

Differences in size selective aerosol sampling for pollen allergen detection using high-volume cascade impactors

M. RIEDIKER, T. KOLLER and C. MONN

Institute for Hygiene and Applied Physiology, Environmental Hygiene Group, Swiss Federal Institute of Technology, Switzerland

Summary

Background Assessment of the allergen content of airborne particles small enough to reach the bronchiolar airways is important for a better understanding of allergic asthma.

Objective In order to test the performance of a high-volume cascade impactor for size-selective sampling of airborne particles, the characteristics of pollen deposition, and the particle size-dependent allergen distribution, were re-examined.

Methods Two cascade impactors with rectangular slots were run in parallel, one with glass fibre filters on all stages, the other with silicone grease on stage 2 (collection of particles of size 4.2–10.2 μm) instead. Pollen was counted using light microscopy and allergens were measured using ELISA techniques.

Results In the impactor without the greased stage 2, a heavy bounce and blow-off for pollen was found. Bounced pollen was deposited mainly on the back-up filter, the sampling stage for particles smaller than 1.4 μm . However, if stage 2 was coated with silicone grease, less than 1% of total pollen was found in the lower stages. On the first stage of the sampler (50% cut-point, 10.2 μm) where all pollen should impact, pollen had a deposition efficiency of only 24–47%, depending on the abundant pollen species. Much less allergen was found in fine particle fractions than in previous studies in which particles were sampled with similar samplers but without grease as a pollen trap.

Conclusions The observed bounce and blow-off of pollen without a greased stage 2 leads to serious mistakes when assessing the allergen content of the fractions collecting particles smaller than intact pollen, i.e., below 10.2 μm . Pollen allergen concentrations in respirable particle fractions are much lower than in the pollen size fraction. This is of great importance both for symptoms of sensitized allergic asthmatics and for research of asthma-related mechanisms.

Keywords: asthma, allergen, pollen, ELISA, high-volume cascade impactor, fine particles, bounce and blow-off, retention efficiency, light microscopy

Clinical and Experimental Allergy, Vol. 30, pp. 867–873. Submitted 23 June 1999; revised 31 August 1999; accepted 22 September 1999.

Introduction

Experimental exposure to pollen allergen loaded fine particles (< about 10 μm) is related to the allergic airway response in pollen-sensitized asthmatics which is not seen for intact pollen [1]. Typical aerodynamic diameters of pollen are between 22 μm (birch) and 100 μm (corn) [2,3]; intact pollen therefore gets mainly deposited in the upper airways. Particles smaller than 10 μm , however, can penetrate

into the bronchiolar tree [4,5]. In order to better understand the symptoms of pollen-sensitized asthmatics during the pollen season, and for preventative measures, it is of interest to determine whether pollen allergens are present in the thoracic particle fraction (< 10 μm , e.g. fragments of pollen, parts of the dehiscing anther, or allergens transferred onto small particles), and to determine the conditions favouring such an occurrence (e.g. weather conditions, air pollution).

During the last three decades, a number of studies has examined the temporal and size-dependent distribution of pollen allergens in particles smaller than pollen [6–18]. In most of these studies, allergens could be detected in the

Correspondence: M. Riediker, Institute for Hygiene and Applied Physiology, Swiss Federal Institute of Technology, Clausiusstrasse 25, 8092 Zurich, Switzerland.

Table 1. Characteristics of the high-volume cascade impactor run with a flow of 565 L/min. Cut-points indicate particle sizes with 50% and 90% retention efficiency on the impaction stage; they are valid for ideally sticking particles [30,35]. A back-up filter after stage 4 collects all remaining particles

Stage no.	Slit width (mm)	Slit length (mm)	No. of slots	Air-velocity (m/s)	50% Cut-point (μm)	90% Cut-point (μm)
1	3.97	111	9	2.37	10.2	14.1
2	1.63	124	10	4.66	4.2	6.9
3	0.91	124	10	8.35	2.1	3.8
4	0.46	124	10	16.51	1.4	1.8

respirable size fraction. However, observations concerning the amount of allergens in small particle fractions are contradictory [11,18,19]. Different samplers and filters, and different methods of allergen detection, were used and therefore, a comparison of the results is difficult.

Frequently, sampling methods based on impaction by inertia are used for examining the allergen load of particles. An air-stream turns at right angles over a surface, and particles above a certain size cannot follow the air stream and become deposited on the surface. By increasing the velocity of the air-stream in subsequent stages of the sampler, smaller and smaller particles are deposited [20]. During the last 50 years, impaction characteristics have been studied theoretically [21–23] and experimentally [24–31]. It has been shown repeatedly that under certain conditions, the collection efficiency is unsatisfactory. A well known problem is the removal of impacted particles from non-sticky surfaces [25]. The most relevant factors for bounce and blow-off are free stream velocity and particle size [21,23,31]. At stream velocities of 3–4 m/s, deposition reductions of 30–60% have been observed for ragweed pollen in wind tunnel experiments [27]. Deposition efficiency is good, however, for small particles ($<1\ \mu\text{m}$) on glass fibre filters, even at high stream velocities [24,28]. These particles are small enough to become trapped between the glass fibres. However, large particles produce massive bounce and blow-off [26,29]. Therefore, the retention efficiencies of impactors have to be evaluated, especially when sampling is done with non-sticky surfaces.

Contrary to the frequently used real impactors, virtual impactors have good retention efficiencies as there is no real impaction surface. Low volume cascade impactors with round orifices are run at a low stream velocity, resulting in reasonable retention efficiencies for large particles [22,24]. However, if, in a certain size fraction, low amounts of allergen are to be detected with good time resolution, a high-volume slotted impactor is needed. In these devices, the high stream velocity appears to promote bounce and blow-off particularly for large particles [21,23,30,31].

Considerably reduced deposition efficiencies for methylene blue particles ($7.7\ \mu\text{m}$) on glass fibre filters were reported for such a sampler; only 29% of the total collected particles were found on stage 1, and 24% were on the back-up filter [26]. When an attempt was made to determine the pollen deposition efficiency in our high-volume cascade impactor, it was realized that fractionation according to size was poor, especially for large particles, as intact pollen was found in all stages and particularly on the back-up filter. In this paper, a technique for reducing pollen bounce and blow-off into lower stages to a minimum is described. The new technique was verified using light microscopy, scanning electron microscopy and an immunological method (ELISA).

Materials and methods

A cascade impactor with rectangular slots was mounted inside the round sampling head of a high-volume sampler (DIGITEL DH77, Digitel AG, Switzerland); Table 1 shows its characteristics. For the experiments, two identical samplers were used. The airflow of the sampler was set to 565 L/min and kept constant over the sampling period with an opto-electronic control device. The flow was calibrated using equipment and procedures from EMPA Dübendorf (Swiss Federal Laboratories for Materials Testing and Research). Cut-points indicate aerodynamic diameters of particles that are kept back by the stage with an efficiency of 50% and 90%. These cut-points are valid for particles sticking ideally to the collection substrate [30]. In the present experiments, particles on stages 1, 3, 4 and the back-up were collected on glass fibre filters (Schleicher & Schuell, Germany; stages 1, 3 and 4: slotted GF50, 10 impaction strips; backup: round through-flow-filter GF9). The collection substrate on stage 2 was either tracing paper coated with a thin ($50\ \mu\text{m}$) layer of silicone grease (Bayer medium vacuum), or a grease layer without tracing paper to provide an efficient adhesive layer.

The samplers were run close to a municipal air monitoring site in central Zurich. Filters were loaded at 0700 h and



Fig. 1. Up-light microscopic picture of the back-up filter of the non-greased sampler from the first experiment. Pollen is clearly visible as coloured (green) spheres.

unloaded at 0700 h the following day. After sampling, the filters were stored at -20°C . For pollen counting, filters were kept at room temperature for a maximum of 30 min. Half-hourly means of relative humidity, temperature and wind-speed were obtained from the municipal air monitoring site in order to analyse any possible influence of climatic factors on the retention efficiency of glass fibre filters on stage 1.

Pollen counting was conducted as follows. In the first experiment, particles were collected in two parallel samplers, one with the grease layer and one, as control, with the usual glass fibre filter on stage 2. One half of each of the 10 impaction strips of the filter (back-up: one quarter) was counted using up-light microscopy ($100\times$ magnification; Zeiss, Germany). Pollen was clearly visible as coloured spheres, especially in the lower stages of the sampler where micron black particles formed the background (see Fig. 1). Filters were also analysed by scanning electron microscopy. Filter pieces of 5 mm diameter were cut, sputtered in argon-vacuum with platinum (30 nm), and analysed using a field emission scanning electron microscope. In following experiments, only the pollen on one sampler was counted. Counting was done by up-light microscopy on a length of 6.2 cm on one strip of the slotted glass fibre filters (stages 1, 3 and 4). On the back-up filter, a radial strip 0.8 cm wide was counted. In one experiment, pollen was counted by through-light microscopy ($100\times$ and $400\times$ magnification) on stage 1 (glass fibre filter, counted length 6.2 cm) and stage 2 (tracing paper coated with grease, counted length 1.49 cm). Through-light microscopy was possible only for these two stages as in the other stages, micron black particles absorbed the light. In a separate experiment, all stages of

the sampler were coated with silicone-greased tracing paper and pollen counted by through-light microscopy.

The allergen load on the glass fibre filters was analysed using ELISA techniques. Filters which had been counted were extracted by an adaptation of the pulverizing elution technique [32,33]. The filters were crushed in a hydraulic press, then eluted using 0.5% Tween-20-containing, phosphate-buffered saline (150 mmol/L Cl^- , 157 mmol/L Na^+ , 3 mmol/L K^+ , 10 mmol/L PO_4^{3-}) with 0.01% NaN_3 as preservative (FLUKA, Switzerland), and microfiltered (Schleicher & Schuell, Germany, $0.45\text{ }\mu\text{m}$ pore size) before analysis. Allergens were analysed using a competitive ELISA against birch allergen based on polyclonal antibodies as described [33]. Briefly, microtitre plates were coated with birch pollen extracts, followed by blocking with milk powder solution. Samples were incubated with IgE-containing serum from a selected allergic patient. Finally, the amount of IgE bound to the plate was measured at 405 nm using labelled anti-IgE antibodies (AKP, Clone G7-26, Pharmingen, USA, and substrate 4-nitrophenyl-phosphate, FLUKA, Switzerland). Allergen concentrations were calculated using a standard dilution curve run in parallel.

Results

The first experiment (18 February 1998) was carried out on a warm and humid winter day (temperature: mean 4.2°C , range $2.0\text{--}7.8^{\circ}\text{C}$, relative humidity: 80.8%, 62.3–91.1%). Pollen was collected in two parallel samplers, one with the grease layer and one, as control, with the usual glass fibre filter on stage 2. As examined by light-microscopy, mostly

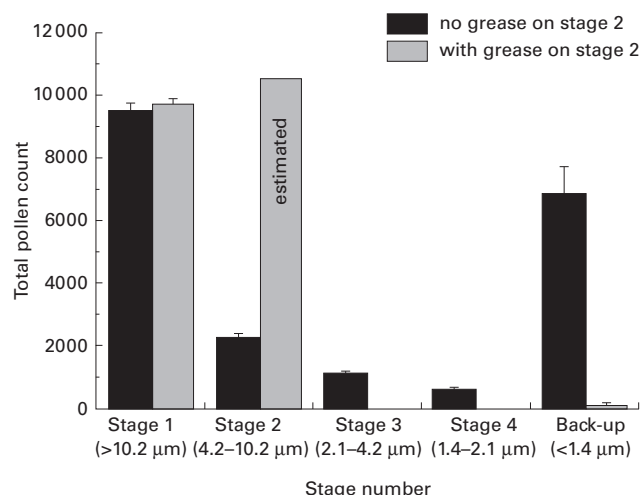


Fig. 2. Comparison of pollen counts of two high-volume cascade impactors run in parallel with and without silicone grease on stage 2. In the original sampler without grease, a considerable pollen load was observed on the stages for particles smaller than pollen, especially on the back-up filter which collects all particles passing the previous stages. Pollen counts on stage 2 of the greased sampler were estimated from the counts of stages 1, 3, 4 and the back-up and from the counts of the non-greased sampler. Differences on stages 3, 4 and the back-up are highly significant ($P < 0.0002$).

alder and hazel pollen were present. Figure 2 shows the results. Total pollen deposition in the non-greased samplers was about 20 500 (sum of all stages). On stage 1 ($> 10.2 \mu\text{m}$), only 47% of the total pollen count was found. Considerable amounts of pollen could be found in all other stages, particularly on the back-up filter (33.5%) (see Fig. 2). In the greased sampler, stage 2 showed a large amount of pollen impacted onto the silicone grease. Stage 2 was used

only as a trap (no filter mounted) to prevent pollen from bounce and blow-off to lower stages (no counting). The proportion of pollen on this stage was estimated to be at least 52% by comparing pollen counts of stages 1, 3, 4 and the back-up of the greased sampler with the total pollen number of the non-greased sampler. The estimated proportions of intact pollen contaminating the stages following the greased surface were 0.04% on stage 3, 0.02% on stage 4 and 0.8% on the back-up filter. In the non-greased sampler, however, the respective proportions were 11.2% on stage 2, 5.6% on stage 3, 3.2% on stage 4 and 33.5% on the back-up filter. The differences in pollen counts between the two samplers for stages 3, 4 and the back-up are highly significant ($P < 0.0002$).

When using scanning electron microscopy, pollen was found on stage 1 of both samplers. On stages 3, 4 and on the back-up filter of the greased sampler, no pollen was found on the arbitrarily chosen areas (5 mm diameter). Visually, particles were well separated by size. In the non-greased sampler, the arbitrarily chosen areas of the back-up filter showed scattered pollen and other large particles. Contrary to the light microscopy of the same filter, which showed a heavy pollen load, quantification of the pollen load on scanning electron micrographs was not feasible because of the small areas visualized compared with light microscopy. Probably, pollen was lost during preparation.

To verify the efficiency of greasing of stage 2 in preventing bounce and blow-off of pollen into lower stages, pollen was counted on eight randomly chosen days during the birch pollen season in spring 1998. Data are summarized in Table 2. Birch and ash pollen accounted for most of the count, but alder, oak and pine pollens were also seen. In agreement with the experiment described above, the proportions of pollen on stages 3, 4 and the back-up were always

Table 2. Deposition of pollen on stages 1, 3, 4 and the back-up filter of the high-volume cascade impactor with grease on stage 2. Sampling was performed on eight randomly chosen days during the spring pollen season at the centre of Zurich. The substrate on stage 2 was tracing paper coated with silicone grease

Sampling date	Total pollen number in impaction stages (50% cut-point)			
	Stage 1 ($10.2 \mu\text{m}$)	Stage 3 ($2.1 \mu\text{m}$)	Stage 4 ($1.4 \mu\text{m}$)	Back-up ($<1.4 \mu\text{m}$)
17 April 1998	20 041	0	0	140
20 April 1998	30 711	0	25	0
22 April 1998	29 616	0	25	175
23 April 1998	27 680	0	0	175
27 April 1998	27 426	0	0	0
29 April 1998	4533	0	0	0
30 April 1998	16 883	0	0	175
6 May 1998	15 763	0	25	0

very low (less than 1%). Hence, the silicone grease-coated stage 2 trapped most of the pollen which should have been retained by stage 1.

In a further experiment, the average retention efficiency for pollen on glass fibre filters on stage 1 was determined. On 12 days in April and May 1998, pollen was counted on stage 1 and the grease-coated stage 2. The relatively large pine pollen formed a major proportion of the deposited pollen. For stage 1, a mean pollen retention efficiency of 24% (range 17% to 36%, SD 5.9%) was found. Daily mean, minimum and maximum of relative humidity, temperature and wind-speed were compared with the retention efficiencies in order to analyse a possible influence of relative humidity, temperature and wind-speed on the retention efficiency of glass fibre filters on stage 1. Relative humidity on the sampling days varied between 40.2 and 70.5% (daily mean), temperature between 11.1 and 23.5 °C (daily mean) and wind-speed between 1.7 and 2.8 m/s (daily mean). However, retention efficiency did not correlate with any of the investigated parameters of relative humidity, temperature and wind-speed of the sampling day.

During two days in 1999 (15–16 March and 25–26 April), all stages of the impactor were coated with silicone grease. Visually, on both days, particles on all stages were well separated by size. More than 99% of the pollen was found on stage 1. By sampling for 24 h in March, a total of 440 000 was collected; 99.4% was on stage 1, 0.6% on stage 2, 0.01% on stage 3, none on stage 4 and 0.003% on the back-up filter. By sampling in April, a total of 173 000 was collected; 99.06% was on stage 1, 0.93% on stage 2, none on stages 3 and 4, and 0.01% on the back-up filter.

Interest was now focused on determining the birch allergen load of the small particle fractions under conditions where bounce and blow-off of pollen are minimized by greasing stage 2. Figure 2 shows the allergen load and the pollen counts of the filters used in the second experiment (mean of eight days in spring 1998 during the birch pollen season). As expected, most of the allergen was found in stage 1, the stage where mostly pollen was deposited. Considerably less allergen was deposited in the lower stages of the sampler. The allergen levels on stages 3 and 4 were approximately 10% of the load of stage 1, whereas pollen counts on the same stages were less than 0.05% (Table 2). On the back-up filter ($< 1.4 \mu\text{m}$), no allergen was detected and pollen counts were 0.4% of stage 1.

Discussion

For pollen, heavy bounce and blow-off from stage 1, which should have retained most of the intact pollen, was observed in the high-volume cascade sampler. This observation is compatible with data from other groups for similar sampling conditions and for particles of pollen size [21,23,26,27,31].

However, no method for inhibiting bounce and blow-off of pollen in high-volume impactors has so far been described.

Our data demonstrate that coating stage 1 ($> 10.2 \mu\text{m}$) or stage 2 ($4.2\text{--}10.2 \mu\text{m}$) with silicone grease very effectively prevents pollen from reaching the lower stages of the impactor. Even the heavy pollen load observed in March 1999 (440 000) did not lead to notable contamination of the following stages. If no measures against bounce and blow-off are taken, very high numbers of pollen will contaminate the fine particle fractions. With respect to the retention efficiencies of stage 1 of the first experiment, where birch and alder pollen were dominant (47% estimated retention), and comparing them with the experiments where the relatively large pine pollen dominated (24% retention), it can be seen that the efficiency of glass fibre filters as sampling surfaces depends very much on the size of the impacted particles. The large pine pollen (diameter $100 \mu\text{m}$ due to two airbags) exposes a much larger surface to the air-stream over the impaction surface and is therefore much more likely to be blown from the glass fibre filters than the smaller birch pollen (diameter $20\text{--}30 \mu\text{m}$). For the present purpose, greasing stage 2 was appropriate as a trap, as the intact pollen fraction (stage 1) was analysed by immunological methods and compared with pollen numbers and allergen load in the fractions of those particles small enough to enter the bronchiolar tree. In addition, pollen counting on the greased stage is facilitated when half-transparent tracing paper is used for supporting the grease as this allows subsequent examination by light microscopy.

Theoretically, small particles could already be trapped by the grease on stage 1 or 2 instead of being deposited on the small particle stages. However, it is thought that this possible error source is of minor importance for the following reasons. Firstly, cascade impactors are designed to separate particles by size, and bounce and blow-off of particles are the only major problems described so far [21,23,31]. Secondly, when analysing both the greased tracing paper and glass fibre filters of the various stages, good visual separation was found for all particles smaller than pollen. On the back-up filter ($< 1.4 \mu\text{m}$), black micronic particles, probably soot particles originating from combustion processes [4], formed the background (see Fig. 1). These black particles were not observed on the previous stages. Thirdly, by greasing stage 1 or 2, the antigenic content of the following stages might be slightly underestimated, and this is preferable to the overestimation which may occur when no grease is used.

Comparison of visualizing methods showed that light microscopy was much more useful than scanning electron microscopy for counting the number of rather large biological aerosols like pollen (but also fungal spores with diameters $> 5 \mu\text{m}$, unpublished observations) on glass fibre filters. The biological compounds showed up well by their

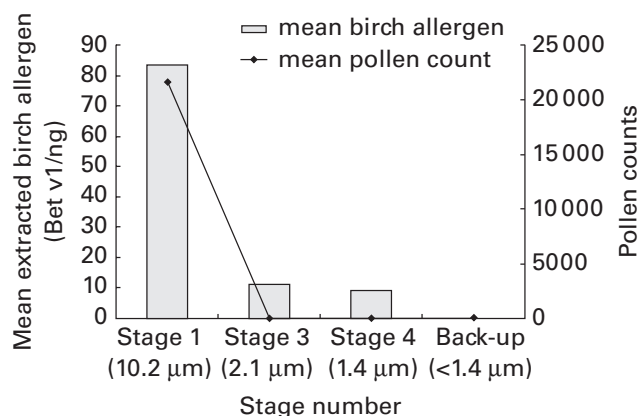


Fig. 3. Pollen counts and birch allergen load on the glass fibre filters loaded stages of the sampler with grease on stage 2. Mean of 8 days during the birch pollen season in spring 1998.

colour and therefore, counting could be performed on a large area of view at low magnification. Scanning electron microscopy had many disadvantages. Pictures obtained were only in grey and thus, detection of pollen was more difficult and time-consuming, and the magnification was too high so that the visualized area was too small for counting. Moreover, the preparation technique is time-consuming and from our experience with specimen preparation for electron microscopy [34], it is speculated that loosely-bound pollen might be lost during the specimen preparation steps. The observed bounce-off from the stage 1 filter is good evidence for weak adhesion of pollen to glass fibre filters.

No influence of humidity, air temperature and wind-speed on the retention efficiency in the impactors tested was observed. However, it cannot be excluded that a possible weak influence is obscured by differences in the species of the collected pollen.

The results obtained by ELISA techniques indicate that the birch allergen load in fine particles is much smaller than the allergen load in the pollen size fraction (Fig. 3). However, the relative allergen content compared with the (contaminating) pollen count is much higher in stages 3 and 4 than in stage 1 with the intact pollen. This is clear evidence that a large proportion of the allergen in stages 3 and 4 is associated with particles of respirable size. These could be either fragments of pollen, soluble allergen adsorbed to air pollutants of various origin, or parts of the dehiscing anther released at the time of pollen shedding as described for other pollen species [16]. Our data are in agreement with previous observations with a low-volume orifice impactor [10] known to produce little pollen bounce-off [7,15], in which only small amounts of allergen were detected in the small particle fraction. However, our data disagree with observations made using slotted high-volume impactors where, on most of the days examined, large amounts of allergen were

found in the fine particle fraction, often even larger than in the coarse fraction with intact pollen [11,13,14,18]. These results might be influenced by pollen bounce and blow-off. Our observations strongly suggest that at least some of the results from the above-mentioned studies, which separated particles by impaction onto glass fibre filters using high-volume cascade impactors, should be interpreted with care. To avoid misinterpretation due to erroneous particle collection, the set-up described here to avoid bounce and blow-off of intact pollen into lower stages of the sampler might be helpful. An alternative would be the development of a high-volume virtual impactor, which would avoid the problem of bounce and blow-off and allow analysis of all sampled fractions.

For the understanding of the influence of pollen allergens on pollen asthma symptoms, the monitoring of allergen-loaded fine particles ($<10\text{ }\mu\text{m}$) capable of penetrating into the lower airways is important [1,4,5,8,9,12]. Previous studies [11,13,14,18] have suggested that pollen allergen amounts in respirable particle fractions are usually in the same range as in the pollen size fraction. However, it is shown here that pollen allergen amounts in the respirable particle fraction are much lower than in the non-respirable particle fraction ($>10\text{ }\mu\text{m}$). Therefore, the observed airway response of pollen-sensitized allergic asthmatics seems to be a response to very low concentrations of allergens, which might be important for better understanding differences between asthmatic and non-asthmatic allergic subjects. Therefore, these findings may be important not only for the better understanding of symptoms of allergic asthmatics, but also for the research of inflammatory mechanisms underlying allergic asthma.

Acknowledgements

The authors thank Sabina Keller (undergraduate biology student) for helping count and determine pollen. They thank Dr M. Muller (electron microscopy service), biology department, ETH Zurich, for helping with the visualization techniques. They would also like to thank Dr A. Meier (laboratory manager) and his staff from the Department of Public Health and Environment of the City of Zurich for providing meteorological data, and Dr R. Gehrig, EMPA Dübendorf (Swiss Federal Laboratories for Materials Testing and Research, Section Air Pollution/Environmental Technology) for the air-flow calibration equipment.

References

- 1 Rosenberg GL, Rosenthal RR, Norman PS. Inhalation challenge with ragweed pollen in ragweed-sensitive asthmatics. *J Allergy Clin Immunol* 1983; 71:302–10.
- 2 Erdtman G, Nilsson S, Praglowski J, eds Erdtmans's Handbook of Palynology. 2nd Edn. Copenhagen: Munksgaard, 1992.

- 3 Di Giovanni F, Kevan PG, Nasr ME. The variability in settling velocities of some pollen and spores. *Grana* 1995; 34:39–44.
- 4 EPA. Air Quality Criteria for Particulate Matter I. Washington: United States Environmental Protection Agency, 1996.
- 5 Schlesinger RB. Comparative deposition of inhaled aerosols in experimental animals and humans: a review. *J Toxicol Environ Health* 1985; 15:197–214.
- 6 Agarwal MK et al. Airborne ragweed allergens: association with various particle sizes and short ragweed plant parts. *J Allergy Clin Immunol* 1984; 74:687–93.
- 7 Busse W, Reed C, Hoehne J. Where is the allergic reaction in ragweed asthma? II. Demonstration of ragweed antigen in airborne particles smaller than pollen. *J Allergy Clin Immunol* 1972; 50:289–93.
- 8 Pehkonen E, Rantio Lehtimäki A. Variations in airborne pollen antigenic particles caused by meteorologic factors. *Allergy: European J Allergy Clin Immunology* 1994; 49: 472–7.
- 9 Rantio Lehtimäki A, Viander M, Koivikko A. Airborne birch pollen antigens in different particle sizes. *Clin Exp Allergy* 1994; 24:23–8.
- 10 Schäppi G et al. Direct determination of allergens in ambient aerosols: Methodological aspects. *Int Arch Allergy Immunol* 1996; 110:364–70.
- 11 Schäppi GF et al. Concentrations of the major birch tree allergen Bet v 1 in pollen and respirable fine particles in the atmosphere. *J Allergy Clin Immunol* 1997; 100:656–61.
- 12 Solomon WR, Burge HA, Muilenberg ML. Allergen carriage by atmospheric aerosol: 1. Ragweed pollen determinants in smaller micronic fractions. *J Allergy Clin Immunol* 1983; 72:443–7.
- 13 Spieksma FTM et al. Evidence of grass-pollen allergenic activity in the smaller micronic atmospheric aerosol fraction. *Clin Exp Allergy* 1990; 20:273–80.
- 14 Spieksma FTM, Nikkels BH, 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; 25:234–9.
- 15 Takahashi Y et al. Existence of exine-free airborne allergen particles of Japanese cedar (*Cryptomeria japonica*) pollen. *Allergy* 1991; 46:588–93.
- 16 Takahashi Y et al. Aerodynamic size distribution of the particles emitted from the flowers of allergologically important plants. *Grana* 1995; 34:45–9.
- 17 Yli Panula E, Takahashi Y, Rantio Lehtimäki A. Comparison of direct immunostaining and electroimmunoassay for analysis of airborne grass-pollen antigens. *Allergy* 1997; 52:541–6.
- 18 Schäppi GF et al. Concentrations of major grass group 5 allergens in pollen grains and atmospheric particles: implications for hay fever and allergic asthma sufferers sensitized to grass pollen allergens. *Clin Exp Allergy* 1999; 29:633–41.
- 19 Schäppi G et al. Analysis of allergens in ambient aerosols: Comparison of areas subjected to different levels of air pollution. *Aerobiologia Int J Aerobiol* 1996; 12:185–90.
- 20 Hinds WC. Aerosol technology — properties, behavior, and measurement of airborne particles. New York: John Wiley & Sons, 1982.
- 21 Braaten DA, Paw UKT. A net deposition model. *Aerosol Technol* 1992; 17:289–302.
- 22 Marple VA, Liu BY, Whitby KT. Fluid mechanics of the laminar flow aerosol impactor. *Aerosol Sci* 1974; 5:1–16.
- 23 Wu Y-L, Davidson CI, Russell AG. A stochastic model for particle deposition and bounceoff. *Aerosol Sci Technol* 1992; 17:231–44.
- 24 Hu JN-H. An improved impactor for aerosol studies — modified andersen sampler. *Environ Sci Technol* 1971; 5:251–3.
- 25 Jordan DW. The adhesion of dust particles. *Br J Appl Phys* 1954; Suppl.3:S195.
- 26 Knuth RH In: Lundgren A ed. Comments on inertial impactor calibration and use aerosol measurement. Gainesville: University Presses of Florida, 1979, 108–16.
- 27 Paw UKT, Braaten David A. Experimental evidence of the importance of rebound in net deposition of particles. *Aerosol Sci Technol* 1992; 17:278–88.
- 28 Rao AK, Whitby KT. Non-ideal collection characteristics of inertial impactors — I. Single-stage impactors and solid particles. *J Aerosol Sci* 1978; 9:77–86.
- 29 Rao AK, Whitby KT. Non-ideal collection characteristics of inertial impactors — cascade impactors. *J Aerosol Sci* 1978; 9:87–100.
- 30 Willeke K. Performance of the slotted impactor. *Am Industrial Hygiene Ass J* 1975; 36:683–91.
- 31 Wu Y-L, Davidson CI, Russell AG. Controlled wind tunnel experiments for particle bounceoff and resuspension. *Aerosol Sci Technol* 1992; 17:245–62.
- 32 Jensen J et al. Immunochemical estimations of allergenic activities from outdoor aero-allergens, collected by a high-volume air sampler. *Allergy* 1989; 44:52–9.
- 33 Riediker M, Koller T, Monn C. Determination of birch pollen allergens in different aerosol sizes. *Int J Aerobiol* 2000; in press.
- 34 Sogo JM et al. Adsorption of DNA molecules to different support films. *J Microscopie* 1975; 104:187–98.
- 35 Marple VA, Liu BYH. Characteristics of laminar jet impactors. *Environ Sci Technol* 1974; 8:648–54.