

Variations in airborne pollen antigenic particles caused by meteorologic factors

Pehkonen E, Rantio-Lehtimäki A. Variations in airborne pollen antigenic particles caused by meteorologic factors.

Allergy 1994; 49: 472–477. © Munksgaard 1994.

High birch pollen antigenic activities in outdoor air samples were found in all particle sizes studied (>7.2 , 2.4 – 7.2 , <2.4 μm and molecular size class, with an ELISA modification). Sampling was done with a low-volume, size-selective bioaerosol sampler (SSBAS) simulating the human respiratory tract in both volume and fractionation. Airborne birch pollen counts for comparisons were obtained from a Burkard trap. No correlations were obtained between antigen concentrations in any particle size fraction and airborne pollen counts. The meteorologic factors studied differed clearly in their effect on antigenicity, depending on the size class studied.

Likewise, the effect of meteorologic factors differed among the three study periods (period I, 4 weeks before the peak pollen season; period II, during the season; and period III, 4 weeks after the season). During the peak pollen period, temperature and relative humidity were the most important meteorologic factors. Before the season, large and very small particles predominated, medium-sized particles being totally absent. The largest size class studied (containing all intact pollen grains) clearly reacted to changes in meteorologic factors; for smaller size classes, these factors were found to be less important, a fact which may make the forecasting of antigen concentrations in the air on the basis of meteorologic data impossible.

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Key words: allergenicity; antigen; birch pollen; ELISA; immunochemistry.

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Accepted for publication 29 November 1993

Pollen antigens in outdoor air have been studied in samplers both with particle size fractionation (12, 14) and without size selection (7). Both high-volume samplers (7, 13) and low-volume samplers, which simulate human respiration (12), have been used. High antigenic activities have been documented in all these studies, and have been found even for submicronic particle sizes (below 1 μm in aerodynamic diameter), where no intact pollen grains or spores are present (11, 12). Correlations with the background count of pollen grains were observed in the studies of Jensen et al. (7), but not in the analyses of Rantio-Lehtimäki et al. (12).

The fractionation of particles into size classes is important for understanding the level of antigen concentrations which may reach different regions of the human respiratory tract. The smallest particle sizes are considered to be the most important ones because they can penetrate most deeply into the human respiratory tract (i.e., to the alveoli), where they may provoke immediate asthmatic reactions.

It has been suggested that traditional pollen reports and forecasts should be replaced by antigen/allergen reports (12). It is essential to investigate

separately the meteorologic conditions under which antigen particles in different size classes increase or decrease before, during, and after the pollination period. Large particles are known to be washed down by rain, but particles of submicronic size may remain airborne, even under unfavorable conditions, for months (6).

Birch pollen allergens have been chosen for more detailed study, because, in Finland, birch pollen is the most important airborne allergen, and 10% of Finnish adolescents and young adults and 5% of children suffer from respiratory pollen allergy (5, 9). The allergenic significance of the family Betulaceae, *Betula* (birch), *Alnus* (alder), and *Corylus* (hazel) is due to the fact that these trees are widespread and shed large quantities of windborne pollen containing allergenic material.

The aims of this study were the following:

- 1) to find out which meteorologic conditions increase airborne antigen concentrations, and which may lower the concentrations or clean the air effectively
- 2) to analyze in detail the changes in airborne birch pollen antigen concentrations in different particle

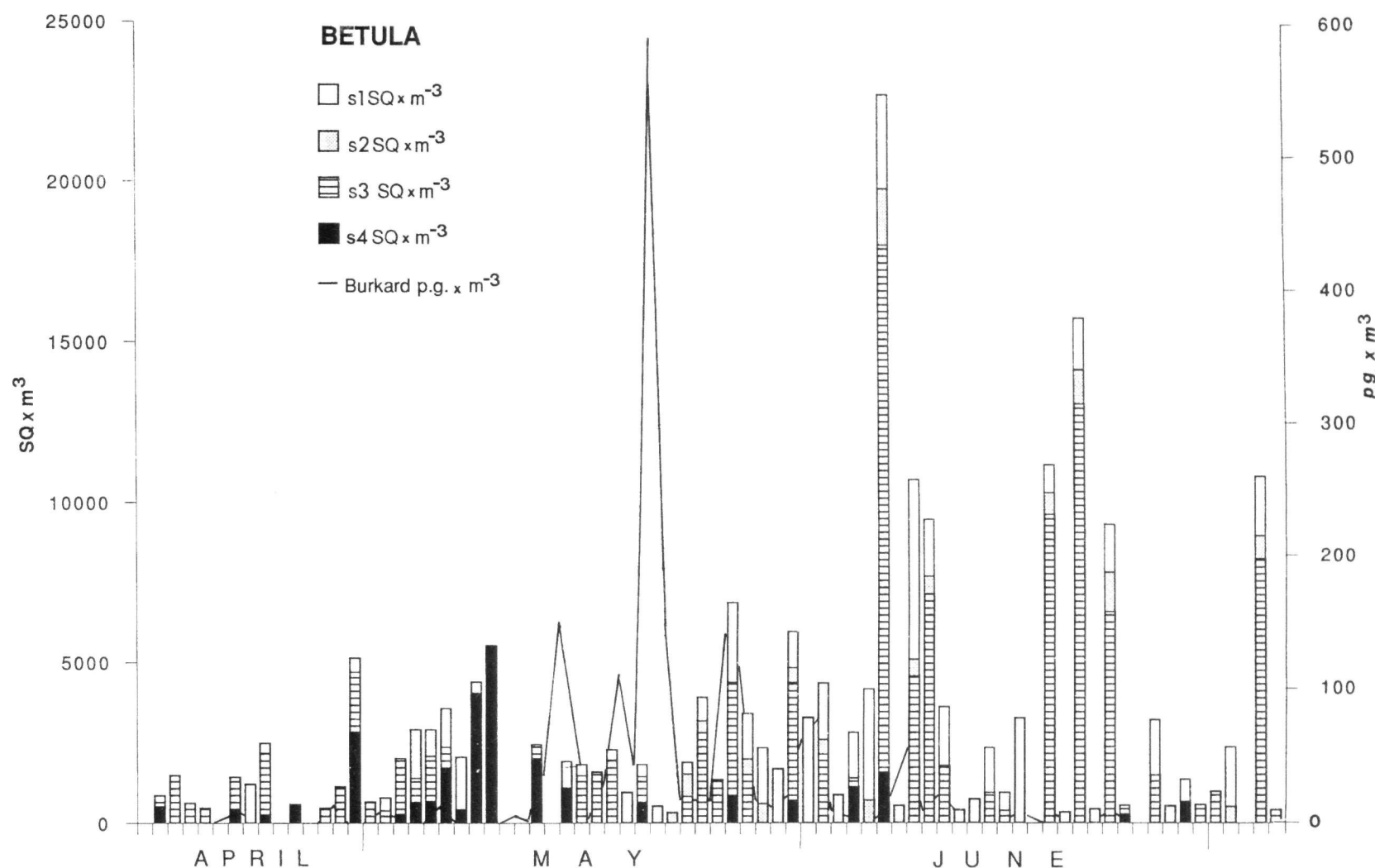


Fig. 1. Comparison between antigenic activities of birch (*Betula*) pollen in air samples (stages 1–4 in SSBAS; bars, in SQ units/m³) and airborne birch pollen counts per cubic meter of air (continuous line, in Burkard samples) from April to June 1992. SSBAS with air-intake volume of 18.5 l/min, divides particles into four size classes stimulating the human respiratory tract: > 7.2 μ m (stage 1), 2.4–7.2 μ m (stage 2), < 2.4 μ m (stage 3), and molecular size (stage 4). Pollen counts were obtained from continuously operating Burkard trap (p.g.: pollen grain).

sizes, and to compare these changes with prevailing background birch pollen counts before, during, and after the peak pollen period.

Methods

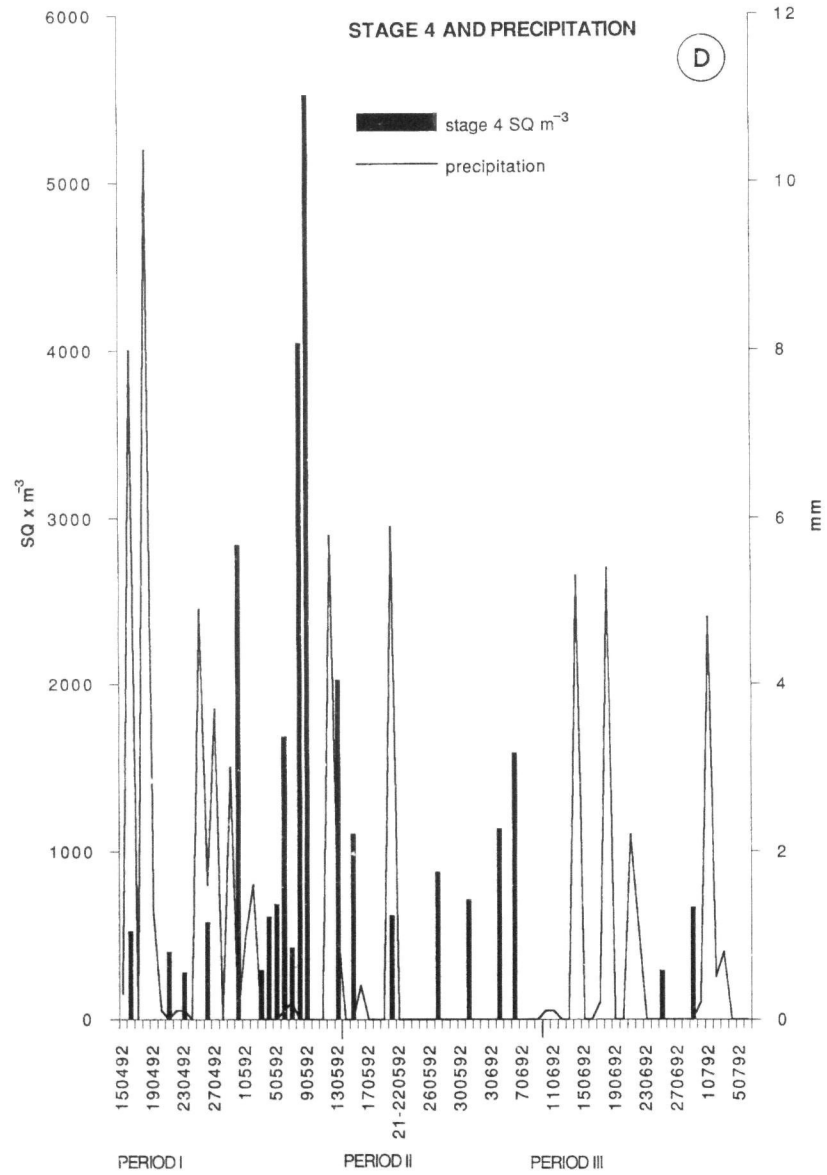
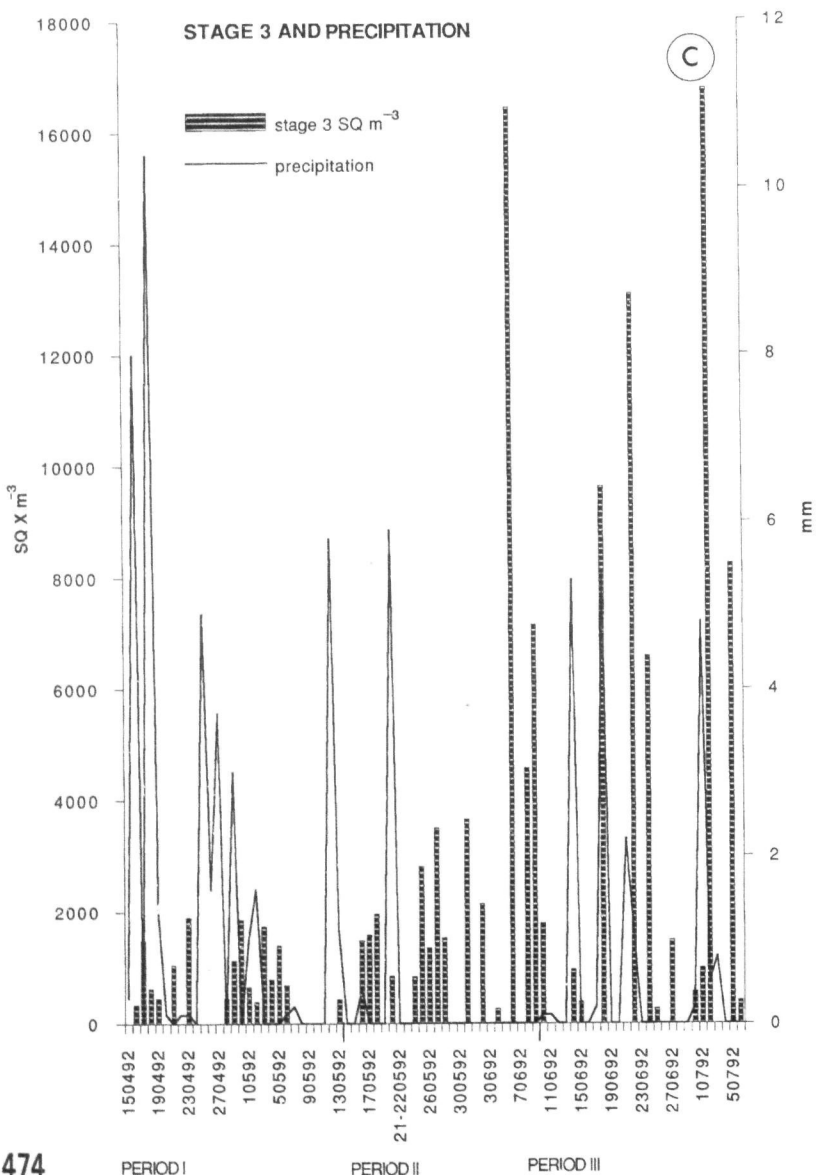
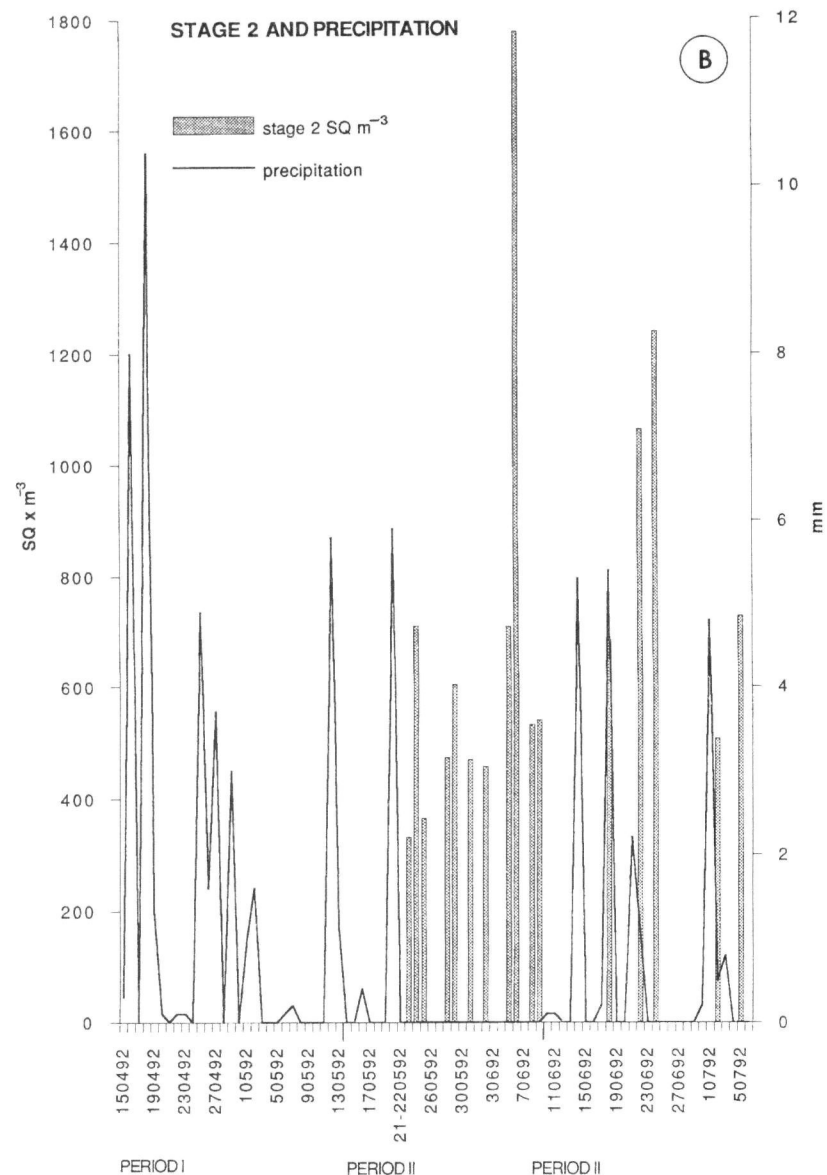
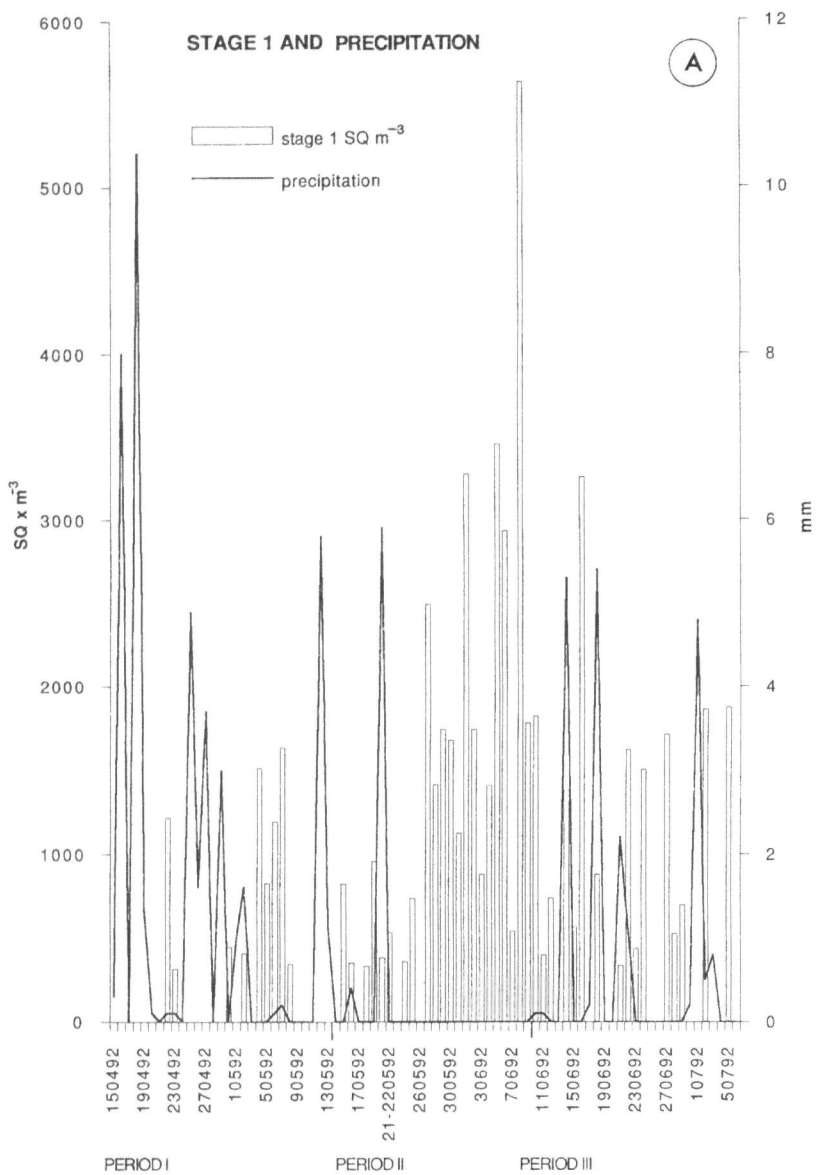
The size-selective bioaerosol sampler (SSBAS) (8) simulates the human respiratory tract in air-intake volume (18.5 l/min) and particle fractionation. It divides particles into four size classes by aerodynamic diameter, as follows: > 7.2 μ m (filter stage 1), 2.4–7.2 μ m (filter stage 2), < 2.4 μ m (filter stage 3), and submicronic antigen-bearing particles, collected in a liquid-cooled (–20°C) condenser unit (stage 4).

The filters (Millipore, type HA; pore size 0.7 μ m in stage 1, 0.45 μ m in stages 2 and 3) were changed daily and stored at –50°C until analyzed. The sample in stage 4 was first thawed and then refrozen on the walls of a round-bottomed flask in a thin layer. The sample was then lyophilized and stored at –50°C in a glass tube.

All air samples from the SSBAS were analyzed by a modification of ELISA (enzyme-linked immunosorbent assay) (12). The filter disks for the procedure were obtained by cutting the filters with a 5-mm cork borer. The disks were immersed in 125 μ l phosphate-buffered saline (PBS, pH 7.4) in microtiter plates (Costar Co), one per well, and incubated overnight at 37°C to allow released birch pollen antigens to bind to the solid phase. The filter disks were then collected and, the microtiter plates were washed three times with PBS, incubated in 125 μ l PBS-BSA-Tw (containing 0.5% BSA + 0.5% Tween-40) 1 h at 37°C, and again washed with PBS. The lyophilized samples were dissolved in 1.5 ml PBS, divided into 125 μ l aliquots (four duplicates), and incubated overnight at 37°C like the filter disks.

The bound birch antigen was detected with 125 μ l/well antibodies against *Betula* pollen produced in rabbit (diluted 1:1000 in PBS-BSA-Tw; ALK, Copenhagen, Denmark). Normal rabbit serum was used as a control. After 2 h incubation at 37°C, the

Fig. 2. A–D. Daily variations in birch pollen antigenic activities in SSBAS air samples (stages 1–4) and precipitation (mm) in three periods: I: 15 April–13 May; II: 14 May–8 June; III: 9 June–6 July, 1992.



plates were washed with PBS. Then HRP (horse-radish peroxidase)-conjugated swine antibodies against rabbit immunoglobulins (DAKO) (100 µl/well of 1:1000 dilution in PBS-BSA-Tw) was added and incubated overnight at room temperature. After three washes with PBS, the enzyme activity of the bound antirabbit-IgG-HRP conjugate was determined by adding *o*-phenyldiamine substrate diluted in citrate buffer (45 mg *o*-phenyldiamine in 15 ml citrate buffer pH 5.5 plus 10 µl H₂O₂) 75 µl/well, incubated at room temperature for about 30 min. The reaction was stopped by adding 125 µl of 1 M HCl.

The optical absorbance was measured at 492 nm by Multiscan[®] microplate reader (Flow Lab.). All filter samples were analyzed in four duplicates. Control filter disks of both pore sizes were used to correct the absorbances (as blanks). The birch pollen extract used for standards was *Betula verrucosa* extract of 100 000 SQ units/ml (ALK, Copenhagen, Denmark). Dilutions of 1:1000, 1:5000, 1:10 000, 1:100 000, and 1:500 000 of birch pollen extracts were used. The antigenic activities of each stage were converted to correspond to SQ units/m³ of air with the intake air volume measured in the flow-control unit of the SSBAS.

For comparison, airborne pollen counts (daily averages per cubic meter of air) were obtained from a continuously operating Burkard trap (Burkard, Rickmansworth, UK), which was situated beside the SSBAS on the roof of a Turku University building (Turku, Finland), 15 m above ground level, approximately 1 km from the town center.

The sampling period was 15 April–6 July 1992, divided into three subperiods as follows: period I, defined as the time up to the point when 1% of the

yearly birch pollen total was reached, extended over the 29 d preceding the peak pollen season; period II, the peak season, lasted for 26 d, from 14 May until 8 June, when a level of 1% of the yearly total was again reached; period III, following the pollen season, was 28 d in duration.

Variations in the birch pollen antigen concentrations were compared with simultaneous airborne pollen counts and with several meteorologic factors. These included *mean temperature* (same day (d), second day before the day of measurement (d-2), and the 3 days combined (d-2, d-1, d)) and *mean precipitation* (daily, second preceding day, and the 3 days combined). Likewise, sun hours, relative humidity, and wind velocity were compared with antigenic activities. All meteorologic data were from the statistics compiled by the meteorologic institute (Turku airport, approximately 6 km away).

Correlation analyses were performed with SAS GLM procedures (13).

Results

Antigenic birch pollen activity was found in all four SSBAS stages. The concentrations were clearly highest at stage 3, which represented an average of 52% of the total activity. The activity was particularly high in the late season after the peak pollen period. Rather high activities were found at stage 4 in the condensed subsample preceding the peak period (Fig. 1). Only some 30% of total activity was detected at stage 1, where intact pollen grains are trapped. Activities at stage 1 were obtained already during period I, before the pollen season, but the highest values were found during the pollen peak (period II) in dry weather (Fig. 2). No antigenic

Table 1. Correlation coefficients among stages (1–4) and meteorologic factors in three periods, 1992. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$

	Before season (period I)				During peak season (period II)				After season (period III)			
	Stage 1 <i>n</i> =27	Stage 2 <i>n</i> =26	Stage 3 <i>n</i> =25	Stage 4 <i>n</i> =26	Stage 1 <i>n</i> =25	Stage 2 <i>n</i> =25	Stage 3 <i>n</i> =17	Stage 4 <i>n</i> =23	Stage 1 <i>n</i> =24	Stage 2 <i>n</i> =24	Stage 3 <i>n</i> =17	Stage 4 <i>n</i> =24
Stage 1			0.129	0.107		0.432*	0.297	0.137		0.424*	0.633**	-0.132
Stage 2							0.799***	0.327			0.783***	-0.150
Stage 3				0.093				0.653**				-0.260
Burkard	0.223		0.090	-0.092	-0.115	-0.208	-0.090	-0.087	0.191	-0.065	-0.094	-0.081
Mean temp	0.331*		0.080	0.296	0.589**	0.354	0.206	0.145	-0.014	-0.516**	-0.700**	0.076
3-d Mean temp	0.392*		0.129	0.481*	0.553**	0.337	0.108	-0.040	0.283	-0.292	-0.087	0.003
Temp 2 d before	0.381*		0.086	0.443*	0.512**	0.320	0.120	-0.055	0.250	-0.225	0.002	0.026
Mean precip	-0.247		-0.118	-0.194	-0.153	-0.139	-0.118	0.153	-0.056	0.077	0.089	-0.135
3-d Mean precip	-0.339*		-0.076	-0.257	-0.356*	-0.116	-0.133	0.119	0.117	-0.030	0.157	-0.181
Precip 2 d before	-0.259		-0.176	-0.288	-0.308	-0.090	-0.038	-0.055	0.547**	0.002	0.243	-0.104
Mean sun hours	0.517**		0.201	0.297	0.017	-0.052	-0.314	0.047	0.144	0.041	-0.231	0.120
3-d Mean sun hours	-0.419*		-0.112	-0.298	0.426*	0.297	0.097	0.023	-0.058	-0.353*	-0.486*	0.316
Mean rel hum	-0.415*		-0.109	-0.142	-0.428*	-0.245	-0.049	-0.253	-0.003	-0.045	0.202	-0.283
3-d mean rel hum	-0.389*		0.194	-0.239	-0.568**	-0.334	-0.110	-0.085	-0.059	0.028	0.347	-0.251
Rel hum 2 d before	-0.092		-0.201	-0.117	-0.486*	-0.318	-0.182	0.047	-0.066	-0.025	-0.230	-0.247
Wind	-0.035		-0.072	-0.432*	-0.281	-0.322	-0.264	-0.315	0.066	-0.041	0.063	-0.393*

activity was detected in stage 2 before the pollen season (period I).

In stage 1, correlations with temperature, sun hours, and relative humidity were obtained; in stage 4, correlations with temperature were observed before the pollen season, but not later (Table 1).

Antigenic activities were not dependent on precipitation of the same day, but activity in stage 1 showed some negative correlation with the mean precipitation of the last 3 days (the 2 preceding days and the same day) or second day earlier (Table 1).

Discussion

An IgG-based method was selected because it requires lower antigen concentrations than IgE-based analyses, where a sampling system based on a high-volume sampler is needed.

For allergic subjects, it is especially important to know that a prominent increase in airborne birch pollen antigen concentrations occurs before the birch pollination period. In the size class of 7.2–2.4 μm , hardly any antigenic activity was recorded; this means that the antigenic particles were either large or very small ($<2.4 \mu\text{m}$). It was particularly worth noting that activities were highest in the submicronic particle sizes (SSBAS stages 3 and 4), which can penetrate deep into the human respiratory tract. This explains allergic symptoms and asthmatic reactions before pollination. For instance, such material may be shed from birch catkins. Antigenic material similar to that found in birch pollen has been detected in the buds, leaves, and bark of birch, especially during flowering (4), and these particles may also be caught by the particle samplers. No antigenic activity has been detected in overwintered catkins (4). Thus, the antigenic material has to come from living trees.

Birch pollen allergens share allergenic determinants with other Betulaceae tree pollens, such as those of alder and hazel (10). The ELISA analysis in use should therefore be tested for these allergens also, to detect possible cross-reactions (especially because it is known that both alder and hazel pollinate before birch), and the conclusions arrived at in this study on the importance of birch pollen antigen concentrations before the birch pollen season should be confirmed.

Even though the activities in stage 1 were not intercorrelated with Burkard pollen counts, the same meteorologic factors which affect pollen release and dispersal, i.e., temperature (positive correlation) and relative humidity (negative correlation), were important during the peak pollen period. After this period, however favorable these factors may be, they can no longer cause pollen shedding. No correlations were

seen in stages 2 and 3 before and during the season. In stage 4, a correlation with temperature was observed before the season. It is possible that the warming of the plant promotes the release of antigenic material before the onset of the pollen season. In warm weather, with upward air movements, these particles may be wafted into higher air layers, and when carried by water molecules they may be trapped in the small-size fraction.

Wetting was an important factor; moisture is presumed to affect the release of antigens, even if this has not yet been proved. Positive correlations with precipitation were obtained for stage 4 during the season, a finding which offers some support for the theory of carriage by water microdroplets. The issue should be studied with, for instance, scanning electron microscopy with different relative humidities under artificial conditions.

The reason that even antigen concentrations in stage 1 did not correlate with the Burkard counts is that the pollen grains are often free of antigen material. They are resuspended in the air from surfaces or may be transported over a long distance and without any activity left.

In sedimentated outdoor dust, antigenic properties remain high much longer than the time during which pollen grains are airborne (11). It is also possible that during a long airborne stage pollen grains release their antigenic content, which continues to fly in another form without the pollen wall.

Meteorologic data obtained from the airport were sufficient, since the most interesting particles remain airborne for a long period and are not dependent on highly localized meteorologic factors (1, 2).

When deposited on the mucous membranes of the human respiratory passages, pollen immediately releases allergenic substances. Baraniuk et al. (3) suggest that rapid sequential release of pollen factors with high initial local solute concentrations and osmolalities is a characteristic feature of pollen hydration on mucous surfaces and in the natural environment, as indicated by studies performed in pollen columns. Although the antigenic properties of allergenic extract components have been characterized, the natural functions of these molecules remain unknown (9). Whole pollen grains may move from the nasal area to the trachea. Smaller antigenic particles can penetrate deeper.

It will be quite difficult to develop methods of predicting airborne antigen concentrations on the basis of meteorologic factors because the only size class which clearly reacted to meteorologic changes was the largest one, which contains the intact pollen grains. Small size classes, which are allergologically more important, seem to be relatively independent of meteorologic changes.

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