NR 2001:2

Airborne birch and grass pollen allergens in driving compartments of coaches

Leif Holmquist and Olof Vesterberg

ARBETE OCH HÄLSA | VETENSKAPLIG SKRIFTSERIE

ISBN 91-7045-589-9 ISSN 0346-7821 http://www.niwl.se/ah/

Arbetslivsinstitutet
National Institute for Working Life

The National Institute for Working Life is Sweden's national centre for work life research, development and training.

The labour market, occupational safety and health, and work organisation are our main fields of activity. The creation and use of knowledge through learning, information and documentation are important to the Institute, as is international co-operation. The Institute is collaborating with interested parties in various development projects.

The areas in which the Institute is active include:

- labour market and labour law,
- work organisation,
- musculoskeletal disorders,
- chemical substances and allergens, noise and electromagnetic fields,
- the psychosocial problems and strain-related disorders in modern working life.

ARBETE OCH HÄLSA

Editor-in-chief: Staffan Marklund
Co-editors: Mikael Bergenheim, Anders Kjellberg, Birgitta Meding, Gunnar Rosén och Ewa Wigaeus Tornqvist

National Institute for Working Life
S-112 79 Stockholm
Sweden

ISBN 91–7045–589–9
ISSN 0346–7821
http://www.niwl.se/ah/
Printed at CM Gruppen, Bromma

Preface

More than ten percent of the Swedish population are allergic to pollen from grass and birch. These allergies often lead to asthma. The medical symptoms of the allergens are especially aggravated when the inhaled air is polluted by engine exhaust gases and particles. Inhalation of air containing even relatively low concentrations of pollen allergens can cause allergic symptoms and may increase the risk of asthma attacks. Pollen allergies cause in addition to allergic disorders high costs for impaired work fitness, sick leave, visit to doctors and treatment. In Sweden the yearly total costs for the society are hundreds of millions of US dollars.

Allergy symptoms are caused by allergens released from pollen grains as small particles. Even moderate winds may cause the allergens to become airborne for long time periods. The allergenicity of the particles may remain for several months out of the pollination season.

We have developed and validated a technique, DOSIS, which now makes it possible to determine the total concentration of allergens in both pollen grains and particles in indoor air. The technique is a prerequisite for mapping airborne allergen concentrations and for elimination efforts.

Coaches constitute a special indoor environment where drivers spend many hours each working day. There are more than 20 000 coach drivers in Sweden and many of them suffer from pollen allergy. We have studied coach drivers in the present project as even mild allergic symptoms like increased lacrimation may impair vehicle drivers performance and increase the risk for traffic accidents.

Stockholm in December 2000

The authors

Correspondence to
Professor Olof Vesterberg
Respiratory Health and Climate Programme
National Institute for Working Life
S-11279 Stockholm, Sweden
Tel. +46 8 730 9601
Fax.+46 8 730 9897

Förord

Mer än tio procent av befolkningen är allergiska mot pollen från gräs och björk. Sådan allergi leder ofta till astma. De medicinska effekterna av allergener blir speciellt besvärliga då den inandade luften ofta är förorenad av motoravgaser och partiklar. Inandning av förorenad luft med även relativt låg halt av pollenallergen kan ge allergisymptom oftare och värre än eljest och öka risken för astmaanfall. Pollenallergier orsakar förutom allergibesvär stora kostnader för sänkt arbetsförmåga, sjukfrånvaro, läkarbesök och behandling. Totala kostnader för samhället är i Sverige flera miljarder kronor per år.

Pollenallergierna orsakas av allergener, som frigörs från pollenkornen som fina partiklar. Måttliga vindar virvlar upp dem och de kan sväva länge i luften. De kan behålla allergeniciten i flera månader. Minimala mängder luftburna allergener kan ge allergisymptom. Vi har utarbetat och validerat en metod, DOSIS, som nu gör det möjligt att i inomhusluft bestämma total halt av allergen både i pollenkorn och partiklar. Metoden är en förutsättning för kartläggning av lufthalter av allergen och saneringsåtgärder och för testning av olika filter för rening av luft.

Bussar utgör en speciell inomhusmiljö där förare vistas många timmar varje arbetsdag. Det finns i Sverige fler än 20 000 bussförare av vilka många besväras av pollenallergi. Skäl för att vi i föreliggande projekt studerat bussförare är att allergiska besvär, även lindrigare sådana såsom ökat tårflöde kan försämra fordonsförares körbeteende och öka risk för trafikolyckor.

Flera personer vid Vägverket och LO, liksom flera företagsläkare har framfört vikten av att problemet belyses.

Stockholm i december 2000

Författarna

Contents

Introduction

The protein content of birch (*Betula pendula*) and timothy grass (*Phleum pratense*) pollen grains might be discharged as allergenic particles with diameters down to 0.1 micrometer. Especially in contact with rainwater, grass pollen on the ground may burst and release small allergenic particles which can become airborne on dry and windy days (Suphioglu *et al.,* 1992; Schäppi *et al.,* 1999). Outdoor air thus may carry a complex mixture of native and empty pollen grains together with small inhalable allergenic pollen particles of low sedimentation rates. It seems reasonable to assume that also the air in many indoor environments is contaminated by inhalable allergenic particles during the pollen producing seasons. Consequently we recently confirmed that the air in a choice of school and office rooms during spring and summer 1997 carried substantial amounts of birch and grass pollen allergens, respectively, predominantly occurring as particles smaller than the pollen grains (Holmquist and Vesterberg, 1999a).

The air in traffic vehicles represents a complex and heterogeneous matrix composed of gases and particulate matter (Miguel *et al.,* 1996; Monn and Koren, 1999; Miguel *et al.,* 1999; Praml and Schierl, 2000). Especially soot and rubber latex particles from engine exhaust emissions and tyre wear, respectively, are mixed with road dust resuspended into the atmosphere by the vehicle traffic. In the birch and grass flowering-seasons large amounts of pollen grains are produced, which can generate airborne strong allergenic protein-containing particles. Further sources of allergenic road dust related to pollen are aerial parts of trees and weeds (Grote and Fromme, 1986; Fountain *et al.,* 1992). Birch and grass pollen allergens have been shown to be fairly resistant to degradation and can retain their allergenic activity at room temperature and humid storing conditions for over a year which might explain the appearance of allergic symptoms occurring long outside the local pollen seasons (Yli-Panula and Ahlholm, 1998). Airborne diesel exhaust and soot particles carrying pollen allergen strongly adsorbed to their surfaces (Ormstad *et al.,* 1997) are expected to be even more stable to proteolytic degradation as compared to native allergens. The allergenic properties of pollen constituents have been shown to be altered by air pollutants in the traffic environment, leading to allergenic particles which might seriously worsen inflammation and allergic reactions related to asthma and hay-fever (Nel *et al.,* 1998; Dias-Sanches *et al.,* 1999; Strand, 1998).

Furthermore, the air pollutant nitrogen dioxide emitted by vehicular traffic has been reported to increase the airway responsiveness to pollen allergens of subjects with asthma as compared to those brought into contact with allergens only (Strand, 1998).

Being exposed to road dust and motor vehicle exhaust during their working days, car, bus and coach drivers allergic to pollen thus represent categories of traffic workers at a high risk of developing acute respiratory symptoms typical of rhinitis and asthma if pollen allergen particle concentrations in the invehicle air reach critical levels. This might seriously affect traffic safety. By means of a newly developed

direct on air sampling filter in solution (DOSIS) technique (Holmquist and Vesterberg, 1999b) we have started to map pollen allergen concentrations in the air in different traffic vehicles to experience if allergen particle elimination measures would be relevant. The present paper reports the results from initial studies of birch and grass pollen allergens in the air in driving compartments of coaches plying the route Stockholm City — Stockholm Arlanda International Airport, Sweden, chosen as authentic models of pollen allergens in the breathing air of traffic vehicle drivers.

Material and Methods

Materials

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 units) 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 generous gifts 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 *(Artemesia 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 and Vesterberg, 1999b). AirChek® 2000 vacuum pumps for air sampling 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 30x15x26 cm (lxwxh) handbag.

Analytical methods

Quantification of pollen grains in outdoor air was kindly made by the staff of the Palynological Laboratory at the Swedish Museum of Natural History in Stockholm Sweden by means of a Burkard Seven Day Pollen Trap (Burkard Manufacturing Ltd. Rickmansworth, UK) positioned about 10 m above the ground 59º30´N and 18º03´E about 5 km and 40 km from the Stockholm city and Arlanda airport coach

terminals, respectively. Pollen allergen was quantified by the direct on sampling filter in solution (DOSIS) double antibody luminescence immunoassay as previously described in detail (Holmquist and Vesterberg, 1999b). 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 chemilumenescent 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 on the floating filters in Step 1 (Holmquist and Vesterberg, 1999b). The humidified box as described in Step 2 (Holmquist and Vesterberg, 1999b) was omitted. Test of endogenous alkaline phophatase 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 o'clock 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^{o}34´N and 18^{o}58´E).

Study design

The study population consisted of articulated coaches each measuring 18x2.6x 3.4 m (lxwxh) and could carry 56 seated passengers. The coaches were fitted with standard filters for the inlet air to the driving compartment. These filters, 341x325x45 mm in size (lxwxh) were designed to eliminate soot, dust and pollen grains from the inlet air and were routinely changed every 4000 km distance covered. The coaches were run on the route Stockholm City — Stockholm Arlanda Airport, Sweden. The distance between the two terminals is about 40 km and includes urban and rural environments. The unidirectional travelling time is scheduled to 35 minutes. Each bus makes four stops in each direction between the terminals for embarking only of passengers in one direction and for disembarking in the other.

Airborne pollen allergens were collected on the PTFE filters, 25 mm in diameter with 1.2 μm nominal pore size, as described by Holmquist and Vesterberg (1999b) by

means of AirChek® 2000 pumps. The flow rate through the sampling filter was 2.0 l per minute. The bag harbouring the pump with the sampling filter facing upwards about 40 cm above the floor was placed behind the driver´s seat. Pollen allergen sampling was made daily in periods of spring and summer 1999 in two coaches run on identical routes and was started in the first coach at about eight o'clock in the morning (run No. 1) and in the second about 10 minutes later (run No. 2). The sampling time was 8.0 hours. The two series of sample filters from run No.1 and that of run No. 2 were treated and analyzed separately. After completed sampling each filter was collected and stored in a sealed polystyrene plastic box at – 70º C for up to 4 months prior to analysis.

Results

Birch pollen allergen

The week prior to and during the one-week airborne birch pollen allergen sampling period, the 17th to the 21st of May 1999, there was no 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 m^3 of air (Figure 1). The profiles of the curves obtained by plotting the birch pollen allergen concentration values of the air in the driving compartments of the totally 10 coaches of run No. 1 and run No. 2, against the sampling days agreed well with the profiles of the corresponding curves produced from both the outdoor 24-h pollen grain counts and the 8-h counts covering the allergen sampling time (Figure 1). The average allergen concentration of the air in the coach compartments over the sampling week was 19±5.6 SQ units per m^3 (mean±SD). The correlation coefficient calculated from pollen allergen concentrations in the invehicle air and those of the paired outdoor pollen grains was 0.51 (Figure 2) with the 95 percent confidence limits – 0.18 and 0.84. No endogenous alkaline phosphatase activity which might give false positive allergen values could be detected on representative sample filters from 4 busses in May 1999 as analyzed by the complete DOSIS procedure but by omission of the primary and secondary antibodies. Blank sampling filters run in parallel with the sample filters on each occasion of analysis yielded allergen concentrations close to zero.

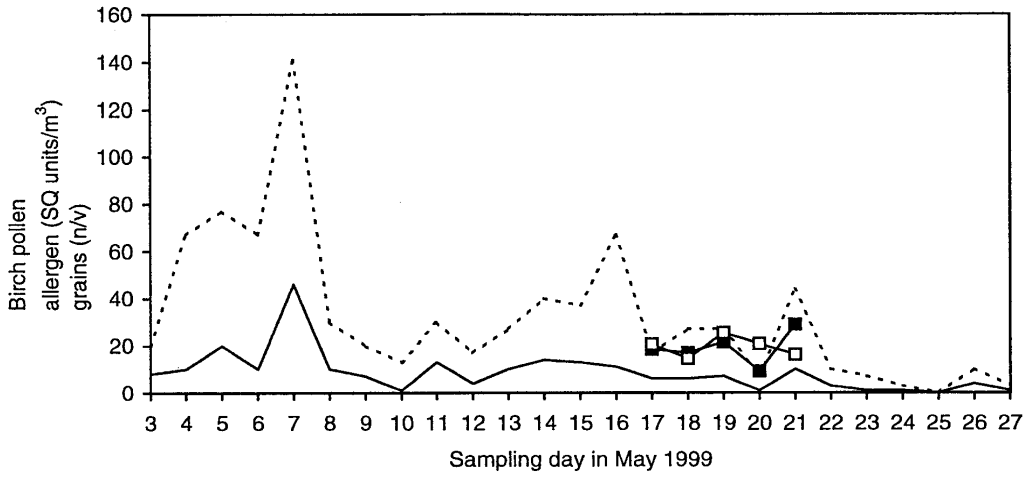

Figure 1. Average concentrations of outdoor air birch pollen grains (n) as obtained with the Burkard trap for the sampling times: 24-h, dashed line (v =1 m^3) and 8-h, solid line (v = m^3/3, the air volume corresponding to 8-h) and of the airborne birch pollen allergens in the driving compartments of the airport coaches of run No. 1 (open squares) and run No. 2 (filled squares) from May 3rd to the 27th 1999.

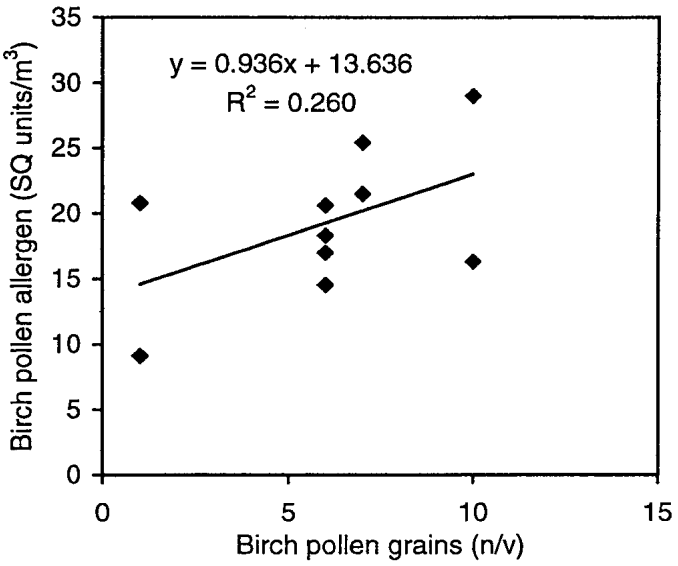

Figure 2. Plot of average 8-h outdoor air birch pollen grain concentrations (n = number of grains; v = m^3/3, the air volume corresponding to 8-h sampling), obtained with the Burkard trap, against paired concentrations of birch pollen allergens in the air in the driving compartments of the airport coaches, as estimated from air samples of May 17th to the 21st 1999.

Grass pollen allergen

The grass pollen production during the flowering-season was high and lasted unusually long, into October 1999. Low, moderate and high concentrations of pollen grains correspond to up to 10, 30 and higher counts per m^3 of air, respectively. During the three sampling periods of five days each (Figure 3) there was no rain in the region between July 5th and 14th. Between July 15th and the 23rd, varying amounts of rain was falling as compiled in Table 1. The curves produced by the grass pollen allergen concentration values of the air in the driving compartments for the first sampling period, the 5th to the 9th of July, of the totally 10 coaches of run No. 1 and run No. 2 plotted against sampling days agreed very well with the corresponding curves from the outdoor 24-h and 8-h pollen grain counts covering the allergen sampling time (Figure 3). The average grass pollen allergen concentration in the air in the driving compartments was 20±5.6 SQ units per m^3 (mean ±SD). The good agreement between the profiles of the pollen allergen and 8-h outdoor pollen grain concentration curves was for the first dry sampling period, reflected by a correlation coefficient of 0.70 with the 95 percent confidence limits 0.13 and 0.92, obtained from these parameters (Figure 4). The corresponding correlation coefficients for the second and third sampling periods including increasing number of rainy days (Table 1) decreased to 0.19 and 0.09, respectively. The average grass pollen allergen concentrations for the second period, July 12th to 16th, showing decreasing pollen grain concentration (Figure 3) was 11±3.4 SQ units per m^3 (mean±SD). The third rainy period, July 19th to 23rd, had a corresponding average allergen concentration of 24±9.3 SQ units per m^3 (mean±SD).

Table 1. Amounts of rain on the sampling days in July 1999; for 24-h, local time from 20:00 to 20:00, with start the day before the denoted sampling day.

Day	Precipitation (l/m^2)
5	0.6
15	1.4
17	1.8
21	4.9
22	6.8
23	1.9

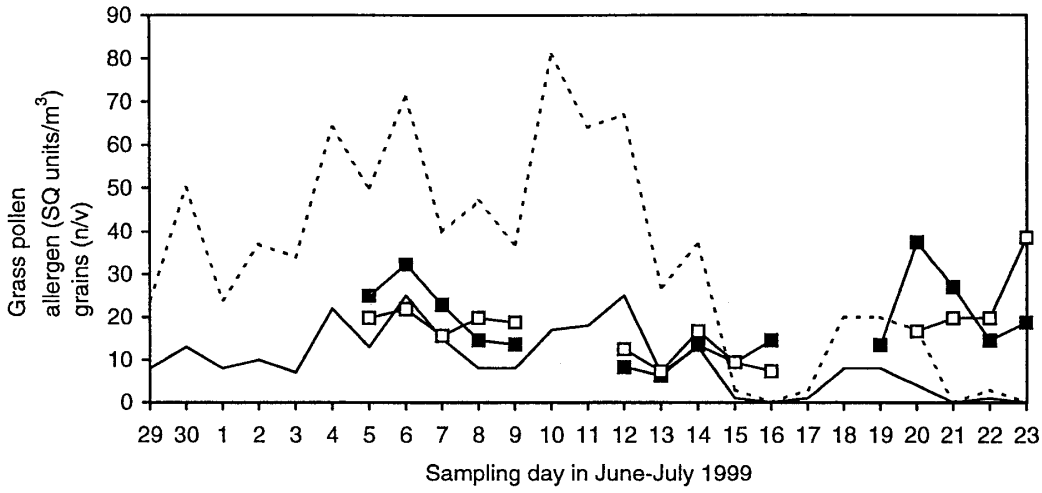

Figure 3. Average concentrations of outdoor air grass pollen grains (n) as obtained with the Burkard trap for the sampling times: 24-h, dashed line (v =1 m^3) and 8-h, solid line (v = m^3/3; the air volume corresponding to 8-h) and of the airborne grass pollen allergens in the driving compartments of the airport coaches of run No. 1 (open squares) and run No. 2 (filled squares) from June 29th to July 23rd 1999.

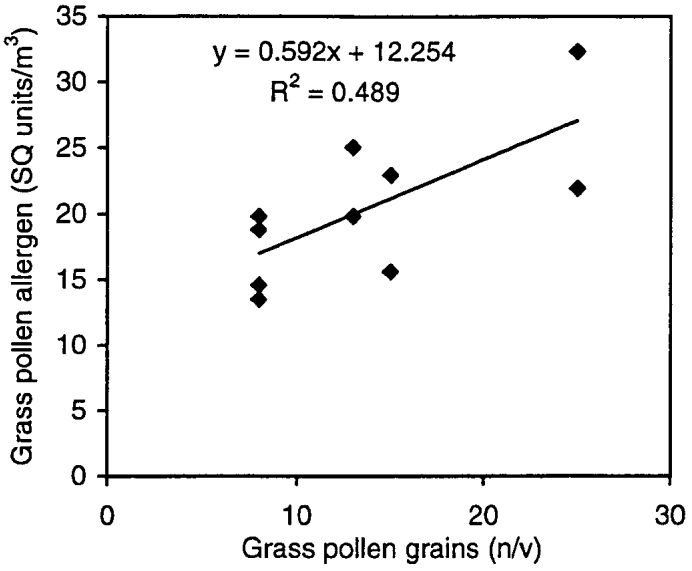

Figure 4. Plot of average 8-h outdoor air grass pollen grain concentrations (n = number of grains; v = m^3/3, the air volume corresponding to 8-h sampling, obtained with the Burkard trap, against paired concentrations of grass pollen allergens in the air in the driving compartments of the airport coaches, estimated from air samples of July 5th to the 9th 1999.

Discussion

The present investigation clearly indicates that birch and grass pollen allergens can contaminate the air in the driving compartments of coaches, equipped with air-cleaning filters, running in urban and rural environments during the pollen producing seasons. Pollen allergens might in addition to the supplied air come into the coaches through open doors and with entering passengers. Although there was no significant cross-reaction between the available polyclonal antibodies and extracts of rubber latex, mugwort, human skin, cat and dog hair, mite and spores, except for the anti-birch immunoglobulins between extracts of birch and mugwort pollen, a contribution to the pollen allergen concentration values from unknown cross-reacting allergens can not be excluded. It has *inter alia* been reported that monoclonal antibodies recognize IgE epitopes of mugwort allergen and cross-react with protein allergens of birch and timothy grass pollen (Grote *et al.*, 1998). Inhibition studies by pre-incubation of sera with extracts of birch and mugwort pollen also displayed IgE-cross-reactivity (Bauer *et al.*, 1996). In addition, timothy grass pollen extracts strongly inhibited IgE binding to latex allergens (Fuchs *et al.*, 1997). Birch pollen antigen penetrated into the compartments even at low to moderate outdoor pollen grain concentrations. The good correlation between invehicle airborne grass pollen allergens and officially reported outdoor pollen grain concentrations during the dry weather period is in accordance with previous findings in school rooms (Holmquist and Vesterberg, 1999a) and for outdoor grass pollen allergens and grains (correlation coefficient 0.69) (Holmquist and Vesterberg, 1999b). The delayed peak of the curve for grass pollen allergen concentration as compared to the corresponding outdoor 8-h and 24-h pollen curves recorded during the rainy period July 15th to the 23rd is consistent with the results obtained at quantification of outdoor grass pollen allergens and pollen grain counts during July 1998 (Holmquist and Vesterberg, 1999b). In previous studies (Suphioglu *et al.*, 1992; Spieksma *et al.*, 1991; Rantio-Lehtimäki *et al.*, 1994; Ong *et al.*, 1995; D'Amato *et al.*, 1998; Schäppi *et al.*, 1997; Schäppi *et al.*, 1999) and in the present, air could be shown to contain substantial amounts of grass and birch pollen allergens even in the absence of or low amounts of pollen grains after light rainfall. This explains the observed disappearance of the correlation between airborne pollen allergens and grains under wet meteorological conditions. Staining of sample filters by reagents specific to the alkaline phosphatase-conjugated antibody-allergen complexes, produced in DOSIS, revealed a particulate nature of the air samples (not shown here) in accordance with earlier findings (Holmquist and Vesterberg, 1999a and 1999b).

Allergic symptoms from birch and grass pollen allergen particles in the air may appear even at concentrations as low as 1 SQ unit/m^3 (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. Thus the present study advocates the need to effectively map airborne pollen allergen concentrations,

and to increase efforts to assure an allergen deficient air in the driving compartments of relevant traffic vehicles.

Acknowledgements

The authors are indebted to Hans-Erik Norbeck for his interest in this work, to Jan Svensson and Martin von Pfaler for instruction of the coach drivers and for supplying pollen count data, respectively, and to Jan Weiner for statistical advice. Economic support was obtained from the Cancer and Allergy Foundation and Stockholms Lokaltrafik AB.

Summary

Holmquist, L & Vesterberg, O. *Airborne birch and grass pollen allergens in driving compartments of coaches.* Arbete och Hälsa 2001:2

Background: Particulate and gaseous air pollutants originating from vehicular traffic have been shown to increase the allergenicity of birch and grass pollen particles and also to aggravate allergic inflammation. Thus vehicle drivers are at a high risk of developing allergic symptoms during pollen producing seasons which may impair traffic safety.

Objectives: to map concentrations of birch and grass pollen allergens in the ambient breathing air of coach drivers.

Methods: Airborne pollen allergens sampled in the driving compartments of coaches, were quantified by luminescence immunoassay. Birch (ten coaches) and grass (30 coaches) pollen allergens were collected in May and June-July 1999, respectively. All coaches were run on the same urban and rural routes.

Results: On average 19±5.6 SQ units per m^3 (mean±SD) of birch pollen allergens were found in the invehicle air. The average airborne grass pollen allergen concentration in the vehicles for the dry period July 5th to 14th was 20±5.6 SQ units per m^3 (mean±SD). The corresponding correlation coefficient was 0.70 for invehicle air grass pollen allergen and outdoor pollen grain concentrations. The correlation disappeared rainy days, when the pollen grains but not the allergens disappeared from the air. Average grass pollen allergen concentrations of the invehicle air for the rainy periods, July 12th to 16th, and July 19th to 23rd, were 11±3.4 and 24±9.3 SQ units per m^3 (mean±SD), respectively.

Conclusions: Although equipped with air inlet filters the driving compartments of the coaches contained substantial amounts of airborne birch and grass pollen allergens. Efforts should be increased to create pollen allergen deficient breathing air for coach drivers at work.

Key words: Allergens; airborne; air sampling; birch; coaches; DOSIS; driving compartments; grass; pollen; quantification.

Summary in Swedish

Holmquist, L & Vesterberg, O. *Airborne birch and grass pollen allergens in driving compartments of coaches.* Arbete och Hälsa 2001:2

Bakgrund: Partikulära och gasformiga luftföroreningar som bildas i trafiken har visats kunna öka allergeniciteten hos björk- och gräspollenallergen och även kunna förvärra allergisk inflammation. Fordonsförare riskerar därför speciellt att drabbas av allergiska symptom under pollensäsongen vilket kan försämra trafiksäkerheten.

Målsättning: Att kartlägga koncentrationer av björk- och gräspollenallergen i bussars förarhytter under verkliga körförhållanden.

Metoder: Luftburna pollenallergen provtogs i bussförarnas andningsluft och kvantifierades medelst kemiluminiscens och användning av specifika antikroppar. Luftburna pollenallergen från björk provtogs år 1999 i maj i tio bussar och från gräs i 30 bussar under juni - juli. Alla bussar körde samma sträckor i stad och på landsbygd.

Resultat: Medelkoncentationen av björkpollenallergen i luften i förarhytter var 19 ± 5.6 SQ enheter per m^3 (medelvärde ± SD). Medelkoncentrationen av gräspollenallergen i luften i förarhytter under den torra perioden 5 - 14 juli var 20 ± 5.6 (medelvärde ± SD). Motsvarande korrelationskoefficient var 0.70 för koncentrationerna av gräspollenallergen i luften i förarhytter och pollen i utomhusluft. Korrelationen försvann regniga dagar då pollenkornen men inte allergenerna försvann från luften. Medelkoncentrationen av gräspollenallergen i luften i förarhytter under de regniga perioderna 12 - 16 juli samt 19 - 23 juli var 11 ± 3.4 respektive 24 ± 9.3 SQ enheter per m^3 (medelvärde ± SD).

Slutsats: Ehuru tilluften i förarhytter var filtrerad innehöll den ansenliga koncentrationer av luftburna pollenallergen från björk och gräs. Åtgärder bör vidtagas för att eliminera halten av sådana allergen i förarhytter.

References

Bauer L, Ebner C, Hirschwehr R, Wuthrich B, Pichler C, Fritsch R, Scheiner O, Kraft D. (1996) IgE cross-reactivity between birch pollen, mugwort pollen and celery is due to at least three distinct cross-reacting allergens: immunoblot investigation of the birch-mugwort-celery syndrome. *Clinical and Experimental Allergy* 26, 1161-1170.

D'Amato G, Spieksma F, Liccardo G, Jäger S, Russo M, Kontou-Fili K, Nikkels H, Wüthrich B, Bonini S. (1998) Pollen-related allergy in Europe. *Allergy* 53, 567-578.

Dias-Sanches D, Penichet Garcia M, Wang M, Jyrala 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* , 1183-1188.

Fountain D W, Berggren B, Nilsson S and Einarsson E. (1992). Expression of birch pollen-specific IgE-binding activity in seeds and other plant parts of birch trees (Betula Verrucosa Ehrh:). *International Archieves of Allergy and Immunology* 98, 370-376.

Fuchs T, Spitzauer S, Vente C, Hevler J, Kapiotis S, Rumpold H, Kraft D, and Valenta R. (1997) Natural latex, grass pollen, and weed pollen share IgE epitopes. *Journal of Allergy and Clinical Immunology* 100, 356-364.

Grote M, Fischer S, et al. (1998) In situ localization of a high molecular weight cross-reactive allergen in pollen and plant-derived food by immunogold electron microscopy. *Journal of Allergy and Clinical Immunology* 101(2 Pt 1), 250-257.

Grote M and Fromme HG (1986) Cross-reactivity of birch anthers and leaves with birch pollen antigens and allergens. A fine-structural immunocytochemical study using the post-embedding protein-A-gold technique. *Journal of Histochemistry and Cytochemistry* 34, 1459-64.

Holmquist L and Vesterberg O. (1999a) Quantification of birch and grass pollen allergens in indoor air. *Indoor Air* 9, 85-91.

Holmquist L and Vesterberg O. (1999b) Luminescence immunoassay of pollen allergens on air sampling polytetrafluoroethylene filters. *Journal of Biochemical and Biophysical Methods* 41, 49-60.

Holmquist L and Vesterberg O. (2000) Miniaturized direct on air sampling filter quantification of pollen allergens. *Journal of Biochemical and Biophysical Methods* 42, 111-114.

Johnsen CR, Weeke ER, Nielsen J, Jensen J, Mosbech H, Frölund L, Madsen F and Poulsen LK. (1992) Aeroallergen analyses and their clinical relevance. II. Sampling by high-volume airsampler 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) Bioaerosols in Ambient Air Particulates: A Review and Research Needs. *Reviews on Environmental Health* 14, 79-89.

Nel A E, Dias-Sanches D, Ng D, Hiura 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.

Ong EK, Singh MB, Knox RB. (1995) Aeroallergens of plant origin: molecular basis and aerobiological significance. *Aerobiologia* 11, 219-229.

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 Archieves of Environmental Health* 73, 209-214.

Rantio-Lehtimaki A, Viander M, and Koivikko A. (1994) Airborne birch pollen antigens in different particle sizes. *Clinical and Experimental Allergy* 24, 23-8.

Schäppi GF, Monn C, Wüthrich B and Wanner HU. (1996) Analysis of allergens in ambient aerosols: comparison of areas subjected to different levels of air pollution. *Aerobiologia* 12, 185-90.

Schäppi F, Suphioglu C, Taylor E, Knox R. (1997) Concentrations of the major birch tree allergen Bet v 1 in pollen and respirable fine particles in the atmosphere. *Journal of Allergy and Clinical Immunol* 100, 656-61.

Schäppi F, Taylor P E, Pain MCF, Cameron PA, Dent AW, Staff I A and Suphioglu C. (1999) Concentrations of major grass group 5 allergens in pollen grains and atmospheric particle: implications for hay fever and allergic asthma sufferers sensitized to grass pollen allergens. *Clinical and Experimental Allergy* 29, 633-641.

Spieksma F Th M, Kramps J A, Plomp A and Koerten HK. (1991) Grass-pollen allergen carried by the smaller micronic aerosol fraction. *Grana* 30, 98-101.

Strand V. *Effects of nitrogen dioxide on airway responsiveness in allergic asthma*. Thesis: Karolinska Institutet, Stockholm, 1998. ISBN 91-628-3068-6.

Suphioglu C, Singh M, Taylor P, Bellamo R, Holmes P, Puy R and Knox R B. (1992) Mechanism of grass-pollen-induced asthma. *Lancet* 339, 569-572.

Yli-Panula E and Ahlholm J. (1998) Prolonged antigenic activity of birch and grass pollen in experimental conditions. *Grana* 37, 180-184.