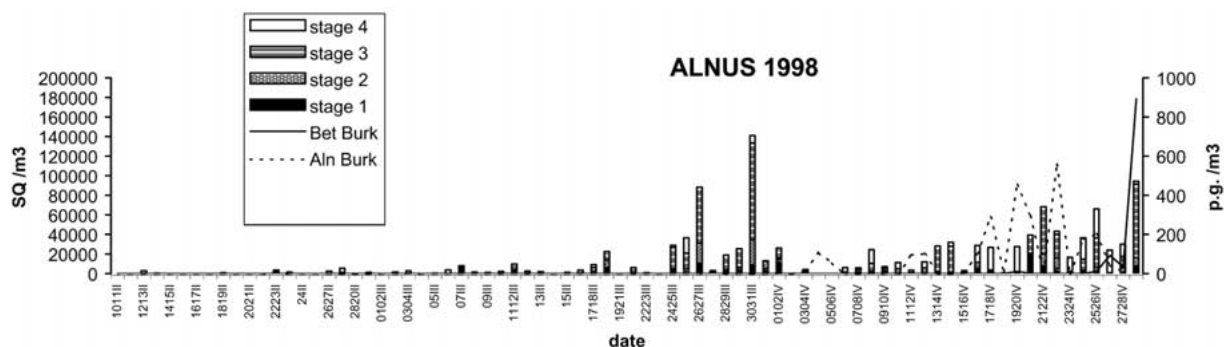


Figure 1. Antigenic activity (SQ units m^{-3} , bars) in IgG ELISA analyses with polyclonal antibodies against *Betula* pollen before and during the alder flowering period. Four particle sizes, stages 1–4: stage 1 $<7.2 \mu m$, 2 $2.4\text{--}7.2 \mu m$, 3 $<2.4 \mu m$, 4 molecular size class. Airborne alder (Aln Burk) and birch (Bet Burk) pollen counts m^{-3} of air in Burkard samples (lines).

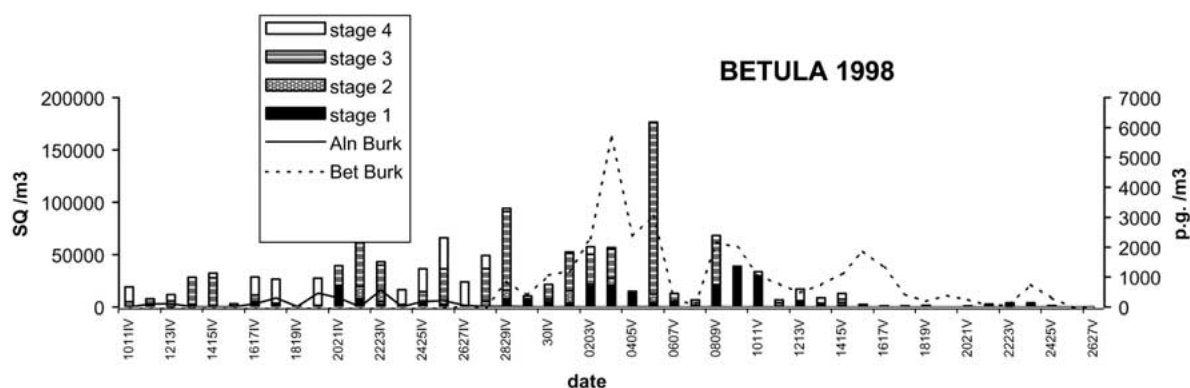


Figure 2. Antigenic activity (SQ units m^{-3} , bars) in IgG ELISA analyses with polyclonal antibodies against *Betula* pollen before and during the birch flowering period. Four particle sizes, stages 1–4: stage 1 $<7.2 \mu m$, 2 $2.4\text{--}7.2 \mu m$, 3 $<2.4 \mu m$, 4 molecular size class. Airborne alder (Aln Burk) and birch (Bet Burk) pollen counts m^{-3} of air in Burkard samples (lines).

Table 2. Spearman correlation coefficients for each variable: Stages 1–4, stage 1 $<7.2 \mu m$, 2 $2.4\text{--}7.2 \mu m$, 3 $<2.4 \mu m$, 4 molecular size class. Air samples analysed with polyclonal antibodies against *Betula* pollen. Stages 1–4 of the SSBAS, pollen counts of alder (Aln Burk) and birch (Bet Burk) in Burkard air samples.

	n	s1	s2	s3	s4	Aln Burk	Bet Burk
s1	100	1.000					
s2	97	0.329**	1.000				
s3	98	0.253*	0.502**	1.000			
s4	85	0.202	0.109	0.021	1.000		
Aln Burk	106	0.050	0.258*	0.201*	0.183	1.000	
Bet Burk	106	0.221*	0.066	0.060	0.253*	−0.069	1.000

**P < 0.01; *P < 0.01

tion systems were linked. Allergen information also continued until the end of the birch pollen period.

Feed-back

Of the 850 questionnaires, 56% were returned. Most of the respondents reported being allergic to birch pollen (36%), but many of them also had cross-reactivities to other Betulaceae-pollen. Most allergic and even non-allergic respondents reported following the daily pollen information regularly. Only 1% reported having no interest in the pollen situation at all. 92% of those with some allergic complications indicated that pollen reports functioned as early reminders to start taking their regular medication. About 38% of them had noticed the new service, and 26% reported that the information service had helped them in the interpretation of early symptoms or in the planning of starting taking medicine.

The most useful media were TV and teletext (37 and 16%) and newspapers (30%); the radio (15%) was third. In 1998, less than 1% made use of on-line services.

Overlapping of birch and alder seasons

We compared nine consecutive years (1990–1998) to find out how often and for what periods alder and birch pollen periods may overlap. In 6 of 9 there was no overlapping; in three years the overlap was 10 days at most (Figure 3). In 1994 and 1995 there was a two week interval between the flowering periods of the two genera. The two flowering periods usually follow each other closely – when alder season is over, the *Betulas* start. The separate pollen periods of alder are due to the occurrence of two species, *A. incana* and *A. glutinosa*. The two important birch species (*B. pendula* and *B. pubescens*) also sometimes peak at slightly different times.

The very early alder pollen findings in some years (January–February) are presumably all the result of long-range transportation. In several years, however, the discontinuity is caused by changeable weather conditions, which are especially common at high latitudes in early spring.

Discussion

This was the first attempt to analyse airborne allergen concentrations on a day-to-day basis and to publish the information regularly. The level of pollen antigens, 500 SQ-units/m³ of air seemed to be feasible phase to start information service, because it was reached at the same time, when the most sensitive subjects reported, they had to start medication.

If only pollen grains are trapped, on the basis of our calculations, even 72% of allergen information is lost, because only 28% of the antigenic activity was measured in the particles of pollen size-class. The replacement of pollen reports with allergen information would be especially useful before the pollen season, certainly in the Nordic countries, where a pollen free period occurs during the snow cover. Variations in the start of alder pollen season at northern latitudes, for instance, may span a period of up to two months. Unfortunately nation-wide daily allergen reports and forecasts are still a matter of the future. No commercial chemicals for all important allergens are available and running several parallel analyses with the methods described here is time-consuming. Thus only the Betulaceae pollen seasons can feasibly be followed using immunochemical methods.

At the moment immunochemical analyses are far more expensive than microscopical counting methods.

Each individual allergen type needs a special set-up. Image analysis of immunostained particles could be an intermediate alternative and is highly recommended for further development (Takahashi et al., 1993). Pollen reports give relevant information during most of the pollen season (i.e. close to the pollen peak period).

Due to the overlapping of the alder and birch pollen periods, polyclonal antibodies against birch pollen were found to be satisfactory for public service. We have shown earlier, that monoclonals against Bet v 1, the main allergen of birch, also pick up Aln g 1, the main allergen of alder, rather well (Pehkonen and Rantio-Lehtimäki, 1995). However, monoclonal antibodies are far more effective in detecting small concentrations. In the beginning of the season, monoclonals might therefore be more suitable. During the entire season, polyclonals are sufficiently sensitive. Monoclonals against alder pollen are unfortunately not commercially available.

Methods of sampling and analysis for immunochemical assays are not standardized. In particular small-sized particles may behave unpredictably under different meteorological conditions (see: Knox et al., 1993; Pehkonen and Rantio-Lehtimäki, 1994; Schäppi et al., 1999). Small-sized particles are important to catch, because they seem to be highly important before the pollen period. Therefore for instance modifications of the Burkard trap are not satisfactory being inefficient in particle size classes below 2 µm in aerodynamic diameter. The ELISA-method may give slightly different results in different laboratories, but it can be recommended as a standard method. The 24-hour delay before getting the results is a disadvantage. At present the analysis takes at least 6 hours. New ideas for reducing the time of analysis have been presented e.g. by Takahashi et al. (1995) and Kawashima et al. (1995). The same delay is of course true with direct pollen counts, especially when the samples are sent by post to the central station as is the practice for instance in Finland.

Expressing changes in the allergen level was problematic. In Finland, general information does not give pollen counts in numbers. Thus we were unable to use nanograms or SQ-units, but could only report that the allergen concentration was low, moderate or high and was expected to increase or decrease. We have no extensive comparative studies between symptoms and allergen concentrations analogous to those we have between symptoms and pollen counts (Viander and Koivikko, 1978).

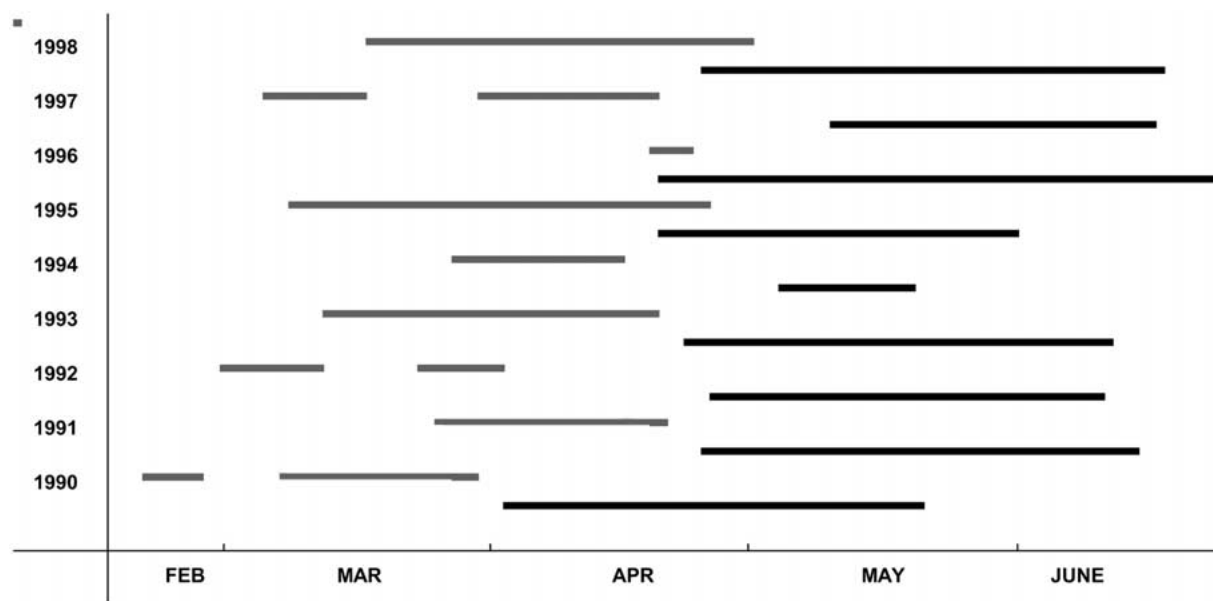


Figure 3. Flowering periods of alder and birch during 1990-1998. Overlapping of the periods in 3 years out of 9.

Teaching clients to utilize the allergen information may be easier than expected. For many people, however, it was not easy to tell the difference between allergen and pollen reports. Unfortunately we have no statistics on the number of persons who experience symptoms before the pollen period. Allergic persons who had paid attention to the new service were able to use it without difficulty and found it useful. The key-factor is the provision of information. Local information should be supplemented by TV service, which was found to be the most popular channel of pollen information provision.

It can be hypothesized that the high proportion of early alder pollen was the result of long-range transportation and does not necessarily contain any allergens. Antigen activities between the stages had high intercorrelations, indicating that airborne allergenic particles may be quite evenly distributed in different size classes. In this particular year it was difficult to predict the start of birch flowering on the basis of a preceding increase in allergen concentrations, because there was no break between alder and birch pollen seasons; this is not usually the case (see Figure 3). In general the occurrence of small-sized particles in the air is a useful tool in predicting the start of the pollen season 1-2 weeks later.

There have been no studies of long-range transport or of the horizontal or vertical distribution of small-sized particles. Considering that the retention

time of small particles in air masses may be months (e.g. Hidy, 1984), long-range dispersal should also be a common phenomenon. Thus we do not know whether the increase in allergenic activity is due to local plants emitting some allergenic particles or to amorphous allergenic material originating from areas where pollination has already started.

We do not suggest that counting pollen should be terminated. Up till now immunochemical analyses have been too labour- and time-consuming to replace the traditional way of analysis, but they are certainly worth developing further. The main problem remains the poor availability of reagents for the immunochemical analyses.

Acknowledgement

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Appendix: Questionnaire sent to members of local Asthma and Allergy Association

1. We have a pollen allergic person in our family.
yes ☐ no ☐
2. If yes, he/she is allergic to
alder ☐ birch ☐ any other pollen ☐

3. Symptoms usually start in
(month) _____; in the beginning of the
month ☐ in the middle ☐ at the end ☐
 4. I have used pollen information service
almost daily ☐ weekly ☐ less than weekly ☐
not at all ☐
 5. If you follow the pollen information service, which
are the media you use. Tick each alternative, which
you make use of:
radio ☐ TV ☐ teletext ☐ newspapers ☐
Internet ☐ Pollen ☐ Bulletin ☐ phone service
☐ other ☐, which:
 6. Do you get information about the pollen situation
too much ☐ enough ☐ too little ☐
 7. Have pollen reports helped you in understanding
the symptoms?
yes ☐ no ☐
 8. Have the pollen reports helped you in timing or
dosage of taking medicine for asthma or allergy
symptoms?
yes ☐ no ☐
 9. Have you noticed the new allergen information for
SW Finland?
yes ☐ no ☐
 10. If yes, has it helped you to recognize the start of
allergic symptoms?
clearly ☐ some ☐ not at all ☐
Wishes or suggestions on pollen and spore infor-
mation in future?
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