

F.Th.M. Spieksma
A.H. Nikkels

Similarity in seasonal appearance between atmospheric birch-pollen grains and allergen in paucimicronic, size-fractionated ambient aerosol

Authors' affiliations:

F.Th.M. Spieksma, A.H. Nikkels, Laboratory of Aerobiology, Department of Pulmonology, Leiden University Medical Centre, Leiden, The Netherlands

Correspondence to:

Dr F.Th.M. Spieksma
Laboratory of Aerobiology
Department of Pulmonology
Leiden University Medical Centre
PO Box 9600
NL-2300 RC Leiden
The Netherlands

Key words: aerobiology; allergen; atmospheric; birch pollen; paucimicronic.

Background: Specific allergen from various pollen types has been found to be carried through the outdoor air, not only by intact pollen grains, but also by much smaller, paucimicronic particles. There is no complete agreement on whether, for birch (*Betula*), the seasonal appearance of atmospheric pollen allergen follows the fluctuation pattern of the pollen season.

Methods: Assessment of birch-pollen allergen in size-fractionated ambient aerosol was performed by descending elution of allergen from impaction strips of a five-stage cascade impactor, and compared with atmospheric birch-pollen concentrations as measured with a volumetric pollen trap.

Results: An overall similarity in the courses of the seasonal presence was found for both birch pollen and allergen in particles of all paucimicronic size fractions, although some airborne allergen was detected before and after the birch-pollen season.

Conclusions: Transfer of pollen allergen to both natural and pollutant environmental dust particles is thought to be the probable explanation of secondary allergen carriage by paucimicronic particles. Until simple techniques for direct and rapid assessment of atmospheric pollen allergen become available, the pollen count gives sufficiently reliable information about the seasonal course of airborne pollen allergen.

Date:

Accepted for publication 28 September 1998

To cite this article:

Spieksma F.Th.M. & Nikkels A.H. Similarity in seasonal appearance between atmospheric birch-pollen grains and allergen in paucimicronic, size-fractionated ambient aerosol.

Allergy 1999; 54, 235–241.

Copyright © Munksgaard 1999

ISSN 0105-4538

It is well established that atmospheric pollen allergen is carried not only by intact pollen grains, but also by smaller particles measuring a few microns, the so-called paucimicronic particles (1). This phenomenon has been shown for several pollen types, such as ragweed (*Ambrosia*) (2, 3), oak (*Quercus*) (4), grasses (Poaceae) (5), Japanese cedar (*Cryptomeria*) (6), and birch (*Betula*) (7). Therefore,

for clinical and experimental studies on pollinosis in which natural exposure to airborne pollen allergen is of essential significance, it is necessary to assess the relationship between the atmospheric pollen-allergen quantities, carried by both pollen grains and paucimicronic particles, and the airborne pollen-grain concentrations, as monitored in many places in Europe (8) and North America (9).

For grass-pollen allergen, it has been shown (10) that the airborne quantities follow the fluctuations of the grass-pollen grain concentrations during the season, with very little atmospheric presence of allergen after the season. Atmospheric appearance of substantial quantities of pollen allergen outside the main pollen season has been reported for ragweed (3), oak (4), and birch (7, 11, 12), although the relationship between birch-pollen grains and allergen was evident in other studies (13–15).

For elucidation of the potential discrepancy in these findings regarding birch and grass pollen, it was decided to examine the relationship between the atmospheric quantities of birch-pollen grains and birch-pollen allergen, in two seasons, 1996 and 1997, at Leiden, The Netherlands, by the same techniques for birch as for grass (5), thus excluding methodological differences (13–16) as the cause of the discrepancy.

Material and methods

For assessment of the atmospheric quantities of pollen grains and pollen allergen, almost identical methods have been used to those described earlier (5), the sampling part of which was similar to that used for the collection of ragweed-pollen allergen in an earlier North American study (3).

Birch-pollen count

Daily atmospheric concentrations of birch pollen were measured with a continuously operated, volumetric pollen trap (Burkard Manufacturing, Rickmansworth, UK), and expressed as average number of pollen grains per m³ over periods of 24 h. The trap was installed on the five-story-high roof of the Leiden University Hospital building. There are no birch trees near the trap.

Particle sampling for allergen assessment

To collect atmospheric particles of various sizes, air was drawn through a five-stage cascade impactor (Andersen

Instruments, Atlanta, GA, USA), mounted on top of a high-volume air sampler (Ecotech Model 2000, Blackburn, Victoria, Australia), which was located approximately 3 m from the pollen trap. The flow rate was set at 1.13 m³ min⁻¹, at which flow, the size fractioning of the sampled particles in the five slot-shaped stages of the cascade impactor is as follows: stage 1: >10 mm; stage 2: 4.9–10 mm; stage 3: 2.7–4.9 mm; stage 4: 1.3–2.7 mm; stage 5: 0.6–1.3 mm. In each of the five stages, nine impaction strips (length 15 cm, width 1 cm), cut from hydrophilic glass-fiber sheets (GF 50; Schleicher & Schuell, Dassel, Germany), were placed. Particles of submicronic sizes are deposited on a bottom filter sheet underneath the cascade impactor rather than on impaction strips. These different formats of deposition make questionable a comparable descending elution procedure in the allergen-assessment technique. Moreover, exposure to allergen carried by submicronic particles is very difficult to estimate, because of the probably more limited deposition rates of these particles inside the airways. Therefore, this study was confined to the effectively size-defined paucimicronic fractions.

In the first observation season of 1996, the duration of individual sampling periods varied between 7.5 and 11 h (Table 1), and took place during daylight, on 11 occasions between 20 April and 4 June, during and after the birch-pollen season. In 1997, sampling periods varied between 9.5 and 11 h (Table 2), and were performed again during daylight, on 15 occasions between 16 January and 8 September, before, during, and after the birch-pollen season. Sampling did not take place on days with fog or rain to avoid wetting of the impaction strips inside the cascade impactor. Mostly, those wet days had low airborne pollen concentrations, and usually caused very few clinical pollinosis symptoms, suggesting low airborne pollen-allergen quantities.

Measurement of allergen on the impaction strips

After removal of the impaction strips from the cascade impactor, they were stored under dry conditions at room temperature for no longer than 2 months. Then, successively, strips from each sampling day were wetted to achieve extraction of the allergen from the dust particles (which were deposited in a narrow band in the middle of the strip), and to allow diffusion of the extracted allergen into the width of the strip. After drying, the allergen on the strip was incubated with pooled serum from pollinosis patients, containing specific anti-birch-pollen IgE antibodies in a combined incubation and descending elution procedure, as described earlier (5, 10). Assessment of birch-pollen aller-

Table 1. Characteristics and results of 11 samplings in 1996. Correlations are between calculated numbers of pollen grains and RAST-inhibition percentages

Sampling	Date	Duration of sampling (h:min)	Calculated number of pollen grains in impactor	RAST-inhibition percentages in atmospheric dust of various size fractions (mm) collected in five cascade-impactor stages				
				1 >10	2 4.9–10	3 2.7–4.9	4 1.3–2.7	5 0.6–1.3
1	20 April	11:10	420 750	80	82	78	72	73
2	24 April	8:00	51 150	60	64	52	55	38
3	25 April	8:55	33 000	49	45	37	14	20
4	27 April	9:40	168 300	83	83	82	78	66
5	28 April	10:40	169 950	78	80	80	73	60
6	2 May	7:50	51 150	58	64	65	56	64
7	7 May	10:10	28 050	40	37	24	9	14
8	15 May	10:15	16 500	42	45	24	16	12
9	21 May	7:25	6600	0	0	0	6	0
10	28 May	10:10	1650	11	23	11	8	6
11	4 June	8:35	0	22	15	14	23	16
Correlation coefficient (r):				+0.72	+0.71	+0.74	+0.73	+0.77
Level of significance (P):				<0.02	<0.02	<0.01	<0.02	<0.01

genic activity was performed by blocking of anti-birch-pollen IgE by the birch-pollen allergen on the strip (either as immobilized complexes in the strip or as soluble complexes

in the eluate), and subsequent measuring of remaining specific IgE activity in the eluate by RAST (RAST inhibition).

Table 2. Characteristics and results of 15 samplings in 1997. Correlations are between calculated numbers of pollen grains and RAST-inhibition percentages

Sampling	Date	Duration of sampling (h:min)	Calculated number of pollen grains in impactor	RAST-inhibition percentages in atmospheric dust of various size fractions (mm) collected in five cascade-impactor stages				
				1 >10	2 4.9–10	3 2.7–4.9	4 1.3–2.7	5 0.6–1.3
1	16 Jan	5:15	0	1	7	4	14	16
2	3 Feb	10:20	0	9	0	3	0	0
3	13 March	10:00	0	34	49	26	22	10
4	26 March	9:40	0	0	0	21	0	6
5	1 April	10:15	9900	76	76	56	72	44
6	8 April	10:15	74 250	54	72	62	46	48
7	9 April	10:15	171 600	65	66	62	63	67
8	10 April	9:25	28 050	17	58	37	48	23
9	17 April	10:25	89 100	73	84	94	77	61
10	21 April	9:55	11 550	33	32	20	29	17
11	23 April	10:25	21 450	73	81	85	72	45
12	2 May	11:00	0	28	44	51	49	42
13	13 May	10:30	0	14	23	22	16	22
14	23 May	10:00	1650	0	1	4	28	0
15	8 Sept	10:00	0	2	4	0	0	7
Correlation coefficient (r):				+0.59	+0.57	+0.60	+0.56	+0.77
Level of significance (P):				<0.05	<0.05	<0.05	<0.05	<0.001

For optimization of the interaction of allergen on the strip with the IgE antibodies, 50 ml pooled serum was applied to the hanging strip. In initial experiments, it was found that with a 1.5-fold dilution of the serum in PBS and strips containing allergen quantities corresponding to the season's expected maximal airborne birch-pollen concentrations, near maximal RAST inhibition was attained. Therefore, in all assessments, 1.5-fold serum dilution was used to enable accurate measurement of RAST inhibition. The elution time was approximately 6 h, during which about 1.0 ml eluate was collected. To enhance and standardize elution time and conditions, the setup of the laboratory equipment was covered with vapor-tight glass containers, keeping air humidity around the hanging strips at a constantly high level.

As controls, and for the calculation of the percent RAST inhibition (5), levels of specific anti-birch-pollen IgE antibodies in the serum pool without application to, and elution from the strip (minimum inhibition) were determined, as well as nonspecific binding of these IgE antibodies to unexposed strips (blank inhibition). For the detection of the remaining free, specific IgE antibodies in the eluate, birch-pollen-allergen-carrying disks (t₃, *Betula verrucosa*, Pharmacia AB, Uppsala, Sweden) were used. Subsequent measuring of the amount of IgE antibodies bound to the disks was performed, at least in duplicate, with Phadebas-RAST (Pharmacia AB, Uppsala, Sweden) in 1996, and with Phadezym-RAST (Pharmacia AB, Uppsala, Sweden) in 1997, always following the manufacturer's instructions.

Results

Airborne birch-pollen concentration

The seasons 1996 and 1997 were quite different in the amounts of airborne birch-pollen grains (Figs. 1a and 2a). In 1996, birch pollen appeared in the atmosphere from 17 April, rising sharply to extremely high daily concentrations up to 1868 pollen grains per m³ on 21 April, returning to lower concentration on 24 April and staying below 200 per m³, and gradually disappearing in the course of May. The birch-pollen season of 1996 was during the second half of April, which is the normal time of the year for the region of Leiden, and was characterized by extremely high concentrations, with the biggest annual sum of daily concentrations of 6383 ever recorded since the start of the observations in 1969 (17). The birch-pollen quantities in 1997 were much lower, with an annual sum of daily concentrations of 1365. This contrast

with the preceding year is a clear reflection of the year-to-year alternating pattern in birch-pollen quantities (17). In 1997, the birch-pollen season was very early, starting on 2 April, with the highest daily concentration of only 176 on 9 April, and ending by 4 May.

From the pollen concentration during the sampling period (which is different from the 24-h averages), taking into account the volume of air drawn by the sampler, the number of pollen grains collected in the cascade impactor could be calculated, and is given in Tables 1 and 2 and shown in Figs. 1b and 2b. Due to the (aerodynamic) size of the birch-pollen grains (20–25 µm), they were deposited on stage 1 of the cascade impactor (5).

Atmospheric birch-pollen allergen

The quantities of birch-pollen allergen, measured as RAST-inhibition percentages, in the five size fractions of atmospheric dust collected by the cascade impactor, are given in the right half of Tables 1 and 2, and shown in Figs. 1c and 2c. The RAST-inhibition percentages from the five stages of the cascade impactor are depicted as stacked bars, illustrating an overall similarity in the seasonal courses of pollen grains and pollen allergen. This similarity can also be expressed as levels of relationship between quantities of pollen grains and allergen, as given on the bottom right hand of Tables 1 and 2.

For the 1996 season, these correlation coefficients (*r*) are very similar for the five cascade-impactor stages. Nor were the coefficients for stages 1 to 4 much different in 1997. This similarity in the correlations of allergen quantities with the sampled amounts of pollen grains can be made visible in scatter diagrams, examples of which for the >10-µm, pollen-grain-containing size fraction in stage 1 and for the 1.3–2.7 µm, pollen-free size in stage 4 are shown in Fig. 3 for 1996, and in Fig. 4 for 1997.

In Figs. 1c and 2c, it can also be seen that in 1996 after the pollen season, and in 1997 before and after the season, the amount of birch-pollen allergen in the atmosphere was small in all size fractions of dust particles. Nevertheless, as illustrated by the asymmetric form of the scatter diagrams in Figs. 3 and 4, there was atmospheric presence of birch-pollen allergen in various size fractions when the airborne birch-pollen grains were very low in number or even absent.

Discussion

The seasonal presence in the air of both birch-pollen grains and allergen showed an overall similarity, as found

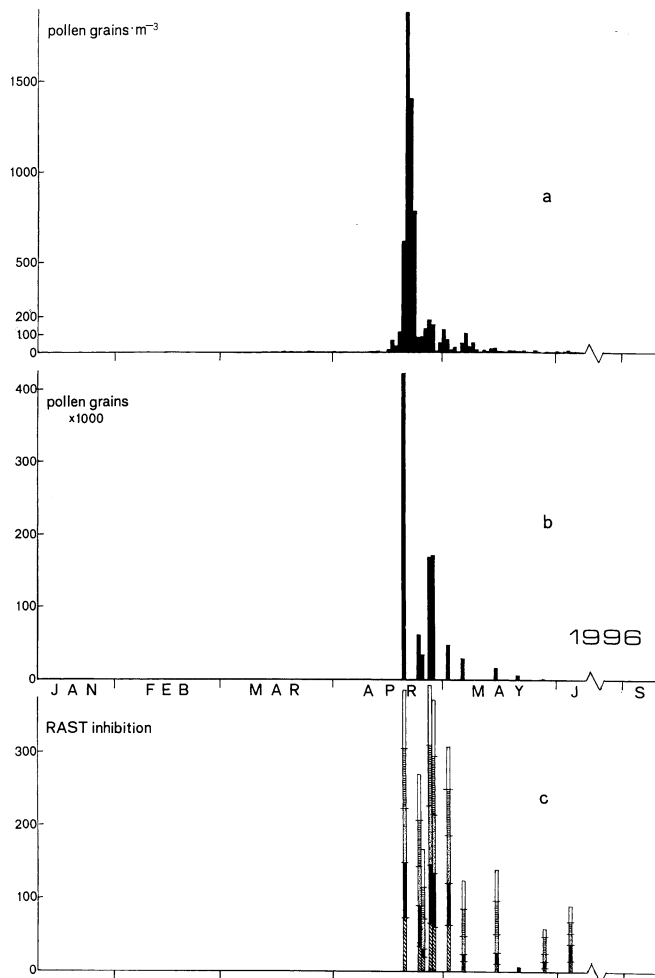


Figure 1. Results for 1996 season. a) Airborne birch-pollen concentration (pollen grains·m⁻³, daily); b) pollen grains (×1000) collected on stage 1 of cascade impactor (on sampling days); c) relative amounts of birch-pollen allergen (RAST inhibition) in five stages of cascade impactor. □: stage 1; ■: stage 2; ▨: stage 3; ■: stage 4; ▩: stage 5. J: June; S: September.

by other authors (15). The relationship, expressed as correlation coefficients for the five size fractions (on average, $r=+0.73$), was higher for the 1996 season, with extremely high birch-pollen quantities, than for the 1997 season (on average, $r=+0.62$), with a rather low birch-pollen concentration. This higher level of relationship in 1996 might be explained by the much wider range of measurement values, particularly for the birch-pollen grains, in that year than for 1997. Another imperfection of the relationship was also caused by the very high birch-pollen amounts in 1996, which led to the RAST-inhibition measurement being restricted by an upper limit of the detection range, which was chosen for the normally expected birch-pollen quantities.

The same kind of relationship between amounts of pollen grains and allergen in the air has been found before with

grass pollen (10), with an even higher correlation coefficient ($r=+0.77$), a correlation which is also in agreement with results obtained by others (14).

In line with the correlations between the quantities of pollen grains and allergen in all size fractions (Tables 1 and 2), the amounts of allergen before and after the pollen seasons were small although not absent (Figs. 1 and 2), as found by other authors (14, 15). Still other authors, however, did find very big quantities of airborne birch-pollen allergen outside the pollen season (7, 11, 12), both in the potentially pollen-containing fraction and in the paucimicronic, pollen-free-size fraction of the ambient aerosol.

To explain the origin of the atmospheric pollen allergen carried by non-pollen-grain particles, several hypotheses have been put forward (1, 10), such as carriage of allergen by nonpollen plant parts (3, 11, 14), by starch granules from ruptured pollen grains (15, 18), by orbicules from the dehiscing anthers which are sometimes carried on the outside of pollen grains (15, 19, 20), by naturally occurring ambient particles (10), or by pollutant particles (21–23) after transfer from the allergen to the particles (21). The relation with air pollution has, however, been questioned by a recent report (24), in which the tree's habitat and genetic background are considered more important for the allergenic potency of the pollen. Meteorologic factors might also play an important role in the atmospheric presence of pollen allergen carried by nonpollen, often paucimicronic particles (11, 13, 15, 25).

Transfer of allergen from pollen grains to environmental, particles, natural or pollutant, of all size fractions might explain the atmospheric presence of pollen allergen after the season, when these secondarily pollen-allergen carrying particles become resuspended in the air by meteorologic events, such as wind or turbulence. The airborne appearance of birch-pollen allergen before the season could be explained by the preceding alder (*Alnus*)-pollen season (12, 13), the allergen of which cross-reacts with birch-pollen allergen. Airborne particles carrying alder-pollen allergen released a few weeks before the birch-pollen season may thus be responsible for the sometimes substantial amounts of atmospheric allergen indistinguishable from the cross-reacting birch-pollen allergen. Particularly in 1997, levels of alder pollen in the air showed some high peaks in the second half of February and the first half of March (data not shown), just preceding the relatively high quantity of allergen on 13 March (sampling no. 3, Table 2).

In conclusion, it can be stated that there is an overall similarity in the atmospheric presence of birch-pollen grains and allergen in the various size fractions of ambient aerosol,

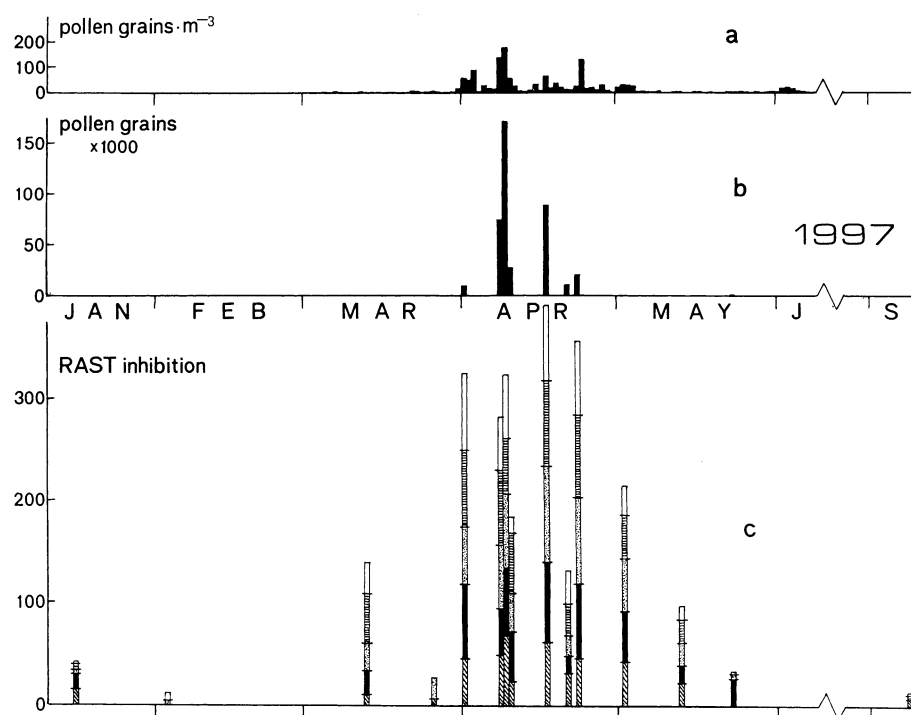


Figure 2. Results for 1997 season. Legend as for Fig. 1.

including the paucimicronic fractions. The results of this study indicate that there is a need for more direct and accurate methods of sampling and measuring airborne allergens, to yield more data about the potential exposure of sensitized subjects. But until such techniques are feasible for continuous observations, the simpler pollen count gives sufficient reliable information, taking into account the atmospheric presence of mostly small quantities of allergen after the pollen season, and of cross-reacting allergen before the season. The processes by which pollen allergen becomes airborne, carried by particles of

various, paucimicronic sizes, are not yet elucidated, but transfer from pollen grains to environmental, natural and/or pollutant particles seems likely.

Acknowledgments The gift by Pharmacia & Upjohn BV Nederland of the reagents for the RAST-inhibition procedure is gratefully acknowledged. We thank Dr P. S. Hiemstra for critical comments, and Mrs C. C. Zuiderduin for the preparation of the manuscript.

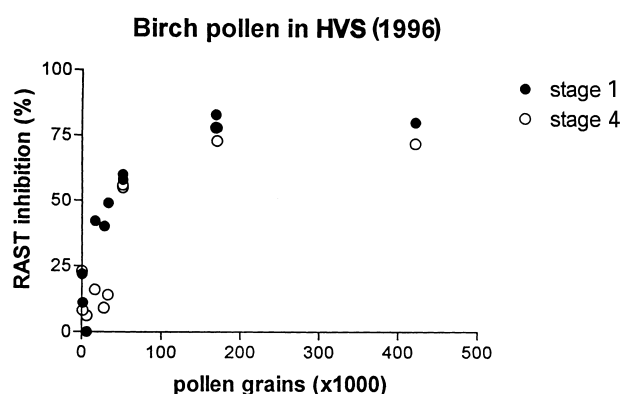


Figure 3. Quantities of birch-pollen grains (collected on stage 1) and relative amounts of birch-pollen allergen (RAST inhibition) on stages 1 and 4 of cascade impactor (1996). HVS: high volume sampler.

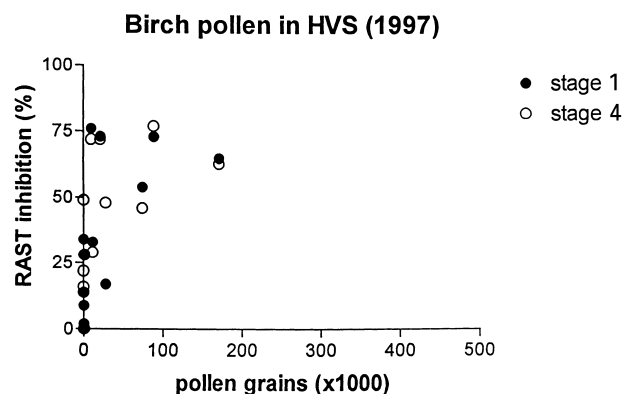


Figure 4. Quantities of birch-pollen grains (collected on stage 1) and relative amounts of birch-pollen allergen (RAST inhibition) on stages 1 and 4 of cascade impactor (1997). HVS: high volume sampler.

References

1. Emberlin J. Plant allergens on pauci-micronic airborne particles [Editorial]. *Clin Exp Allergy* 1995;**25**:202–205.
2. Solomon WR, Burge HA, Muilenberg ML. Allergen carriage by atmospheric aerosol. I. Ragweed pollen determinants in smaller micronic fractions. *J Allergy Clin Immunol* 1983;**72**:443–447.
3. Agarwal MK, Swanson MC, Reed CE, Yunginger JW. Airborne ragweed allergens: association with various particle sizes and short ragweed plant parts. *J Allergy Clin Immunol* 1984;**74**:687–693.
4. Fernandez-Caldas E, Swanson MC, Pravda J, Welsh P, Yunginger JW, Reed CE. Immunochemical demonstration of red oak pollen aeroallergens outside the oak pollination season. *Grana* 1989;**28**:205–209.
5. Spieksma FThM, Kramps JA, van der Linden AC, et al. Evidence of grass-pollen allergenic activity in the smaller micronic atmospheric aerosol fraction. *Clin Exp Allergy* 1990;**20**:273–280.
6. Takahashi Y, Sakaguchi M, Inouye S, Miyazawa H, Imaoka K, Katagiri S. Existence of exine-free airborne allergen particles of Japanese cedar (*Cryptomeria japonica*) pollen. *Allergy* 1991;**46**:558–593.
7. Rantio-Lehtimäki A, Viander M, Koivikko A. Airborne birch pollen antigens in different particle sizes. *Clin Exp Allergy* 1994;**24**:23–28.
8. Spieksma FThM. Regional European pollen calendars. In: D'Amato G, Spieksma FThM, Bonini S, editors. *Allergenic pollen and pollinosis in Europe*. Oxford: Blackwell Scientific, 1991:49–65.
9. Lewis WH, Dixit AB, Ward WA. Distribution and incidence of North American pollen aeroallergens. *Am J Otolaryngol* 1991;**12**:205–226.
10. Spieksma FThM, Nikkels AH, Dijkman JH. Seasonal appearance of grass pollen allergen in natural, pauci-micronic aerosol of various size fractions. Relationship with airborne grass pollen concentration. *Clin Exp Allergy* 1995;**25**:234–239.
11. Pehkonen E, Rantio-Lehtimäki A. Variations in airborne pollen antigenic particles caused by meteorologic factors. *Allergy* 1994;**49**:472–477.
12. Pehkonen E, Rantio-Lehtimäki A. Sensivity of IgE-ELISA for detecting airborne pollen antigens of Betulaceae. *Grana* 1996;**34**:350–356.
13. Ekebom A, Vesterberg O, Hjelmroos M. Detection and quantification of airborne birch pollen allergens on PVDF membranes by immunoblotting and chemiluminescence. *Grana* 1996;**35**:113–118.
14. Schäppi GF, Monn CH, Wüthrich B, Wanner HU. Analysis of allergens in ambient aerosols: comparison of areas subjected to different levels of air pollution. *Aerobiologia* 1996;**12**:185–190.
15. Schäppi GF, Suphioglu C, Taylor PHE, Knox RB. Concentrations of the major birch tree allergen Bet v 1 in pollen and respirable fine particles in the atmosphere. *J Allergy Clin Immunol* 1997;**100**:656–661.
16. Kauppinen EI, Jäppinen AVK, Hillamo RE, Rantio-Lehtimäki AH, Koivikko AS. A static particle size selective bioaerosol sampler for the ambient atmosphere. *J Aerosol Sci* 1989;**20**:829–838.
17. Spieksma FThM, Emberlin JC, Hjelmroos M, Jäger S, Leuschner RM. Atmospheric birch (*Betula*) pollen in Europe: trends and fluctuations in annual quantities and the starting dates of the seasons. *Grana* 1995;**34**:51–57.
18. Suphioglu C, Singh MB, Taylor PH, et al. Mechanism of grass-pollen-induced asthma. *Lancet* 1992;**339**:569–572.
19. Takahashi Y, Sasaki K, Nakamura S, Miki-Hirosige H, Nitta S. Aerodynamic size distribution of the particles emitted from the flowers of allergologically important plants. *Grana* 1995;**34**:45–49.
20. El-Ghazali G, Takahashi Y, Nilsson S, Grafström E, Berggren B. Orbicules in *Betula pendula* and their possible role in allergy. *Grana* 1995;**34**:300–304.
21. Behrendt H, Becker WM, Friedrichs KH, Darsow U, Tomingas R. Interaction between aero-allergens and airborne particulate matter. *Int Arch Allergy Immunol* 1992;**99**:425–428.
22. Hirotaka I, Shunkichi B, Kazunori M. The relationship between Japanese cedar pollinosis and air pollutants deposited on the pollen. *Aerobiologia* 1996;**12**:37–42.
23. Knox RB, Suphioglu C, Taylor P, et al. Major grass pollen Lol p 1 binds to diesel exhaust particles: implications for asthma and air pollution. *Clin Exp Allergy* 1997;**27**:246–251.
24. Helander ML, Savolainen J, Ahlholm J. Effects of air pollution and other environmental factors on birch pollen allergens. *Allergy* 1997;**52**:1207–1214.
25. Venables KM, Allitt U, Collier CG, et al. Thunderstorm-related asthma – the epidemic of 24/25 June 1994. *Clin Exp Allergy* 1997;**27**:725–736.