

# Birch-pollen antigenic activity of settled dust in rural and urban homes

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Concentrations of birch-pollen antigens were measured in 10 homes in southwestern Finland, four in urban and six in rural areas. Dust samples were collected once a week with a vacuum cleaner equipped with a special collection device (ALK, Copenhagen) combined with an exchangeable glass microfiber filter in a filter dish. Control samples were taken from horizontal surfaces outdoors. All samples were analyzed by a modification of the IgG-ELISA procedure. The birch-pollen antigenic activity in indoor settled dust was lower than that in dust outdoors. The mean concentration of antigenic activity indoors peaked 3 weeks later than outdoors. The lag indicates that the most important means whereby antigens are carried indoors is via footwear and clothes, rather than, for instance, ventilation. Antigenic activity was still detected 2 months after the peak pollen period. As a source of antigens, both indoor and outdoor dust may be an important cause of pollen-allergy symptoms after the season.

E. Yli-Panula, A. Rantio-Lehtimäki

Aerobiology Unit, Department of Biology,  
University of Turku, FIN-20500 Turku, Finland

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Dr E. Yli-Panula  
Aerobiology Unit, Department of Biology  
University of Turku  
FIN-20500 Turku  
Finland

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Pollen grains are generally considered to be a risk factor for pollen allergy only outdoors. Allergic subjects are therefore advised to stay indoors during peak pollen periods. However, we know very little about how high concentrations of pollen grains originating from allergenic plants, especially submicronic particles, reach the indoor environment. In outdoor air, up to 50% and more of birch-pollen antigenic material has been shown to occur in small size classes (below 2.4  $\mu\text{m}$  in aerodynamic diameter) (9, 15).

O'Rourke & Lebowitz (8) observed that, in early spring, the floor sweepings from homes (with open windows) in Tucson, Arizona, USA, contained total pollen concentrations as high as 5.5 million pollen grains  $\text{g}^{-1}$  of house dust. Pollen in settled indoor dust thus may contribute more significantly to pollen exposure than is generally suggested (11), and exposure to indoor allergens may be a major cause of asthma (2). Empty pollen grains do not contain allergenic protein material; the (immuno)chemical study of the composition of indoor dust is therefore important.

Studies of birch-pollen antigenic activity in settled dust outdoors (19) have suggested that this activity remains high long after the peak pollen period, especially in dry weather. Since conditions indoors are even more stable and dry, the antigenicity may be expected to be at least as persistent as in the outdoor environment. Frequent cleaning indoors eliminates

the largest particles but may not have a particularly great effect on particles in the submicronic size classes. Vacuum-cleaning actually resuspends settled particles in the air (7).

If the main source of the antigenic activity of indoor dust is settled dust outdoors, the source does not decrease in strength for a long time after the peak pollen period (19). The present study thus has the following aims:

- 1) to compare changes in the concentrations of birch-pollen antigens in settled dust in urban homes
  - a) with settled dust concentrations in rural homes
  - b) with outdoor settled dust concentrations in urban areas;
- 2) to compare variations in birch-pollen antigenic activity in dust settled outdoors
  - a) with simultaneous birch-pollen counts in outdoor air samples
  - b) with various weather factors;
- 3) to compare airborne pollen counts with changes in birch-pollen antigenic activity indoors in both urban and rural homes.

## Material and methods

The dust samples for birch-pollen antigen analyses were collected in 10 homes in southwestern Finland: four situated in a city (Turku, 163 000 inhabitants),

and six in rural areas (within a 60-km radius of Turku). The samples were taken in randomly chosen single-family homes with no mechanical ventilation. Dust samples were collected once a week (about 15 min each time) on the ground floor, with a vacuum cleaner equipped with a special collection device (ALK, Copenhagen) with a glass microfiber filter (diameter 7 cm, Whatman) in a filter dish. The samples varied greatly in physical appearance, ranging from small amounts of fine dust to large quantities of hair, fuzz, and other debris. The largest particles (such as bread crumbs and gravel) were picked off before the analyses.

Control samples were collected from settled outdoor dust with a suction sampler (vacuum pump of the Andersen 2000 type, with an air-intake volume of  $28 \text{ l min}^{-1}$ ), on each occasion from the same open, smooth window ledges (3.5 m above ground). The sampler consists of a sampling tube (6 cm in length, 1.5 mm in diameter), a sampling unit with a valve preventing particles from passing through, and a collar to which an airtight, sterile, 10-ml plastic tube can be attached. The samples were collected directly into a tube once a week before noon.

For comparisons, airborne birch-pollen counts (daily averages per cubic meter of air) were obtained from a continuously operating Burkard trap, a modification of the Hirst pollen and spore trap (4), situated on the roof of a Turku University building, 15 m above ground level.

The sampling period lasted from 12 April (week 15) to 15 August (week 32). The peak birch-pollen period started in week 16, peaked in week 18, and ended in week 24. The period was considered to have ended when the daily level contributing 10% of the yearly total was reached (10). Eight of the 18 samplings were carried out after the birch-pollen period.

Dust samples were analyzed by a modification of the IgG-ELISA (enzyme-linked immunosorbent assay) method developed for outdoor air analyses of birch-pollen antigen (13); 10 mg of each dust sample was weighed; mixed with 1 ml of phosphate-buffered saline (PBS), pH 7.4; and centrifuged at 6000 rpm for 15 min. All samples (dilution series of 1:1 to 1:100 000 in PBS) were analyzed in quadruplicate in 96-well microtiter plates (EIA/RIA plates; Costar®). The bound birch-pollen antigen was detected by means of polyclonal antibodies against *Betula* pollen produced in rabbit (ALK A/S, Copenhagen, Denmark, diluted 1:1000 in PBS bovine serum albumin (BSA) (0.5%)-Tw (0.5%)), and HRP (horseradish peroxidase)-conjugated swine antibodies against the rabbit IgG (DAKO A/S, 125  $\mu\text{l}$ /well of 1:1000 dilution in PBS-BSA-Tw). The enzyme activity of bound anti-rabbit-IgG-HRP (with *o*-phenyldiamine as a substrate) was measured as optical absorbance at

492 nm by a Multiscan® microplate reader (Flow Lab, Inc).

The *Betula verrucosa* pollen extract (100 000 SQ  $\text{ml}^{-1}$ ) used as a standard (dilutions of 1:1000, 1:10 000, 1:100 000, and 1:1 000 000) was provided by ALK, Copenhagen. The antigenic activities of the samples were expressed in SQ (standard quality) units (ALK, Copenhagen) per gram of dry dust.

Variations in the antigenic activity of settled outdoor dust were compared with the rain sum and average relative humidity from 1–7 previous days and the mean temperature of the sampling day. The concentrations of birch-pollen antigenic activity in urban indoor dust were compared with those in outdoor dust. Cross-correlations were plotted with the SYSTAT program library (16). Pearson's correlation coefficients were calculated for the mean antigenic concentrations of urban and rural homes by the SAS GLM procedures (14).

## Results

Birch-pollen antigenic activity was found in urban settled dust both outdoors (Fig. 1) and indoors

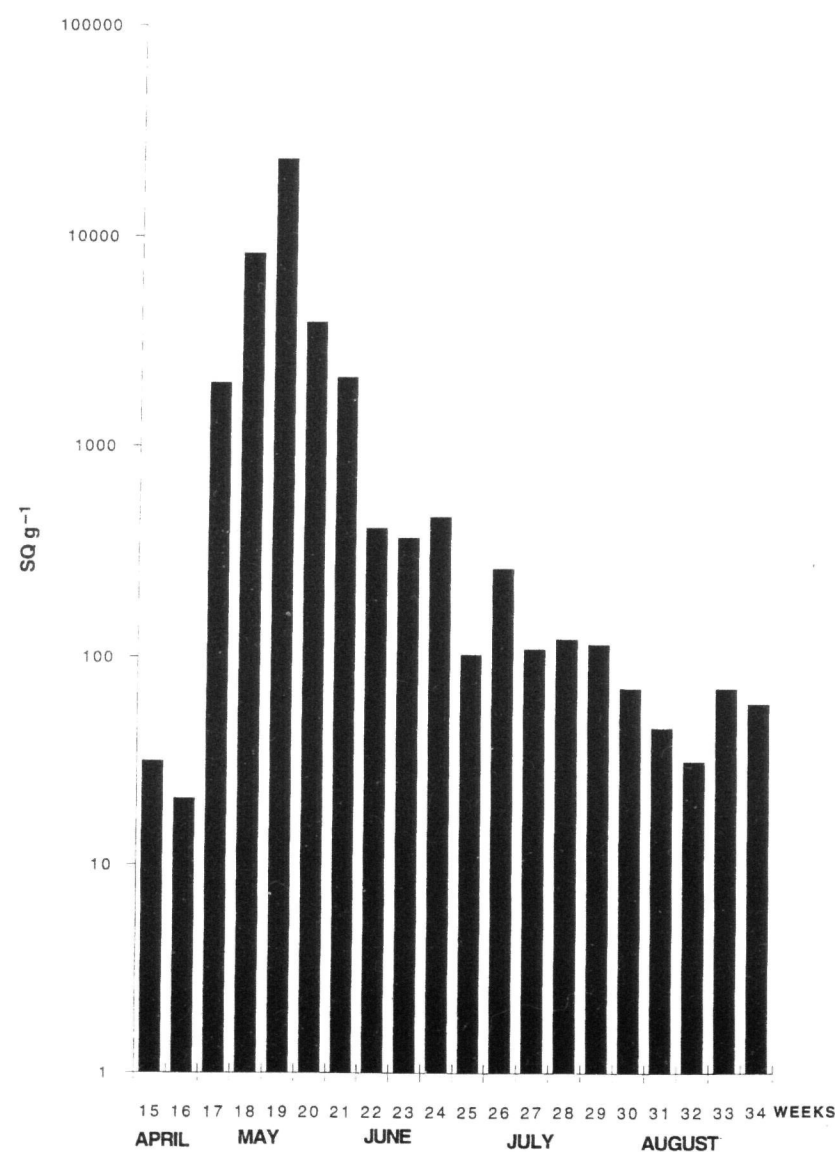


Fig. 1. Birch-pollen antigenic activity (SQ units  $\text{g}^{-1}$  of dust on window ledges) analyzed weekly in outdoor settled dust in summer 1993.

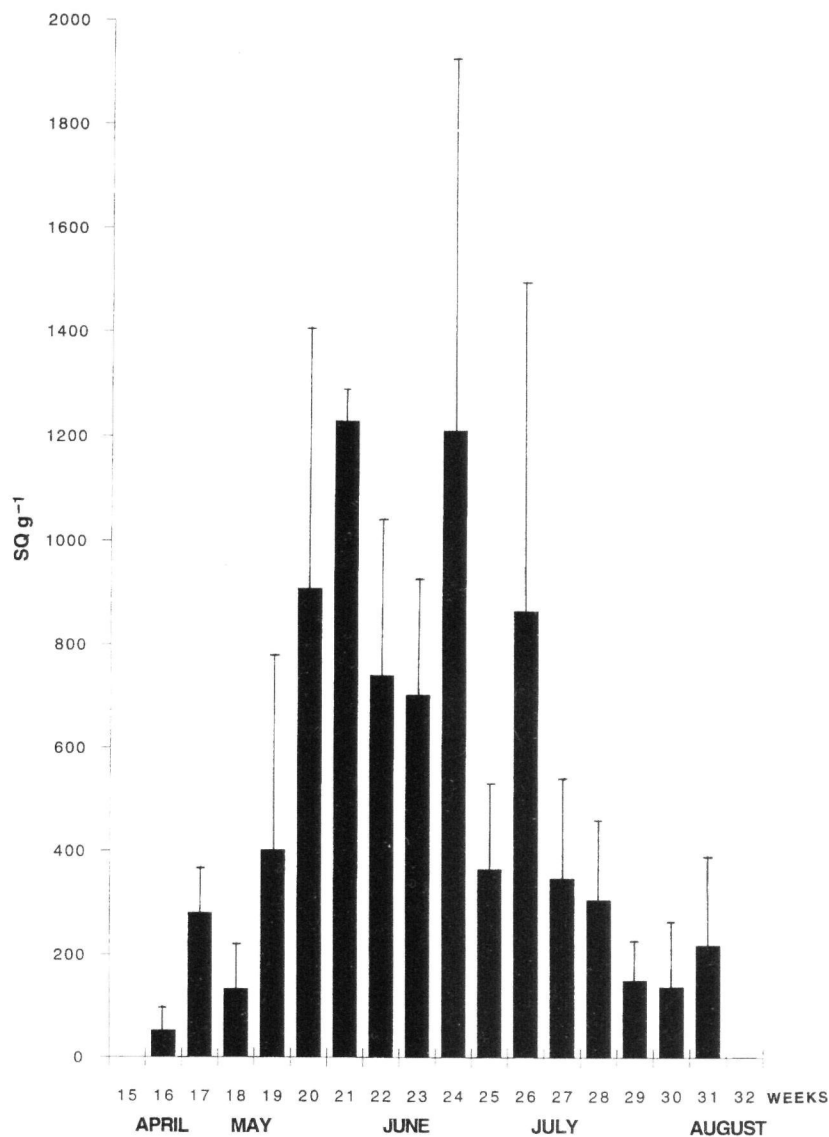


Fig. 2. Mean  $\pm$  SE of total birch-pollen antigenic activity (SQ units  $g^{-1}$  of dust) analyzed weekly in urban homes in summer 1993 ( $n = 4$ ).

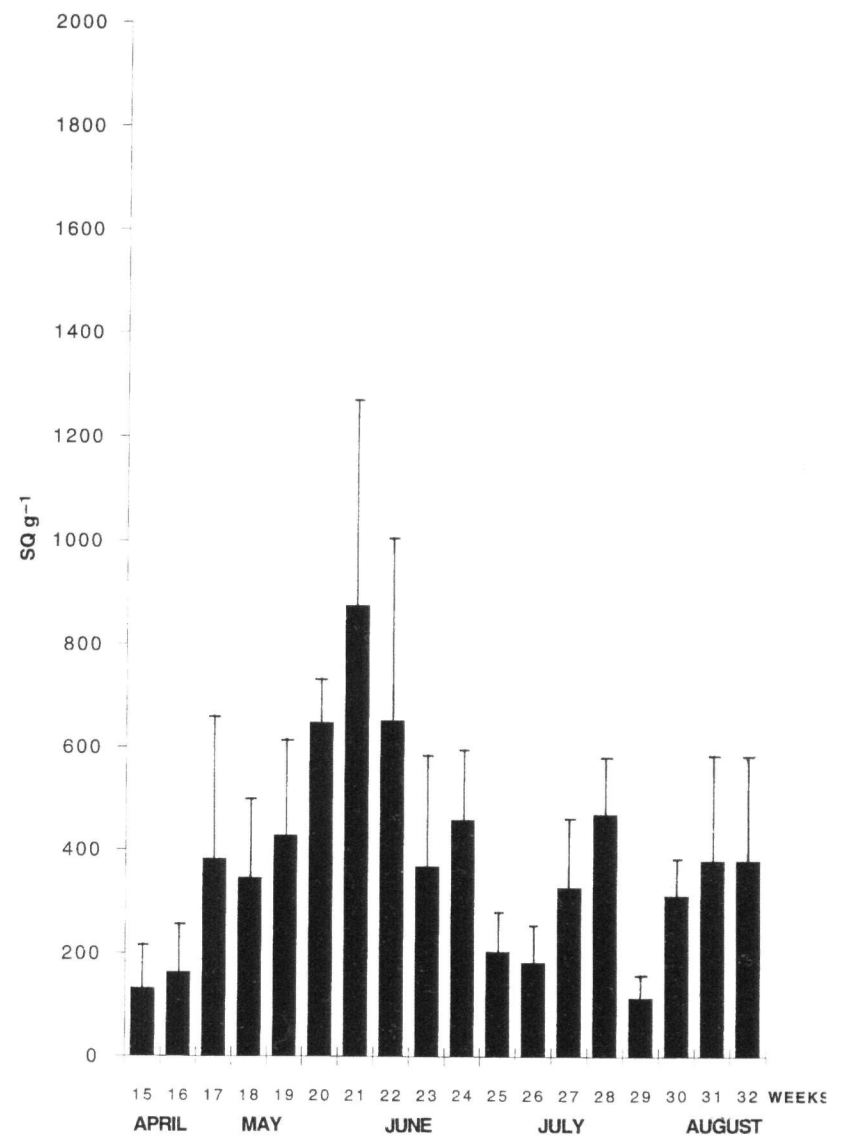


Fig. 3. Mean  $\pm$  SE of total birch-pollen antigenic activity (SQ units  $g^{-1}$  of dust) analyzed weekly in rural homes in summer 1993 ( $n = 6$ ).

(Fig. 2), as well as in rural homes (Fig. 3), throughout the study period. Activity was clearly higher in outdoor than in indoor dust, the highest concentrations of antigenic activity (23 300 SQ units outdoors, 2800 SQ units indoors) being found 1–4 weeks after the pollen peak. Some activity was still detected 2 months after the peak pollen period; toward the end of our sampling period, it fell to zero, but only in urban residences. The mean concentration of birch-pollen antigenic activity was slightly but not significantly higher in urban than in rural homes.

The peak birch-pollen periods for 1992 and 1993 differed greatly both in duration and in the total quantity of birch pollen (Fig. 4). Both indoor and outdoor birch-pollen antigenic activity cross-correlated with the Burkard birch-pollen counts for the 7 days preceding (BBP7). The lag in the rise in antigenic activity compared to BBP7 varied by 1–3 weeks (Table 1). The cross-correlation was highly significant in two out of four cases in urban homes (UH) and significant in five out of six cases in rural homes (RH) (Table 2). In one out of five rural homes, antigenic activity negatively correlated slightly significantly with the Burkard birch-pollen counts for

the 7 previous days. Antigenic activity in outdoor settled dust also correlated with the simultaneous Burkard counts (Table 1).

Birch-pollen antigenic activity in settled dust on outdoor surfaces (SQS) cross-correlated with the activity of indoor dust in two urban homes (Table 1). The activity on outdoor surfaces was correlated with the mean relative humidity of the 2 weeks preceding

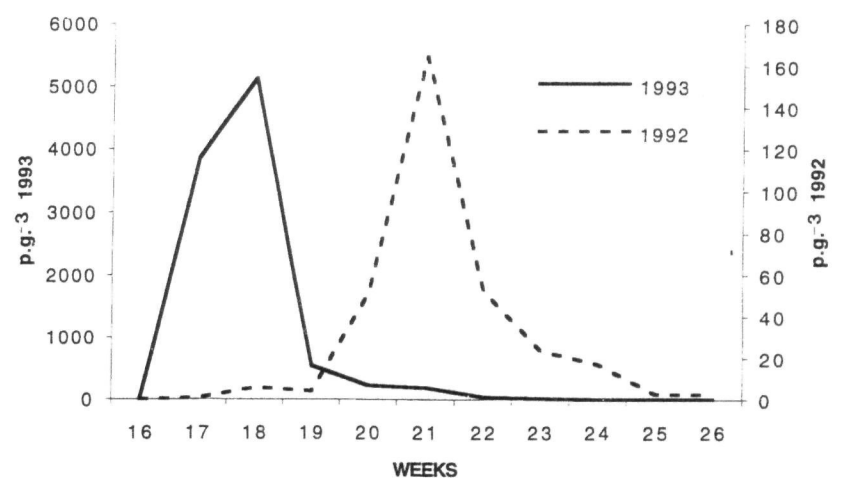


Fig. 4. Mean weekly airborne birch-pollen counts in Turku in 1992 and 1993 (1).

Table 1. Cross-correlation coefficients of birch-pollen antigenic activity (SQ units) in settled dust outdoors (SQS) and indoors (SQUH=urban home) with Burkard counts from 7 previous days (BBP7), and birch-pollen antigenic activity in outdoor settled dust with activity indoors in urban area. Differences significant at 5% (\*), 1% (\*\*), and 0.1% (\*\*\*) levels; coefficients ( $r$ ) ( $n=18$ )

|      | SQS          | SQUH1        | SQUH2 | SQUH3        | SQUH4  |
|------|--------------|--------------|-------|--------------|--------|
| BBP7 | 0.881<br>*** | 0.709<br>*** | 0.360 | 0.686<br>*** | -0.180 |
| SQS  |              | 0.659<br>**  | 0.403 | 0.835<br>*** | -0.256 |

Table 2. Cross-correlation coefficients of birch-pollen antigenic activity in indoor settled dust (SQRH=rural home) with Burkard birch-pollen counts from 7 previous days (BBP7). Differences significant at 5% (\*), 1% (\*\*), and 0.1% (\*\*\*) levels; coefficients ( $r$ ) ( $n=18$ )

|      | SQRH1       | SQRH2 | SQRH3        | SQRH4       | SQRH5       | SQRH6        |
|------|-------------|-------|--------------|-------------|-------------|--------------|
| BBP7 | 0.651<br>** | 0.514 | 0.693<br>*** | 0.640<br>** | -0.543<br>* | 0.761<br>*** |

( $n=18$ ;  $r=0.617$  (significant at 1% level)). No correlation was found with the rain sum of the 7 days preceding.

The mean concentrations of birch-pollen antigenic activity indoors in the city and in rural areas were also correlated ( $n=18$ ;  $r=0.655$  (significant at 1% level)).

## Discussion

The peak birch-pollen period for 1993 was exceptionally long and intensive compared to that for 1992, which was close to the average for 17 years (1); however, no essential differences were detected in the concentrations of antigen activity of outdoor dust between 1992 and 1993. It can therefore be postulated that the high pollination rate was not the crucial factor in the high antigenic activities obtained in either indoor or outdoor dust. As a source of birch antigens, either indoor or outdoor dust may be an important trigger for pollen-allergy symptoms in any year.

In 1993, birch pollen was presumably not released during the entire period, as calculated on the basis of the general threshold value of 10%; pollen resuspension toward the end of and after this period, however, was so massive that airborne pollen counts remained high for several weeks. The most important source of this "late pollen" is dust settled on various surfaces. Another source, of course, is long-range transportation (3), which in a year such as 1993 is also an important factor, due in part to the very high amounts of pollen occurring in neighboring countries south of Finland.

The lag of 3 weeks in the rise in indoor concen-

trations of birch-pollen antigenic activity compared to outdoor ones was surprisingly long. It is explained partly by antigenic dust being carried, for instance, on shoes and clothing; partly by penetration through natural ventilation. Ventilation, however, is not a very obvious medium of passage; sedimentation is expected to be more important in indoor environments, where air currents are weaker and resuspension is less important than in outdoor environments. The delay should thus be at most a few hours; in fact, antigenic activity ought to peak indoors and outdoors roughly at the same time.

Birch-pollen antigenic activity in indoor dust in the city was slightly higher than in rural homes, but the number of homes was too small to draw any definite conclusions. However, the number of samples was sufficient to determine the variations in antigenic activity in indoor dust, and the main question posed in this study, concerning the presence of birch-pollen antigenic activity in house dust, has been answered. The density of birch trees in the surroundings did not seem to explain the differences; on the other hand, birch pollen is dispersed fast and far (5) and the allergenicity is long-lasting (18).

Cleaning methods (dry or wet) can cause variations in the antigen concentrations on the floor. If only dry cleaning is used, vacuum-cleaning may release antigen material in small size classes back into the air (7).

The total count of birch-pollen grains did not seem to account for the variations in birch-pollen antigen concentrations in the air (9). Correspondingly, information concerning concentrations of settled indoor antigenic material is more important than counting the number of pollen grains in the dust sample. First of all, the pollen walls may retain their form and structure for years, and pollen concentration in the dust thus does not reveal anything about the amount of antigenic material present. Secondly, intact pollen grains do not penetrate deep into the respiratory tract but are retained in the upper airways, where they mainly cause rhinitis and nasal or ophthalmic irritation. Small micronic particles, on the other hand, penetrate into the alveolar part of the lungs, where they may cause immediate asthmatic reactions (12, 15).

Very little is known about the structure and characteristics of submicronic particles. Knox et al. (6) have shown that the airborne concentration of starch grains of the grass *Lolium perenne* increases after rain. These grains are supposed to be released from grass pollen when it is wet. Knox et al. (6) have also found positive IgE-RAST reactions to these starch granules. Takahashi (pers. comm.) analyzed the high antigenic activity of Japanese-cedar (*Cryptomeria japonica*) pollen in particles 1  $\mu\text{m}$  in size, 99% of which were identified as Übisch bodies. So far, no

studies have been made of the relation between birch starch granules or Übisch bodies and antigens. Nor have submicronic particles in indoor air been studied thus far.

SQ units are arbitrary; they can be compared with one another but not transformed into antigen quantity per air volume. For this purpose, monoclonal antibodies and IgE analysis should be used (17).

In the future, it is important to determine the best way of cleaning antigenic material from indoor surfaces and how to avoid the carriage of antigen material into the house. Analyses of airborne antigen concentrations indoors are also needed. We do not know how high concentrations of antigens found in settled dust may rise into the air under different circumstances. The results of experimental and case studies would be useful in epidemiologic studies of pollen allergy. The small particle size enables penetration through ventilation filters, especially if they are not in perfect condition and correctly adjusted. The efficiency of different ventilation filters should also be studied. It is also not known whether the relationship between antigenic activity and the amount of pollen grains varies from year to year.

In conclusion, birch-pollen antigenic activity was found to occur indoors in concentrations which may cause symptoms in allergic subjects. The assumption that indoor dust quickly loses its antigenic activity was shown to be incorrect.

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