

Aeroallergen analyses and their clinical relevance

II. Sampling by high-volume airsampler with immunochemical quantification versus Burkard pollen trap sampling with morphologic quantification

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A comparison was made between the amount of airborne pollen collected by Burkard airsampler and the allergenic activity of particles trapped on glass fibre filters in an Accu-Vol high-volume airsampler. The comparison was made throughout the pollen seasons 1986 to 1989. Both airsamplers were operated 24 h a day. They were placed less than 5 m apart, and estimation of the pollen amount was made on a day-to-day basis during the pollen seasons, and on a weekly basis outside the seasons. The occurrence of the 3 clinically most important allergenic types of pollen, birch, grass, and mugwort, was analysed, and close correlations between the 2 sampling techniques were found (r_s 0.5–0.8, $p < 0.001$). The detected range of counted pollens/m³ was: birch 0–1075, grass 0–156, and mugwort 0–44. By immunochemical analysis we found the corresponding amounts to be 0–80, 0–8, and 0–1 SQ-U/m³, respectively. Pollen counts and immunochemical estimation were compared with the symptom score recordings of allergic persons for birch season 1989 and for grass seasons 1986, 1988, and 1989. A close correlation was found for both sampling techniques for the grass seasons in 1986 and 1989 (r_s 0.51–0.61, $p < 0.001$ – 0.0001), but a less significant correlation was found for the 1988 grass season, and for birch in 1989 (r_s 0.24–0.34, $p < 0.001$ – 0.05).

Key words: aerobiology; aeroallergens; birch; Burkard pollen trap; grass; high-volume airsampler; IgE RAST-inhibition; mugwort; pollen; pollinosis.

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Airborne pollens in the outdoor environment are the main cause of rhinitis and asthma in pollen-allergic persons. The symptoms can be elicited by fragmented pollen grains as well as whole pollens (2, 21, 22). Since 1977, the Aerobiological Group of the

Danish Asthma-Allergy Association has continuously recorded airborne pollens in Denmark (9, 18, 27). Different kinds of high-volume airsampling and evaluation of particle size distribution have revealed that pollen-allergenic activity can be found in particle sizes smaller than whole pollen grains (3, 5, 20, 23). These particles may reach the lower airways, thus causing asthma. Other data suggest that particles of sub-pollen size are clinically important: symptoms do not always correlate with pollen counts (25) and the sub-pollen size particles cannot be classified by morphological detection. Therefore immunochemical detection of pollen allergens seems important.

Abbreviations: NHS: normal human serum pool; PBS: sodium phosphate 10 mmol/l, sodium chloride 150 mmol/l, sodium azide 100 mg/l; PBSE: PBS with addition of ϵ -amino-n-caproic acid 0.1 mol/l (BDH 37014); PBST: PBS buffer pH 7.4 with addition of Tween 20 0.01% v/v; SDS-PAGE: sodium dodecyl sulfate – poly acrylamide gel electrophoresis; STE: Sørensen phosphate buffer 1/15 pH 7.0, sodium azide 200 mg/l, Tween 20 0.5% v/v, ϵ -amino-n-caproic acid 0.1 mol/l; SQ-U: standardized quality units.

In a previous short-term study (14), a close correlation was found between the comparison of morphological and immunochemical analyses. However, pollen allergenic activity was seen in periods without morphologically detectable pollens. The aim of this study was therefore to compare, during extended sampling periods, the results from microscopical and immunochemical analyses used and, subsequently, to relate the results to patient-recorded symptom scores. The clinically most important pollens, birch (*Betula* spp.), grass (*Poaceae*) and mugwort (*Artemisia vulgaris* L.), were chosen for the study.

Material and methods

Burkard airsampling

The Burkard® seven day recording volumetric spore trap (Burkard) (Burkard Manufacturing Co. Ltd., Richmansworth, Hertfordshire, UK), a modified version of the Hirst's spore trap (10), was used for collecting pollen grains. Briefly, the airborne pollens are trapped on an adhesive tape by sucking air at a flow of 10 l/min through a 2 × 14 mm slit in the air-sampler. The tape is mounted on a rotating drum driven by clockwork. This enables recording of the time variance during the day. In this study, the drum was replaced with another drum at 24 h intervals in the pollen season and weekly outside the season. The exposed tape is mounted on a slide and the trapped pollen grains are classified and counted by light microscopy. Each strip represents every 2nd hour during the sampling periods. The amount of pollen is expressed as number of pollen grains/m³ air (16, 18). Throughout the year, 48 different taxonomic species or groups have been counted by the pollen counters. In this study the comparison has been made for tree pollen (*Betula* spp., *Corylus avellana*, *Ulmus glabra* Hudson, *Alnus glutinosa* (L.) Gaertner), grass pollen (*Poaceae*), and mugwort (*Artemisia vulgaris* L.).

HIVOL airsampling

The ACCU-VOL® high-volume air sampler (HIVOL) (GMWS-2310, General Metal Works, Inc., Cleves, Ohio, USA) was used for collection of particles. The air sampler was operated at a flow of 962 l/min, and mounted with glass fibre filter sheets 20 × 25 cm (GMS-810 (General Metal Works, Inc.), retaining with a 95% efficacy particles > 0.3 µm in diameter). After a sampling period of 24 h, filter sheets are replaced daily during the pollen season and weekly outside the pollen season. After sampling, the sheets are folded once with the exposed side inward, and stored at -20°C until elution and analysis. At the time of analysis, the filter sheets are refolded twice without opening the filter, transferred

to vellum envelopes and pulverized at 350 kg/cm² (COMPAC® DP10, Juelsminde, Denmark) in a hydraulic press. The pulverized filter material is transferred to test tubes, and 6 ml of Sørensen/Tween/ε-aminocaproic acid buffer (STE) added. Elution takes place overnight on a vibrator. The eluates are isolated by centrifugation (161 × g for 20 min), and the allergenic activity in the supernatant, the test sample, is then analysed by immunochemical estimation, as described below.

For sampling purposes the Burkard and HIVOL air samplers were placed 15 m above the ground, and < 5 m apart, on the roof of the Danish Meteorological Institute in central Copenhagen. The sampling periods were parallel for both air samplers.

Allergens

Lyophilised allergen extracts (Aquagen®, ALK, Hørsholm, Denmark) of birch (*Betula verrucosa* Ehrh.), timothy grass (*Phleum pratense* L.), and mugwort (*Artemisia vulgaris* L.) were reconstituted in PBS/ε-aminocaproic buffer (PBSE) to a stock solution of 1 000 000 SQ-U/ml. ≈ 2 mg/ml. Lyophilised allergen extracts (Aquagen®, ALK, Hørsholm, Denmark) of hazel (*Corylus avellana*) and wych-elm (*Ulmus glabra* Hudson) were reconstituted in PBSE buffer to a stock solution of 100 000 SQ-U/ml. Alder (*Alnus glutinosa* (L.) Gaertner) (Soluprick® (ALK, Hørsholm, Denmark) 1:20 w/v) was diluted 1:10 in PBSE. Hazel, elm, and alder allergen extracts were used to reveal unspecificity of the birch serum pool.

Serum pools

Sera from patients allergic to either birch (*Betula verrucosa* Ehrh.), grass (*Phleum pratense* L.), or mugwort (*Artemisia vulgaris* L.) were chosen. The patients all had positive medical histories of reaction to the allergen, positive skin prick tests, and RAST class 4 for the allergen in question. Further, they had a RAST class < 2 for any other allergen. None of the patients had been undergoing immunotherapy. Reactivity of each serum sample against individual allergens in each extract was evaluated by SDS-PAGE and immunoblotting (13). In brief, SDS-PAGE was performed with 16% separation gels and 5% stacking gels in an electrophoresis chamber. Samples were prepared by boiling in Laemmli sample buffer without mercaptoethanol. Pollen extracts were loaded onto one gel and subjected to immunoblotting. Molecular weights were determined with prestained molecular weight markers, and staining performed with Coomassie brilliant blue. Electroblothing was made with a semi-dry electroblotter, and proteins were transferred to nitrocellulose in a discontinuous buffer system. The nitrocellulose strips

were cut into sizes of 0.5×11.5 cm and blocked in Tris-buffered saline, containing Tween and powdered milk. Serum samples, serum pools, normal human serum pool (NHS) and buffer were applied to the nitrocellulose strips and incubated overnight. Nitrocellulose-bound IgE was detected by autoradiography after incubation overnight with ^{125}I -labelled rabbit antihuman IgE. All sera had a positive band for the major allergens and individual bands for minor allergens. No bands were observed for NHS or buffer. The observed molecular weights for major [and minor] allergens were: 1) birch – 17 kD [29 and 36 kD], 2) grass – 28 and 33 kD [10, 11 and 65 kD] and 3) mugwort – 33 kD (4, 8, 11, 13). From the results of the individual RAST and immunoblotting analyses, 3 IgE reference sera pools were made: birch containing 13 sera, grass containing 17, and mugwort containing 4.

Maxisorp IgE RAST-inhibition methodology

For each of the sera pools, dose-response studies were performed to establish optimal dilutions of allergens for coating of Maxisorp® test tubes (Nunc, Roskilde, Denmark) and dilution of sera pools for RAST-inhibition (15, 19). Maxisorp test tubes were coated by addition of 300 μl allergen diluted in PBS (birch and grass 1:50, and mugwort 1:100) and incubated overnight at 4°C . RAST-inhibition was performed at 4°C by overnight incubation of 400 μl of the 3 sera pools with 400 μl of allergen dilutions or test samples. Sera pools were all diluted 1:50 and allergen/antibody complexes spun down at $3000 \times g$ for 20 min. The remaining free specific IgE antibodies in the inhibition mixtures were transferred to test tubes and analysed in the IgE Maxisorp® RAST according to the method described by Poulsen et al. (19).

In brief, 300 μl of the supernatant was transferred to an allergen-coated Maxisorp test tube and incubated overnight at 20°C on a vibrator. After incubation, 300 μl of anti-IgE ^{125}I (Pharmacia AB, Uppsala, Sweden), diluted 1:12 (about 10 000 cpm) was added, incubated overnight at 20°C and counted for 5 min in a gamma-scintillation counter. Between each step test-tubes were washed 3 times in PBST. Results were obtained from duplicate analyses and means were expressed as:

$$\% - \text{inhibition} = 100 - 100 \times \left(\frac{\text{Sample} - \text{Blank}}{\text{Uninhibited} - \text{Blank}} \right)$$

Allergen extracts of birch, grass, and mugwort standardized for allergen activity according to the procedures by ALK in standardized quality units (SQ-U) were used as reference allergens for stan-

dard curves. Standard curves were made by analysing 10-fold dilutions in PBS of stock allergen extract solutions, and the amount of allergen in the samples was interpolated from the standard curves. RAST-inhibition curves of dilutions of reference allergens and eluates were parallel. The amount of sampled allergen was expressed in SQ-U/ m^3 air.

Symptom score

Patients sensitive to birch and grass pollen were investigated. The diagnosis was confirmed by skin prick test, RAST and seasonal symptoms. Patients were all living within the range of the HIVOL and Burkard airsamplers, and no more than 10 km from the sampling site. By means of a daily questionnaire, each patient was asked to estimate conjunctival, nasal, and bronchial symptoms and the consumption of anti-allergic drugs. Symptoms were recorded for grass-allergic patients in 1986 ($n=51$), in 1988 ($n=60$), and in 1989 ($n=70$), and for birch-allergic patients in 1989 ($n=37$). Symptoms were graded from 0 to a maximum value of 3, and summarized with a weighted score for the drug used by the patients (6, 17, 18, 28). The symptom recording forms, birch 1989, originate from a follow-up study by B. Nüchel Petersen et al. (unpublished data) on tree pollen extract immunotherapy patients (17).

Statistics

Correlations between pollen counts, immunochemical estimations and clinical symptoms were analysed by Spearman's rank correlation coefficient, r_s , corrected for ties.

For each pollen type, 3 yearly seasons were defined; a pre-season, mid-season, and post-season. The pre-season finishes on the date when 2.5% of the total yearly amount of pollen have been collected. The post-season begins on the date after which 97.5% have been collected. The mid-season constitutes the period between these dates (9).

Results

The detected ranges of counted pollens/ m^3 were birch 0–1075, Wych elm 0–215, alder 0–74, hazel 0–21, grass 0–156, and mugwort 0–44. The corresponding amounts of allergens in SQ-U/ m^3 were birch 0–80, grass 0–8, and mugwort 0–1.

The results from 3 years' collection of birch pollens/allergens and a summation of alder and hazel pollens are given in Fig. 1A–1C. For each year, the mid-pollen birch season is shown in the figures as an insert. The results of the summing up of symptom score and the weighted consumption of drugs for birch-allergic patients are shown in Fig. 1C. The re-

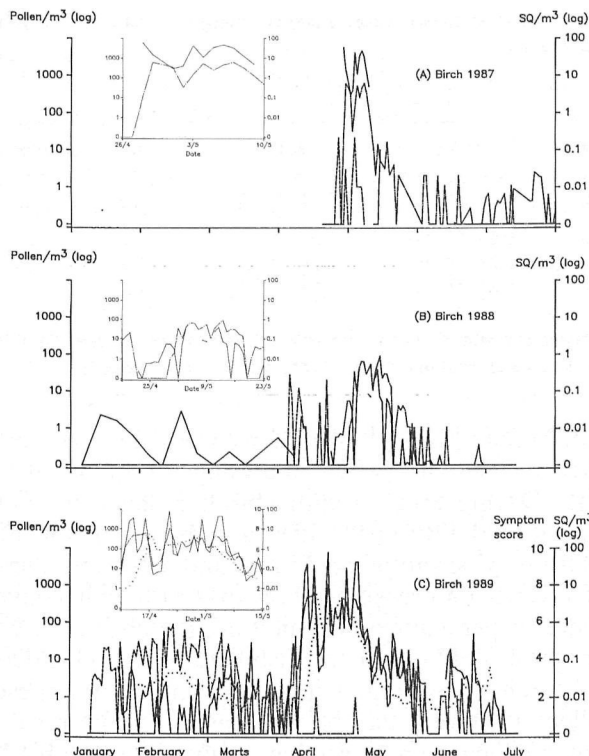


Fig. 1. Comparison between daily quantification of birch pollen and birch aeroallergen activity sampled by high-volume airsampler and immunochemical quantification (—) and Burkard airsampling and morphological counting (— · — · —). Values for A (1987), B (1988), and C (1989). Values are expressed/m³ air sucked through the airsamplers for a period of 24 h. Inserted diagrams represent mid-pollen seasons as described in Material and methods. For 1989 the mean symptom scores for birch-allergic patients are shown (· · · · ·). The sum of alder and hazel pollen counts are shown for comparison (— · — · —).

sults of the 4 years' sampling of grass pollens/allergens are given in Fig. 2A–2D with inserts representing grass mid-pollen season. The results of the summing up of symptom score and weighted consumption of drugs for grass-pollen allergic patients are given in Fig. 2A, 2C, and 2D. The results from 3 years' measurements of mugwort allergens and pollens are given in Fig. 3A–3C. Disconnected curves in Figs. 1, 2, and 3 represent periods with operational malfunction of the airsampler.

The statistical analysis comparing results from airsamplers and symptom scores, and airsamplers mutually, are given in Tables 1 and 2.

Discussion

In this study we have chosen an IgE-RAST-inhibition assay to quantify collected pollen allergens. In a previous work (14), subclass-IgG₄-RAST-inhibition assay was used for quantification. Using a lower detection limit defined as a 20% inhibition, we found equal sensitivity between the 2 immunochemical as-

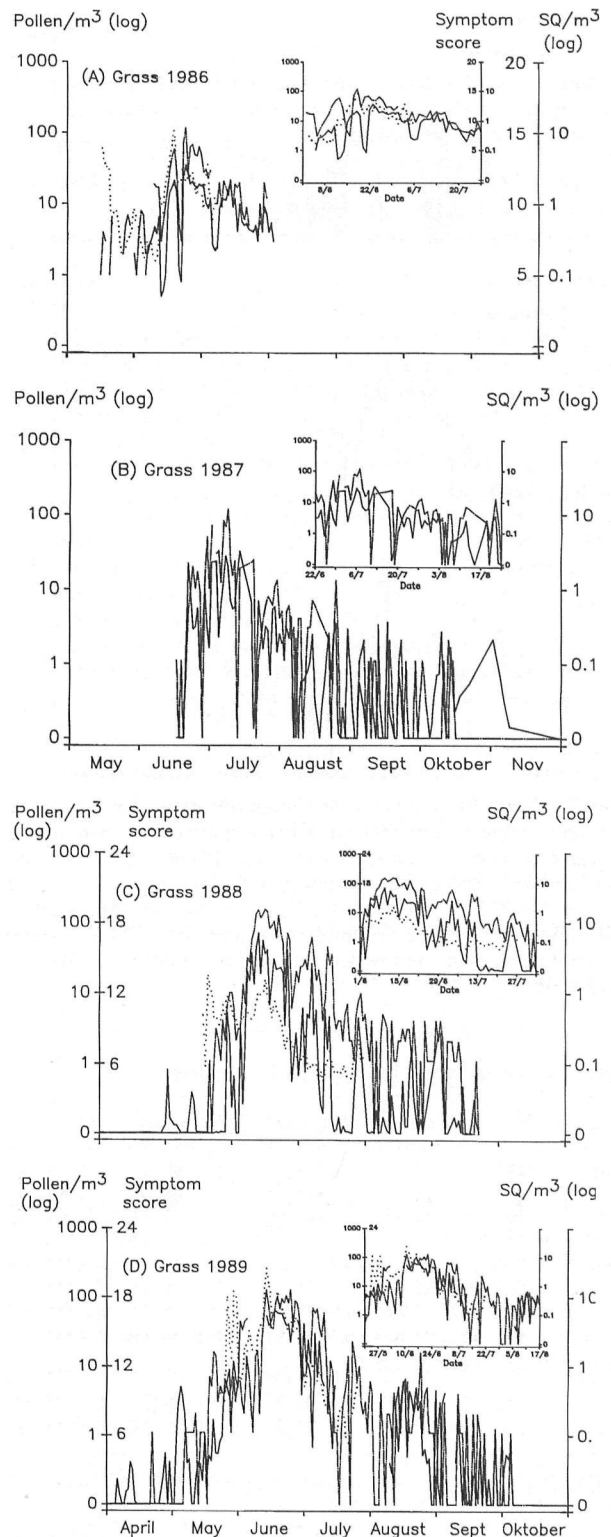


Fig. 2. Comparison between daily quantification of grass pollen and grass aeroallergen activity sampled by high-volume airsampler and immunochemical quantification (—) and Burkard airsampling and morphological counting (— · — · —). Values for A (1986), B (1987), C (1988), and D (1989). Values are expressed/m³ air sucked through the airsamplers for a period of 24 h. Inserted diagrams represent mid-pollen season as described in Material and methods. For 1986, 1988 and 1989 the mean symptom scores for grass-allergic patients are shown (· · · · ·).

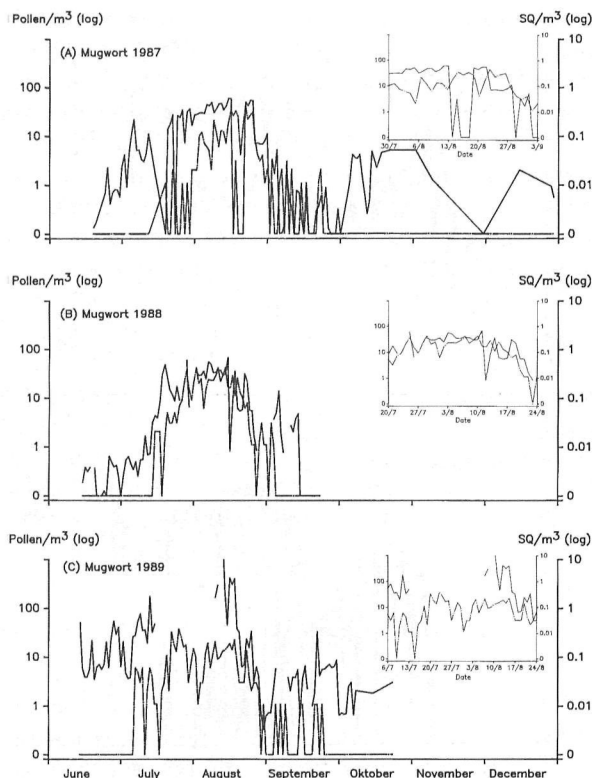


Fig. 3. Comparison between daily quantification of mugwort pollen and mugwort aeroallergen activity sampled by high-volume air sampler and immunochemical quantification (—) and Burkard air sampling and morphological counting (---). Values for A (1987), B (1988), and C (1989). Values are expressed/m³ air sucked through the air samplers for a period of 24 h. Inserted diagrams represent mid-pollen seasons as described in Material and methods.

Table 1. Comparison between Burkard and HIVOL air sampling

Pollen	Year	Pre-season	Mid-season	Post-season	Whole period
Birch	1987	NA	0.9 * (11)	0.3 NS (50)	0.5 *** (61)
	1988	NA (5)	0.8 *** (23)	0.2 NS (50)	0.5 *** (88)
	1989	0.3 * (77)	0.7 *** (30)	0.5 *** (53)	0.7 *** (160)
Grass	1986	NA	0.8 *** (51)	NA	0.7 *** (58)
	1987	0.7 NS (6)	0.5 *** (51)	0.1 NS (54)	0.6 *** (111)
	1988	0.4 * (44)	0.9 *** (54)	0.3 * (37)	0.8 *** (135)
	1989	0.4 ** (60)	0.6 *** (57)	0.4 ** (49)	0.8 *** (166)
Mugwort	1987	0.5 ** (33)	0.6 ** (28)	-0.2 NS (37)	0.6 *** (98)
	1988	0.6 *** (36)	0.3 NS (27)	-0.1 NS (21)	0.7 *** (84)
	1989	0.4 * (26)	0.6 ** (22)	0.1 NS (39)	0.5 *** (87)

Coefficients of correlation (r_s) were calculated using Spearman's rank correlation corrected for ties. Number of days compared (n) in brackets.

NS=not statistically significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NA=not applicable.

says at 10 SQ-U/ml eluate for all allergens. This is comparable to other allergen testing systems (15, 24). We decided to proceed with IgE-RAST-inhibition because of the disputable, allergenically significant role of IgG₄ subclasses, and because relevant IgE sera are more readily obtained.

Table 2. Comparison between Burkard and HIVOL air sampling mutually compared with symptom score

Pollen	Correlation to symptoms		Year	No. patients
	HIVOL	Burkard		
Birch	0.28 ** (141)	0.34 ** (154)	1989	37
Grass	0.60 ** (32)	0.51 ** (50)	1986	51
	0.27 * (65)	0.24 * (72)	1988	60
	0.61 *** (43)	0.53 *** (63)	1989	70

Coefficients of correlation (r_s) were calculated using Spearman's rank correlation. Number of days compared in brackets. * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$.

In January through March, we were able to detect allergenic activity, when analysing for birch allergens. During these months birch pollens are not present on the Burkard slides, and the reason for the results may be sampling of hazel and alder aeroallergens. Data not presented show that, although it gave a smaller percentage of inhibition, the chosen birch sera pool could not distinguish between hazel, alder, and birch. Cross-reactivity is well known for these pollens (29) and may be the reason for the result. Elm allergens could not be analysed using birch serum pool. By Burkard sampling and morphological detection, hazel and alder pollen seasons are found in the first 3 months of the year (9), and if for comparative purposes we add hazel and alder pollen countings, we find a good correlation to HIVOL (dash-dot-dot lines in Fig. 1 A–C represent the sum of alder and hazel pollen countings).

In general, we found a close correlation between the 2 sampling methods for the whole season and, especially during mid-season, for all 3 allergens and for all the years (Table 1). The data revealed pre- and post-seasonal differences with a tendency towards allergenic activity outside the counted pollen period, particularly for birch and mugwort. In agreement with the findings of others (3, 12, 26) we observed large day-to-day variations in both pollen counts and the amount of aeroallergens. The start and duration of the mid-season varied for each pollen species, as seen in previous studies (9, 18). Since 1978, the scale shown in Table 3 has been used with the purpose of forecasting daily amounts of pollen in

Table 3. Symptom-related frequencies and number of allergenic pollen/m³ in the air, 24 h mean

Scaling of pollen	Birch	Grass	Mugwort	Patients with allergic symptoms reacting
Low	0–10	0–10	0–10	Most sensitive patients only ↑ All patients
Moderate	10–100	10–30	10–30	
High	> 100	> 30	> 30	

the air. The scale is based on clinical experience related to symptoms, pollen counts for earlier periods and meteorological data. When this scaling is transformed to the presented results, it shows a high correlation to the severity of symptoms recorded, counted pollens and pollen allergen analysis. A tendency towards exacerbation of birch and grass symptoms could be seen when the SQ-load in the air was higher than 1 SQ-U/m³. The recording of symptoms correlated to the 1989 birch season was also performed pre- and post-seasonally. For the grass season, symptom recording was only undertaken for a short period during mid-season. For this reason it is not possible to give any correlation outside the season in order to differentiate between the sensitivity/specificity of the 2 sampling techniques concerning symptoms. Further studies elucidating this problem are in progress. For the birch-allergic patients, mild symptoms were present outside the "birch season", but these may have been ascribable to other allergenic pollens with cross-reacting allergens such as hazel and alder.

Taking into account the different principles of sampling and counting/analysis of pollens, a high correlation was found for the 2 methods (Table 1). Both airsamplers are placed at high levels for the purpose of trapping pollens representative for a large geographical area. Theoretically, the particles smaller than whole pollen grains should stay airborne for a longer period. In contrast to studies performed by others (1, 2, 22, 23), our study did not differentiate between the sampled particle size, since the HIVOL when operated without an impaction head does not separate the particles according to size. Further studies must be conducted to demonstrate whether the detection of aeroallergens outside the "counted pollen" season is ascribable to sub-pollen particles present in the outdoor environment, as described by Fernandez-Caldas et al. (7).

Conclusions

Both methods could be used for counting pollen concentrations relevant for clinical symptoms in pollen-allergic patients. Burkard pollen sampling and morphological recognition require less effort and are less expensive and more useful in forecasting because of the shorter time needed to count pollen, as compared with immunochemical quantitation.

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References

1. AGARWAL MK, SWANSON MC, REED CE, YUNGINGER JW. Immunochemical quantitation of airborne short ragweed, *Alternaria*, antigen E, and Alt-I allergens: a two-year prospective study. *J Allergy Clin Immunol* 1983; 72: 40–5.
2. AGARWELL 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: 687–93.
3. AGARWAL MK, YUNGINGER JW, SWANSON MC, REED CE. An immunochemical method to measure atmospheric allergens. *J Allergy Clin Immunol* 1981; 65: 194–200.
4. ARNTZEN FC, WIEHLMSEN TW, LÖWENSTEIN H, et al. The international collaborative study on the first international standard of birch (*Betula verrucosa*) pollen extract. *J Allergy Clin Immunol* 1989; 83: 66–82.
5. BUSSE WW, REED CE, HOEHNE JH. Where is the allergic reaction in ragweed asthma? Demonstration of ragweed antigen in airborne particles smaller than pollen. *J Allergy Clin Immunol* 1972; 50: 289–93.
6. D'SOUZA MF, EMANUEL MB, GREGG J CHARLTON J, GOLDSCHMIDT J. A method for evaluating therapy for hay fever. *Clin Allergy* 1983; 13: 329–35.
7. FERNANDEZ-CALDAS E, SWANSON MC PRAVDA J, WELSH P, YUNGINGER JW, REED CE. Immunochemical demonstration of red oak pollen aeroallergens outside the pollination season. *Grana* 1989; 28: 205–9.
8. GJESING B, JÄGER L, MARSH DG, LÖWENSTEIN H. The international collaborative study establishing the first international standard for timothy (*Phleum pratense*) grass pollen extract. *J Allergy Clin Immunol* 1989; 72: 258–67.
9. GOLDBERG C, BUCH H, MOSEHOLM L, WEEKE ER. Airborne pollen records in Denmark, 1977–1986. *Grana* 1988; 27: 209–17.
10. HIRST JM. An automatic volumetric spore trap. *Ann Appl Biol* 1952; 39: 257–65.
11. IBSEN H, FORMGREN H, LÖWENSTEIN H, INGEMANN L. Immunochemical and biological characterization of a mugwort (*Artemisia vulgaris*) pollen extract. *Allergy* 1985; 40: 289–94.
12. ICKOVIC MR, BOUISSIUD-CORBIERES F, SUTRA JP, THIBAUDON M. Hay fever symptoms compared to atmospheric pollen counts and floral phenology within Paris suburban area in 1987 and 1988. *Aerobiologia* 1989; 5: 30–6.
13. JAROLIM E, MATTHIESEN F, STAHL SKOV P, TEJL M, ØSTERBALLE O, POULSEN LK. Hyposensitization therapy with whole pollen extract or purified allergens monitored by immunoblotting. *Int Arch Allergy Appl Immunol* 1990; 92: 254–64.
14. JENSEN J, POULSEN LK, MYGIND K, WEEKE ER, WEEKE B. Immunochemical estimation of allergenic activities from outdoor aero-allergens, collected by a high-volume air sampler. *Allergy* 1989; 44: 52–9.
15. JOHNSEN CR, ABRAHAMSEN L, POULSEN LK, STAHL SKOV P. Aeroallergen analyses and their clinical relevance. I. Immunochemical quantification of allergens by RAST-inhibition, Mab-ELISA, basophil histamine release and counter current immuno electrophoresis. *Allergy* 1991; 46: 492–501.
16. KÄPYLÄ M, PINTTINEN A. An evaluation of the microscopical counting methods of the tape in the Hirst-Burkard pollen and spore trap. *Grana* 1981; 20: 131–41.
17. NÜCHEL PETERSEN B, JANNICHE H, MUNCH EP, et al. Immunotherapy with partially purified and standardized tree pollen extracts. I. Clinical results from a three-year double-blind study of patients treated with pollen extracts either of birch or a combination of alder, birch and hazel. *Allergy* 1984; 43: 353–62.

18. NÜCHEL PETERSEN B, LARSEN L, EGELUND E, BUCH H, JÜRGENSEN F, GRAVESEN S. Pollen and mould spore counts in Denmark. Description of methods. Hillerød, Denmark: Pharmacia, 1986.
19. POULSEN LK, PEDERSEN MF, MALLING H-J, SØNDERGAARD I, WEEKE B. Maxisorp RAST – a sensitive method for detection of antigen-specific human IgE in culture fluids. *Allergy* 1989; 44: 173–80.
20. SOLOMON WR. Sampling airborne allergens. *Ann Allergy* 1984; 52: 140–7.
21. SOLOMON WR. Airborne allergens associated with small particle fractions. *Grana* 1986; 25: 85–7.
22. 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: 443–7.
23. SPIEKSMATM, KRAMP JA, VAN DER LINDEN AC, et al. Evidence of grass-pollen allergenic activity in the smaller micronic atmospheric aerosol fraction. *Clin Exp Allergy* 1989; 20: 273–80.
24. VAN METRE TE, MARSH DG, ADKINSON, NF, et al. Dose of cat (*Felix domesticus*) allergen 1 (*Fel dI*) that induces asthma. *J Allergy Clin Immunol* 1986; 78: 62–75.
25. VIANDER M, KOIVIKKO A. The seasonal symptoms of hyposensitized and untreated hay fever patients in relation to birch pollen counts: correlation with nasal sensitivity, prick test and RAST. *Clin Allergy* 1978; 8: 387–96.
26. VON WAHL PG, PULS KE. The emission of mugwort pollen (*Artemisia vulgaris* L.) and its flight in the air. *Aerobiologia* 1989; 5: 55–63.
27. WEEKE ER, JØRGENSEN G, PETERSEN BN, TORP U, BUCH H. Pollenregistrering i København sommeren 1977 (Pollen registration in Copenhagen in summer 1977). *Ugeskr Laeg* 1979; 141: 1194–9.
28. WILKINSON P, TAUDORF E. Clinical trial of hyposensitisation in hay fever: two methods of relating symptom scores to daily pollen counts. *Allergy* 1984; 39: 463–8.
29. YMAN L. Botanical relations and immunological cross-reactions in pollen allergy. 2nd ed. Uppsala, Sweden: Pharmacia Diagnostics AB, 1982.

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