

Airborne Birch and Grass Pollen Allergens in Street-Level Shops

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Abstract Polluted urban outdoor air may be enriched with large amounts of submicronic respirable pollen allergen particles that penetrate into street-level shops. The objectives of the study were to map concentrations of birch and grass pollen allergens in indoor air of street-level shops and to explore the effect of electrostatic air cleaning under authentic working conditions, indoor air samples were collected in May and July 1999 in two shops. Allergens were quantified by a direct on sampling filter in solution (DOSIS) luminescence immunoassay. The average concentration of airborne indoor birch pollen allergen in the shop with air cleaning was estimated to be 20 ± 9 SQ/m³ (mean $\pm$ SD) compared to 31 ± 17 SQ/m³ (mean $\pm$ SD) of that without. The air cleaner reduced the indoor air birch pollen allergen concentration by on average 26 to 48% ($P < 0.05$). Corresponding figures for airborne indoor grass pollen allergen concentrations were 14 ± 7 SQ/m³ and 17 ± 8 SQ/m³, indicating a statistically non-significant (t -test) average 18% reduction of allergen by air cleaning. Excluding two observations with poor fit to the statistical model a significant ($P < 0.05$) average 27% reduction was obtained. Substantial amounts of airborne birch and grass pollen allergens may occur in street-level shops during flowering seasons.

Key words Allergy; Allergen; Pollen; Grass; Birch; Indoor air; Shops; Air cleaner.

Practical Implications

This paper demonstrates that the application of a direct on sampling filter in solution method (DOSIS) for analysis of airborne birch and grass pollen allergens, can be used to map concentrations of these allergens in the air of exposed indoor environments, before and after air cleaning measures.

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Introduction

During their working-days the staff in urban street-

level shops are at risk to be exposed to airborne road dust constituting a very heterogeneous mixture of fine particles derived from motor vehicle exhaust, tyres and brake linings and of *inter alia* soil, biologic material and heavy metals (Miguel et al., 1996; Miguel et al., 1999; Monn et al., 1999; Praml et al., 2000). In the birch and grass pollen-flowering seasons, the polluted outdoor air may also be enriched with large amounts of submicronic respirable pollen allergen particles being released from the pollen grains under different meteorological conditions. The allergenicity of these particles might even be aggravated when adsorbed to soot particles from motor vehicle emissions (Dias-Sanches et al., 1999; Ormstad et al., 1997; Nel et al., 1998). The quality of indoor air obviously is a result of different arrangements for filtering or cleaning the inflowing outdoor air (Jamriska et al., 1999). Pollen allergens in the ambient air can lead to serious symptoms typical of rhinitis and asthma in afflicted subjects and have recently been demonstrated to be a risk factor for daily cardiovascular and respiratory-disease mortality (Brunekreef et al., 1999). It thus seems important to authentically map and control pollen allergen levels in the air of exposed indoor environments.

By means of a direct on air sampling filter technique (DOSIS) (Holmquist & Vesterberg, 1999a) we recently confirmed that the air in office and school rooms (Holmquist & Vesterberg, 1999b) and in the driving compartments of coaches (Holmquist & Vesterberg, 2001) contained substantial amounts of birch and grass pollen allergens during the corresponding pollen producing seasons. Herein we report the results from analysis of particulate airborne birch and grass pollen allergen concentrations in two adjacent street-level shops, chosen as authentic models of staff exposed to indoor air pollen allergens.

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Material and Methods

Material

Water extracts of pollen from birch (*Betula pendula*) and timothy grass (*Phleum pratense*) for skin prick testing, Soluprick SQ[®], each containing 100,000 SQ units/ml (manufacturer's specification) were purchased from ALK-Abelló A/S (Hørsholm, Denmark) and used as reference allergens for the standard curves. The birch and grass pollen extracts contained 23 µg Bet v 1 and 25 µg Phl p 5 protein/ml (100,000 SQ) as major allergens, respectively, according to the manufacturer. Polyclonal primary antibodies, rabbit IgG anti-*Betula* and rabbit IgG anti-*Phleum* raised against water extracts of birch pollen and purified Phl p 5, respectively, were from the same company. Rabbit IgG from normal non-immunized animals was purchased from Dakopatts (Gentofte, Denmark). Water extracts of latex (*Hevea brasiliensis*), cat hair, dog hair, mugwort (*Artemisia vulgaris*) pollen, mould spores (*Cladosporium herbarum* and *Alternaria alternata*), and mite (*Dermatophagoides pteronyssinus*) were obtained as Soluprick[®] preparations from ALK-Abelló A/S and a standardized extract of human skin (code No. 77/633) was from NIBSC (Hertfordshire, UK). No significant cross-reaction between these extracts and the primary antibodies above could be demonstrated with exception of the mugwort extract which cross-reacted with the anti-*Betula* antibody preparation. All other reagents and materials including the sample filter holder, further modified by omission of its silicone gasket for increased sampling filter area, were the same as previously described for quantification of pollen allergens by DOSIS (Holmquist & Vesterberg, 1999a). Air sampling PTFE filters (polytetrafluoroethylene), 25 mm in diameter with 1.2 µm nominal pore sizes were from Sartorius (Göttingen, Germany). AirChek[®] 2000 vacuum pumps for air sampling at 2.0 l per min of pollen allergens were purchased from SKC Inc. (Eighty Four, PA, USA). Each pump equipped with a piece of tubing for the sampling filter holder was wrapped in foamed plastic and placed in a 30×15×26 cm (l×w×h) handbag. Air samples were taken using one sampling filter in each shop for 8 h, between 09:15 and 17:15. The total sampling time each day was recorded by the pumps.

The air cleaners were an Office Air Cleaner prototype working on the principle; a bundle of free-hanging 2.5 to 6 kV electrostatically charged 150 to 400 mm long polypropylene fibers which attracts all types of particles independently of their charge. The flow rates of the air through each air cleaner were 252 and 135 m³/h at full and reduced effect, respectively. Our original intention was to run the air cleaners every second day except for weekend days. During the study they were operated on the days shown in the figures.

Analytical Methods

Quantification of pollen grains in outdoor air was made by the staff of the Palynological Laboratory at the Swedish Museum of Natural History in Stockholm, Sweden by means of a Burkardt Seven Day Pollen Trap (Burkardt Manufacturing Ltd., Rickmansworth, UK) positioned about 10 m above the ground 59°30'N and 18°03'E, about 10 km from the study shops. Pollen allergen was quantified by the direct on sampling filter in solution (DOSIS) double antibody luminescence immunoassay as previously described in detail (Holmquist & Vesterberg,

1999a). Briefly, the allergens firmly adsorbed to a polytetrafluoroethylene (PTFE) sampling filter are reacted with specific antibodies conjugated with alkaline phosphatase, generating a matrix-bound allergen-antibody-phosphatase complex. The filter is then floated on a solution of a chemiluminescent phosphatase substrate. Aliquots of the reaction mixture are withdrawn at defined time intervals and light emitted by the product of the enzyme activity, recorded by a luminometer, is linearly related to the amount of allergen over a large mass range. The method was further simplified by pipetting the dilutions of the standard allergens (antigens) as droplets directly onto the floating filters in Step 1 (Holmquist & Vesterberg, 1999a). The humidified box as described in Step 2 (Holmquist & Vesterberg, 1999a) was omitted. Test of endogenous alkaline phosphatase activity of selected whole sample filters was performed by running the complete DOSIS procedure but with exclusion of the primary and secondary antibodies. Estimation of non-specific background related to the sample matrix was made by running DOSIS on two halves of a relevant sample filter using rabbit IgG from normal non-immunized animals (negative control) as primary antibody on one of the halves, prior to incubation with the secondary antibody, swine anti-rabbit IgG conjugated with alkaline phosphatase. Blank filters were run simultaneously on each occasion of analysis of standard and sample filters.

Meteorological Parameters

Meteorological parameters were recorded each day between 20:00 and 20:00 local time for 24-h periods which started the day before the pollen allergen sampling day. This was performed by the Swedish Meteorological and Hydrological Institute at Stockholm, Sweden (59°34'N and 18°58'E).

Statistical Methods

Multiple linear regression analysis was performed with indoor allergen concentrations as dependent variable. Independent variables that were tested in the regression model were outdoor pollen grain concentration, shop, air cleaner (y/n) and amount of rain during the 24-h measurement periods. Rain was categorized as 0, 1 or 2 corresponding to 0, 1 to 2 and more than 3 mm of rain, respectively.

The statistical analyses were performed using the statistical package SAS/STAT User's Guide, (SAS Institute Care, NC, USA).

Study Design

Two study shops separated by a distance of 80 m, were chosen in a satellite town, Solna, Sweden (59°21'N and 18°04'E), which is situated about 10 km north of the city of Stockholm. Between the shops there was about 1000 m² of lawn cut several times monthly and a few tall birches were growing about 90 m from the shops. Opposite the paved road with public traffic harbouring the shops, a shopping mall was situated.

Shop No. 1, a driving school, was frequented during business hours by customers ordering time for driving lessons and for performing written traffic examinations. The room had the dimensions 6.5×5.5×3.5 m (l×w×h). The entrance door facing the paved street was positioned in one of the short sides of the room. The opposite side had

two door openings each facing an adjacent room. The room was ventilated through natural ventilation. The air cleaning device was placed in the room alongside the entrance door about 2 m above the floor. When operating, the air cleaner was run at reduced effect during the working day from 08:30 to 17:00 and with full effect from 18:00 the preceeding evening to 08:30 the following morning. The air sampling filter was positioned 0.8 m above the floor along the same wall as the entrance door. The diagonal distance between the sampling filter and the air cleaner was 3 m.

Shop No. 2 was managed by an optician selling glasses, spectacle frames and related services. The room had the dimensions 12×4.5×4 m (l×w×h) and had an entrance door in the middle of the long side facing the busy paved street. The room was ventilated through natural ventilation. The air cleaner was positioned opposite the entrance door 0.9 m above the floor and was run as described above for room No. 1. The sampling filter was placed at the wall 7 m from and alongside the entrance door, about 0.9 m above the floor. The diagonal distance between the sampling filter and the air cleaner was 6 m.

Results

Birch Pollen Allergen

The days prior to the birch pollen allergen sampling period of May 1999, there was little rain in the sampling region. The outdoor daily 24-h birch pollen counts were unusually low for the month with only

few values exceeding moderate counts ranging from 10 to 100 grains per m³ of air. In shops No. 1 and No. 2 birch pollen allergens could be recorded on sampling days both with and without active air cleaner, as shown in Figure 1A and B. The concentrations of the airborne allergen varied between 16 and 70 SQ/m³ with a mean and $\pm$ SD of 36 and 23 SQ/m³ of air, respectively, in shop No. 1 for sampling days without air cleaner and between 15 and 33 SQ/m³ with mean and $\pm$ SD of 26 and 7 SQ/m³ of indoor air, respectively, for the sampling days with active air cleaning. The corresponding allergen concentration values for shop No. 2 were 13 to 34 SQ/m³ with 27 ± 9 SQ/m³ and 5 to 17 SQ/m³ with 13 ± 5 SQ/m³. Taken together all sampling days of the two shops, showing an average allergen concentration in indoor air of 20 ± 9 SQ/m³ (mean $\pm$ SD) when the air cleaners were running, a statistically significant 35% lower average allergen concentration of the birch indoor air allergen could be demonstrated, compared to days without air cleaning (31 ± 18 SQ/m³; mean $\pm$ SD) ($P<0.05$) as analyzed by one-sided *t*-test. Multiple linear regression analysis showed no statistically significant effect of outdoor air birch pollen grain concentration, shop or rain on indoor air allergen concentration. However, using this statistic model a significant 15 SQ units/m³ or 48% average reduction of

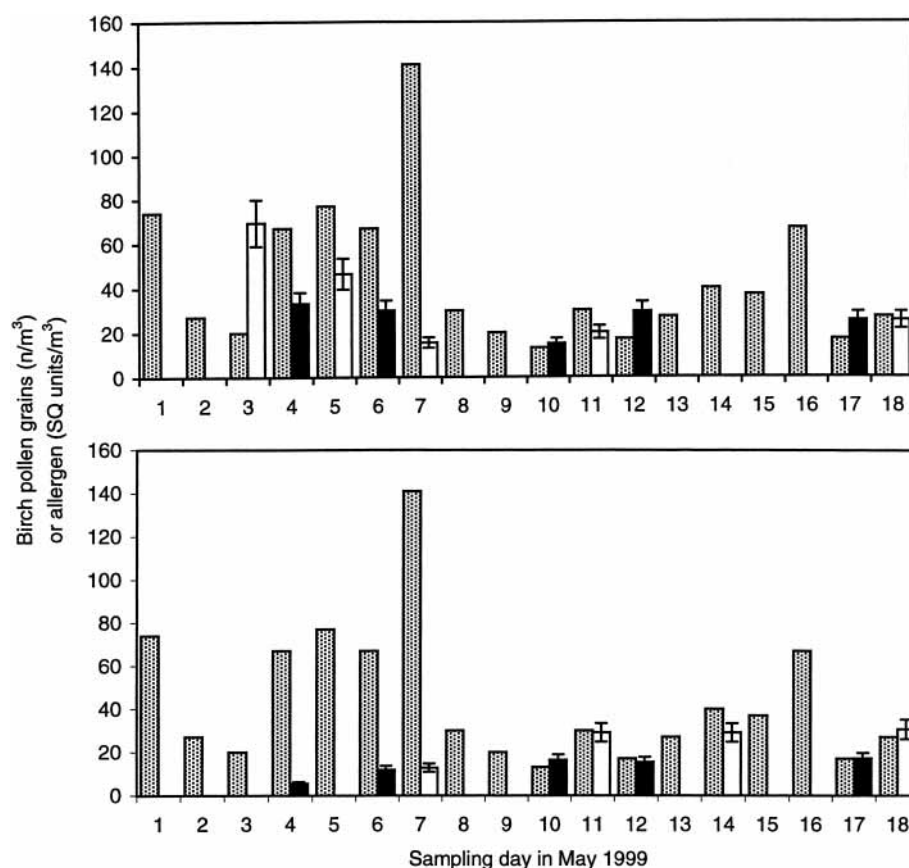


Fig. 1 Bar charts showing the concentrations of outdoor air birch pollen grains obtained with a Burkard trap, reported as average values from 24-h sampling periods (dotted bars) and of indoor air birch pollen allergens quantified by the DOSIS technique of the traffic school (upper panel) and the optician's shop (lower panel). Black and white bars represent concentrations of allergens in indoor air of days with and without active air cleaner, respectively

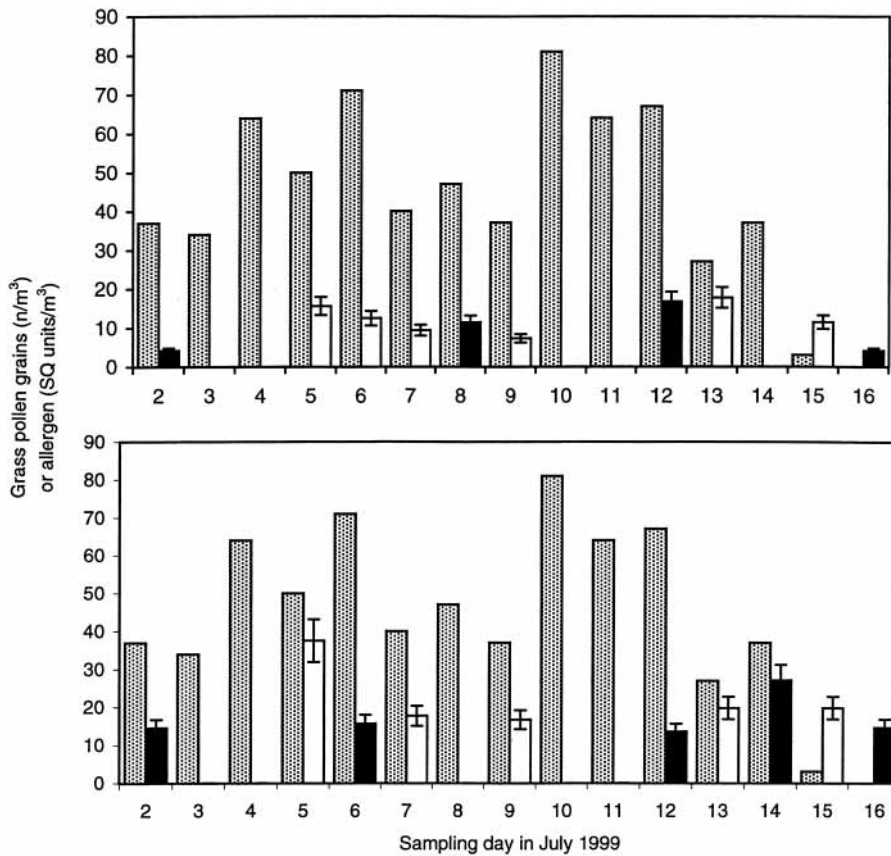


Fig. 2 Bar charts showing the concentrations of outdoor air grass pollen grains obtained with a Burkard trap, reported as average values from 24-h sampling periods (dotted bars) and of indoor air grass pollen allergens quantified by the DOSIS technique of the traffic school (upper panel) and the optician's shop (lower panel). Black and white bars represent concentrations of allergens in indoor air of days with and without active air cleaner, respectively

indoor airborne birch pollen allergen concentrations, with the 95% confidence limits -27 and -2 SQ units/ m^3 , could be assigned to the use of air cleaner as compared to days without. This effect was strongly dependent on one observation with a rather poor fit to the regression model. Excluding that observation the effect of the air cleaner was almost halved to 8 units/ m^3 , or about 26% reduction.

Grass Pollen Allergen

The grass pollen production during the flowering season was high compared to average years. Low, moderate and high concentrations of pollen grains correspond to up to 10, 30 and higher counts per m^3 , respectively.

During the three sampling periods little rain was falling only on two days in the region. Grass pollen allergens were abundant in the indoor air of shops No. 1 and No. 2 on sampling days with and without internal air cleaning as shown in Figure 2A and B. The concentrations of the airborne indoor allergen varied between 7 and 18 SQ/ m^3 with a mean and $\pm$ SD of 12 and 4 SQ/ m^3 of air, respectively, in shop No. 1 for sampling days without air cleaner and between 4 and 17 SQ/ m^3 with mean and $\pm$ SD of 9 and 7 SQ/ m^3 of indoor air, respectively, for the sampling days with active air cleaning.

The corresponding allergen concentration values for shop No. 2 were 17 to 38 SQ/ m^3 with 22 ± 9 SQ/ m^3 and 14 to 27 SQ/ m^3 with 17 ± 6 SQ/ m^3 of sampling days without and with active air cleaner, respectively.

Using concentration values from all sampling days of the two shops, a non-significant average 18% reduction effect on indoor air grass pollen allergen concentration could be assigned to the air cleaner by a *t*-test. Multiple linear regression analysis, showed that on average a non-significant 5 SQ units/ m^3 or 29% reduction of airborne indoor grass pollen allergen could be assigned to the use of the air cleaner as compared to days without. The regression model indicated statistically significant higher allergen concentrations in shop No. 2 compared to shop No. 1. If two observations with poor fit to the model were deleted this resulted in somewhat lower figures, 4 SQ units/ m^3 reduction or 27%, which however became statistically significant ($P < 0.05$) as a result of decreased residual variation. It was not possible to fit a model including rain as a variable to the data.

Discussion

The present study unambiguously shows that the indoor air in street level shops with natural ventilation can be contaminated with substantial amounts of birch

and grass pollen allergens during pollen-producing seasons. This is in accordance with previous findings in school rooms (Holmquist & Vesterberg, 1999b). However, the officially recorded 24-h concentration of pollen grains of the outdoor air was not significantly correlated to the paired indoor air concentrations of allergens. A reason for this might be that the shops were surrounded by neighbouring pollen-producing plants and weeds which can result in a local pollen allergen load on the shops. In contrast, the officially recorded 24-h pollen counts were derived from a source 10 m above the ground 10 km from the study objects. Lack of close correlation between outdoor air birch pollen grain counts and antigen concentrations has been reported by Rantio-Lehtimäki et al. (1994).

The shops represent indoor environments, the air of which should be subjected to forceful cleaning efforts. In addition to pollen allergens penetrating into the shops with the inlet air and through the entrance doors, allergens may be derived from customers clothing and from resuspension of settled dust. The levels of indoor air birch and grass pollen allergen concentrations observed in the present work, ranging from about 4 to 38 and 5 to 70 SQ/m³, respectively, were of the same order of magnitude as found in previous investigations in school and office room and in the driving compartments of coaches (Holmquist & Vesterberg, 2001). It has been demonstrated that allergic symptoms from birch and grass pollen allergen par-

ticles in the air might appear at concentrations down to 1 SQ/m³ (Johnsen et al., 1992). However such data can only be interpreted under certain well-defined conditions specifying allergen concentration in the air and exposure time.

Our pilot attempts to eliminate the pollen allergen in the indoor air in the two shops by air cleaner were partly successful for birch allergen which makes further more extensive air cleaning measures promising. The less evident reduction of grass pollen allergens in the indoor air of the two shops by the air cleaner might be explained by high summer temperatures which led to frequent days with open entrance doors for ventilation. This could result in a load in excess of the capacity of the air cleaner used in this study. In addition the air cleaners had to be run with reduced effect during the sampling days due to their disturbing noise. In conclusion, our results indicate that it would be possible using the DOSIS technique to map and reduce or eliminate pollen allergen particles in the air of problematical indoor environments by combined controlled inlet air filtration and internal air cleaning.

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References

- Bruneekreef, B., Hoek, G., Fischer, P. and Spijksma, F.T. (2000) "Relation between airborne pollen concentrations and daily cardiovascular and respiratory-disease mortality", *The Lancet*, **355**, 1517–1518.
- Dias-Sanches, D., Penichet Garcia, M., Wang, M., Jyrälä, M. and Saxon, A. (1999) "Nasal challenge with diesel exhaust particles can induce sensitisation to a neoallergen in the human mucosa", *Journal of Allergy and Clinical Immunology*, **1999**, 1183–1188.
- Holmquist L. and Vesterberg O. (1999a) "Luminescence immunoassay of pollen allergens on air sampling polytetrafluoroethylene filters", *Journal of Biochemical and Biophysical Methods*, **41**, 49–60.
- Holmquist, L. and Vesterberg, O. (1999b) "Quantification of birch and grass pollen allergens in indoor air", *Indoor Air*, **9**, 85–91.
- Holmquist, L. and Vesterberg, O. (2001) "Airborne birch and grass pollen allergens in driving compartments of coaches", *Arbete och Hälsa* **2**, 1–13.
- Jamriska, M., Thomas, S., Morawska, L., and Clark, B.A. (1999) "Relation between indoor and outdoor exposure to fine particles near a busy arterial road", *Indoor Air*, **9**, 75–84.
- Johnsen, C.R., Weeke, E.R., Nielsen, J., Jensen, J., Mosbech, H., Frolund, L., Madsen, F. and Poulsen, L.K. (1992) "Aeroallergen analyses and their clinical relevance. II. Sampling by high-volume air sampler with immunochemical quantification versus Burkard pollen trap sampling with morphologic quantification", *Allergy*, **47**, 510–516.
- Miguel, A. G., Cass, G. R., Glovsky, M. and Weiss, J. (1999) "Allergen in paved road dust and allergen particles", *Environmental Science and Technology*, **33**, 4159–4168.
- Miguel, A. G., Cass, G. R., Weiss, J. and Glovsky, M. (1996) "Latex allergens in tire dust and airborne particles", *Environmental Health Perspectives*, **104**, 1180–1186.
- Monn, C. and Koren, H. S. (1999) "Bio-aerosols in ambient air particulates: a review and research needs", *Reviews on Environmental Health*, **14**, 79–89.
- Nel, A. E., Dias-Sanches, D., Ng, D., Hira, T. and Saxon, A. (1998) "Enhancement of allergic inflammation by the interaction between diesel exhaust particles and the immune system", *Journal of Allergy and Clinical Immunology*, **102**, 539–554.
- Ormstad, H., Johansen, B. V. and Gaarder, P.I. (1997) "Airborne house dust particles and diesel exhaust particles as allergen carriers", *Clinical and Experimental Allergy*, **28**, 702–708.
- Praml, G. and Schierl, R. (2000) "Dust exposure in Munich public transportation: a comprehensive 4-year survey in buses and trams", *International Archives of Environmental Health*, **73**, 209–214.
- Rantio-Lehtimäki, A., Viander, M. and Koivikko, A. (1994) "Airborne birch pollen antigens in different particle sizes", *Clinical and Experimental Allergy*, **24**, 23–8.