

Seasonal appearance of grass pollen allergen in natural, pauci-micronic aerosol of various size fractions. Relationship with airborne grass pollen concentration

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Summary

In a study during the 1993 grass pollen season at Leiden, the relationship between atmospheric pollen allergen carried by five size fractions of pauci-micronic (few microns) particles and the grass pollen count was investigated. Sampling was carried out on dry days, and atmospheric pollen allergen in the particle fractions was assessed by a RAST-inhibition assay while grass pollen quantities were measured with a volumetric pollen trap. It appears that the atmospheric presence of grass pollen allergen in all size fractions is restricted mainly to the period of presence of grass pollen grains. Before and after the grass pollen season atmospheric grass pollen allergen quantities are generally very low. It is concluded that a routinely performed grass pollen count is a reliable measurement for the estimation of the amount of atmospheric grass pollen allergen, also in the pauci-micronic particle fraction.

Clinical and Experimental Allergy, Vol. 25, pp. 234–239. Submitted 7 March 1994; revised 7 June 1994; accepted 17 June 1994.

Introduction

The airborne presence of pollen allergens outside the pollen grains has been demonstrated for several pollen types, such as ragweed (*Ambrosia*) [1–3], birch (*Betula*) [4], oak (*Quercus*) [5], Japanese cedar (*Cryptomeria*) [6], and grasses (*Poaceae*) [7–11]. In some of these studies, using size fractionating sampling devices, the allergenic activity was shown to be carried by particles measuring a few microns or less, i.e. of pauci- or even sub-micronic sizes [1–6,10,11].

The nature and origin of these smaller micronic pollen-allergen carrying particles is not yet known. It has been suggested [11] that osmotic bursts of humidified rye-grass pollen grains creates a natural aerosol of starch granules of pauci-micronic size, carrying a specific allergen *Lolium perenne* (*Lol p V*), which is responsible for the rise in airborne grass pollen allergen following episodes of rain [7,8]. This finding implies a more or less direct relationship between the airborne presence of pollen grains and allergen carrying micronic particles. On the other hand,

for ragweed [3] and for oak [5] the presence of pollen allergen outside, particularly after the pollen season has been reported, incriminating non-pollen plant parts as possible origin of the airborne allergenic activity, just as has been described for *Parietaria* and cocksfoot [12]. Also, the possibility of transfer from pollen grains to environmental (pauci-)micronic particles, by an extraction process under humid conditions, has been put forward [13]. This study does not aim at an answer to these questions about the origin of the small particles, as it was limited to dry weather conditions.

Worldwide, in many places, airborne quantities of allergenic pollen grains are monitored, to collect knowledge about levels of allergen to which pollen allergic patients are exposed to by inhalation. Because in Europe, grass pollen is the major cause of pollinosis, it is of great clinical interest to determine the airborne presence of grass pollen allergen in relation to the atmospheric quantities of grass pollen grains. More specifically, it is important to know if grass pollen allergen carried by particles other than grass pollen grains is present in the outdoor air outside the grass pollen season, in significant amounts capable of provoking hay fever symptoms.

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Methods

The methods used for measuring airborne quantities of pollen grains and for assessing the presence of airborne grass pollen allergen are similar to those described *in extenso* earlier [10]. Therefore, only a summary of these techniques is presented here, together with the minor changes.

Particle sampling

Particle sampling was carried out on the roof of the Leiden University Hospital building which is five storeys high, in the west of the city of Leiden, in a semi-urban environment, with private houses with gardens and university facilities with parks, and with some wasteland.

As usual, airborne pollen-grain concentrations, expressed as the mean number of pollen grains per m³ over periods of 24 h, were measured continuously with the volumetric Burkard pollen trap (Rickmansworth, UK). To collect the airborne particles to be studied for their allergen carrying properties, a high volume air sampler (Ecotech Model 2000, Blackburn, Victoria, Australia) was used. This instrument has the same sampling characteristics as the one used in the earlier observation [10]. To be able to detect relatively small amounts of allergen, the running time was extended from approximately 8 to approximately 12 h compared with the earlier study. Sampling with the high volume sampler was carried out on 18 occasions, covering the grass pollen season from beginning to end, on days with dry weather to prevent the intake of rain or fog droplets and thus the wetting of the impaction filters.

A five-stage cascade impactor (Andersen Instruments Inc., Atlanta, GA, USA) was mounted on top of the high volume sampler. Also as described earlier, with the flow rate of the air sampler set on 1.13 m³ min⁻¹, the size fractions containing 80% of the mass were: Stage I: >10 µm; stage II: 4.9–10 µm; stage III: 2.7–4.9 µm; stage IV: 1.3–2.7 µm; and stage V: 0.6–1.3 µm. For this study the back-up filter underneath the five impaction stages was left out of consideration.

The impaction strips were cut, and placed in the cascade impactor, again as described earlier [10], from sheets of glass fibre with hydrophilic properties (GF 50; Schleicher & Schuell, Dassel, Germany), necessary for the extraction, incubation and elution of the allergen in the allergen measuring procedure.

Measurement of allergen

The glass-fibre impaction strips need to be hydrophilic to

allow moistening, and thus extraction of the allergen from the impacted dust particles, and release of the allergen into the strip. After drying, the allergen in the strip was incubated with specific anti-grass-pollen IgE containing pooled serum during a descending elution procedure, exactly as described earlier [10]. Detection of grass pollen allergenic activity was established by blocking of anti-grass pollen IgE antibodies by the allergen in the strip, and subsequent measuring of remaining specific IgE reactivity in the eluate by RAST ('RAST-inhibition'), using *Dactylis* pollen allergen discs (G3; Kabi Pharmacia Diagnostics AB, Uppsala, Sweden). Levels of specific anti-grass-pollen IgE antibodies in the serum pool without prior application to, and descending elution from the strip (minimum inhibition), non-specific binding of these IgE antibodies to clean, un-exposed strips (blank inhibition) and baseline, non-specific inhibition by serum without anti-grass pollen IgE antibodies were routinely performed, and used for the calculation of the percentage RAST-inhibition as a measure of the (relative) amount of allergenic activity on the strips. Minor changes in the earlier described incubation and elution technique, which were caused by the use of a different type of impaction glass-fibre substrate, were the application on the strip of 50 µl (instead of 100 µl) serum, the collection of 1.0 ml (instead of 0.5 ml) eluate from the strip, and the longer elution time of 6 h (instead of 60 min). To enhance and standardize elution time and conditions, this set up of the laboratory equipment was covered by vapour tight glass containers to keep air humidity around the strips at a constantly high level.

Results

Pollen quantities

In the Netherlands, the 1993 grass pollen season, as assessed with the pollen trap at the University Hospital Leiden, was characterized by an early start from mid-May, and by an extraordinarily high and rather early peak period during the first 11 days of June (Fig. 1a). The second half of June showed relatively low airborne grass pollen concentrations, whereas in July, August, and September the levels were decreasing and low, as is usual for the west European grass pollen season.

Pollen grains were also collected by the high volume air sampler which was located at 2 m distance from the pollen trap. The airborne grass pollen concentrations (numbers of grains per m⁻³) during the operation of the high volume sampler were derived from the trap measurements, and served for the calculation of the quantity of pollen grains collected by the high volume sampler. Sampling was performed on 18 occasions between the

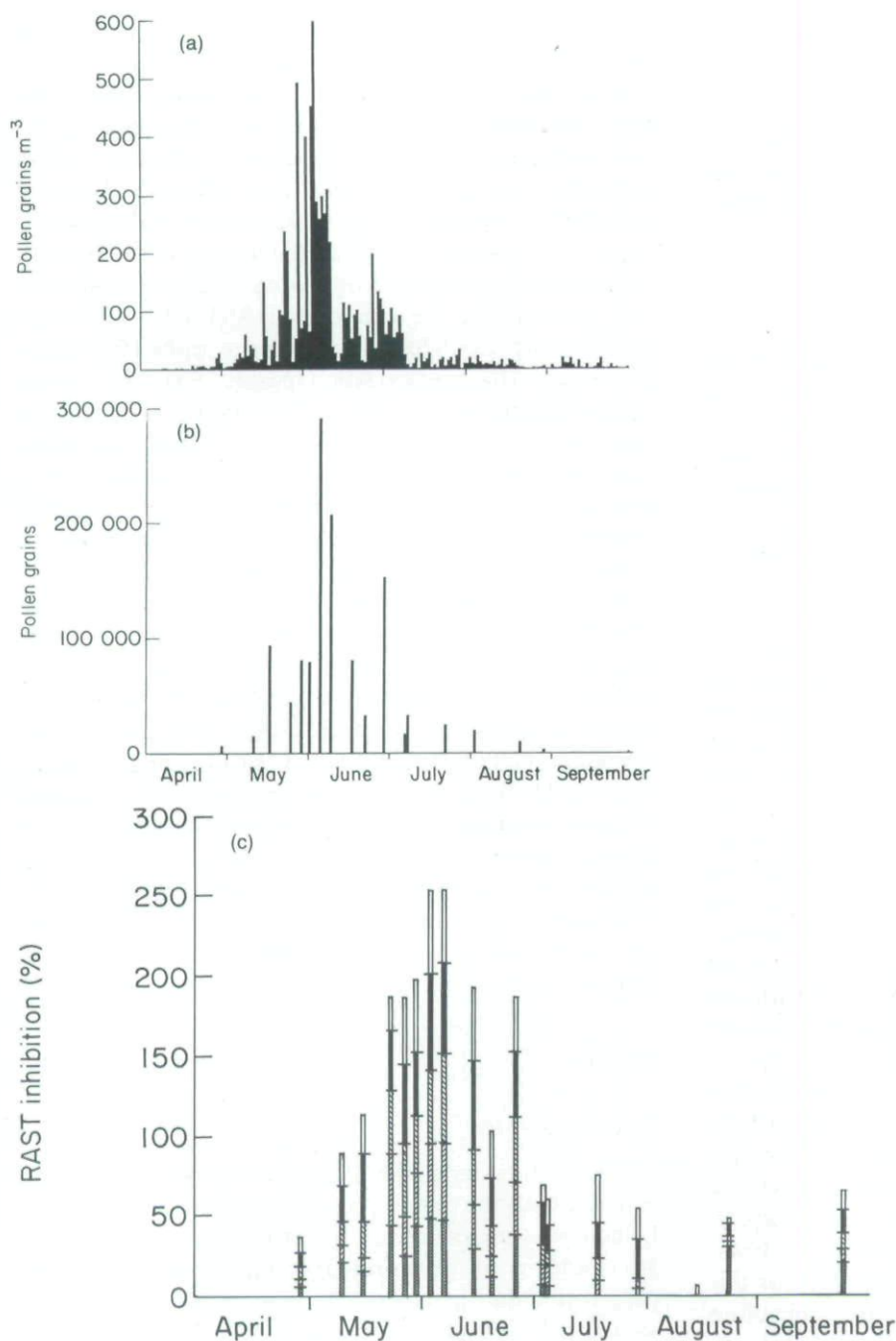


Fig. 1. Seasonal appearance of airborne grass pollen and grass pollen allergen, at Leiden, in 1993. (a). Daily airborne grass pollen concentration. (b). Calculated quantities of grass-pollen grains collected by the high volume sampler, on 18 occasions, before, during and after the grass pollen season. (c). Sums of RAST-inhibition percentages as measure for relative amounts of grass pollen allergen in five size fractions (I–V) of atmospheric dust, presented as stacked bars. □. Stage I. ■. Stage II. ▨. Stage III. ▩. Stage IV. ▧. Stage V.

end of April and of September, with intervals of 3–7 days during the main grass pollen season, and of 12–20 days after the main season. The sampling times and durations, and the calculated quantities of collected pollen grains are given in the left part of Table 1. For this calculation, that time area of the pollen-sampler slide is counted which corresponds with the operation time of the high volume air sampler, and then the pollen count is multiplied by the volume of air (m³) sampled. These quantities of grass pollen grains are also shown in Fig. 1(b). It is

obvious that these quantities, collected by the high volume sampler during a part of the day, do not always have the same ratio to the average airborne grass-pollen concentrations during 24 h. But generally there appears to be a good relationship ($r = +0.89$) between the amounts of grass pollen grains collected by the pollen trap and those collected by the high volume sampler. Due to their aerodynamic size, grass pollen grains will be accumulated selectively in the top stage I of the cascade impactor [10].

Airborne allergen

The relative amounts of grass pollen allergen in the atmospheric dust material collected in the five impaction stages of the cascade impactor, measured as RAST-inhibition percentages, are given in the right half of Table 1, and shown in Fig. 1c. To be able to show the results of the assessment of pollen-grain quantities and relative allergen amounts on an equal time scale, the sum of the RAST-inhibition percentages of the five stages is presented in stacked bars, so that the theoretical maximum values of 100 may be exceeded. From the results shown in Fig. 1 it becomes clear that the amount of atmospheric grass-pollen allergen is very similar to the airborne grass-pollen concentration. This appears to be true not only for the bigger particle dust fraction of stage I which contains the pollen grains, but there is also a strong correlation between the airborne pollen concentration and the amount of allergen in the dust of the pollen free pauci-micronic size fractions collected in stages II to V of the cascade impactor. The coefficients of correlation between the quantities of

collected pollen grains and the amount of allergen in the five dust fractions are given at the bottom of Table 1. Figure 2 shows two examples of these correlations, for stage I, containing the pollen grains; and for stage IV with small, pauci-micronic particles of 1.3–2.7 μm . For the other stages the results are very similar. It is also clear from Fig. 1 that before and after the main grass pollen season, in April, and in August and September, atmospheric grass pollen allergen is not completely absent on days with dry weather conditions.

Discussion

The results of the observations on the airborne presence of grass pollen allergenic activity presented here, confirm and corroborate the earlier findings with only two samplings [10]. One of the questions raised in that earlier discussion was whether a relationship exists between the airborne allergen levels and the airborne pollen concentration.

The aim of this study was to investigate the seasonal

Table 1. Dates and durations of high volume air samplings, calculated quantities of collected grass pollen grains, and RAST-inhibition percentages as measure for relative amounts of grass pollen allergen in five size fractions of atmospheric dust, with the coefficients of correlation (r) between the quantities of pollen grains and RAST-inhibition percentages

Sampling	Date 1993	Duration of sampling (h:min)	Calculated quantity of collected pollen grains	RAST-inhibition percentages in dust in five cascade impactor-stages				
				I >10	II 4.9–10	III 2.7–4.9	IV 1.3–2.7	V 0.6–1.3
1	28/29 April	18:20	6 600	10	9	7	6	5
2	11 May	11:30	14 850	19	22	16	10	21
3	17 May	10:45	94 050	25	43	23	8	15
4	25 May	7:10	44 550	22	37	40	44	44
5	29 May	11:40	80 850	43	48	46	24	25
6	1 June	11:30	79 200	45	39	37	33	43
7	5 June	10:20	292 050	53	59	46	47	48
8	9 June	11:45	207 900	47	57	56	48	47
9	17 June	11:10	80 850	48	53	36	28	28
10	22 June	12:15	33 000	32	28	20	13	11
11	29 June	12:45	153 450	36	40	41	33	37
12	7 July	11:55	16 500	11	27	13	11	7
13	8 July	11:40	33 000	16	16	4	18	6
14	22 July	12:25	24 750	31	21	14	9	0
15	2 August	11:40	19 800	20	24	6	4	0
16	19 August	11:45	9 900	5	1	0	0	0
17	28 August	11:05	3 300	5	6	4	3	30
18	29 Sept.	11:55	1 650	13	13	11	8	20
Correlation coefficient (r):				+0.78	+0.81	+0.79	+0.79	+0.69
Level of significance (P):				<0.001	<0.001	<0.001	<0.001	<0.002

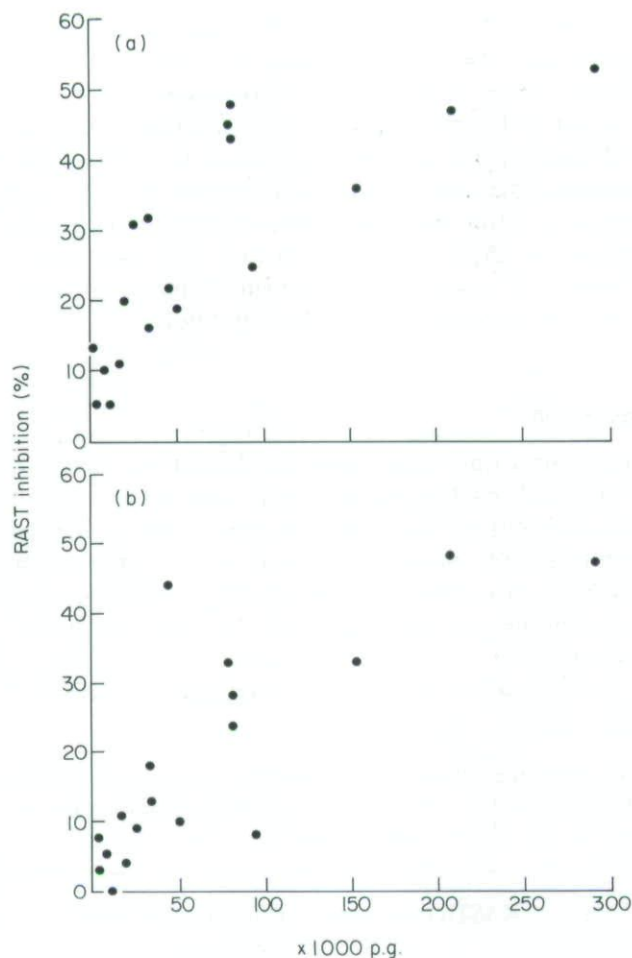


Fig. 2. Two examples of the relationship between RAST-inhibition percentages as measure for relative amounts of grass pollen allergen in atmospheric dust collected on stage I (particle sizes $> 10 \mu\text{m}$) and on stage IV (particle sizes $1.3\text{--}2.7 \mu\text{m}$) of a cascade impactor, and different quantities of collected grass pollen grains (p.g.), on 18 occasions before, during and after the grass pollen season. (a) Stage I, $r = +0.78$. (b) Stage IV, $r = +0.79$.

course of the atmospheric grass pollen allergen amount in relation to the airborne grass pollen concentration, in order to judge the value of the routinely performed, so-called pollen count as a measure for atmospheric pollen-allergen amounts. It is not surprising that such a relationship ($r = +0.78$) exists for the dust fraction collected in stage I of the cascade impaction, where the pollen grains are accumulated (Fig. 2). But it is noteworthy that these same high levels of relationship also exist between quantities of pollen grains and allergen amounts in the pollen-free, pauci-micronic fractions collected in stages II to V. For a biological phenom-

enon, the relationship between quantities of airborne pollen grains and atmospheric pollen allergen amounts in all size fractions is remarkably high, and the similarity in the seasonal courses (Fig. 1) is also rather great. This is illustrated, for instance, by the simultaneous drop in both the airborne pollen concentration and the atmospheric allergen amount on 22 June (sampling nr. 10).

Shortly before, and long after the main grass pollen season, during sampling nr. 1 on the night of 28–29 April, and more evidently during sampling nrs 16, 17 and 18 in August and September, atmospheric grass pollen allergen is not completely absent, but the level is rather low in all size fractions (except in stage V, after the season). These low levels of natural allergen exposure are probably too low to provoke symptoms of pollinosis. The conclusion is that, within an acceptable range of biological variation, the routinely performed assessment of airborne grass pollen concentration is a reliable measurement of the amount of atmospheric grass pollen allergen. This also holds for the smaller size, pauci-micronic dust fraction, which is thought to be capable of penetrating into the lower regions of the respiratory tract [10].

This conclusion is not in agreement with those of Fernandez-Caldas *et al.* [5] for atmospheric oak-pollen allergen, and of Rantio-Lehtimäki *et al.* [4] for birch-pollen allergen. Possibly, the difference in the conclusion is caused by the difference in the length of the periods considered, and with the amount of variations in both the pollen counts and allergen assessment which is accepted as not essential. It is true that both for the grasses and the birches the relationship between atmospheric pollen concentrations and allergen amounts during the pollen season is not complete on every individual day, and big aberrations are sometimes observed. It is also true that both for the grasses and the oaks there are pollen-allergen carrying particles in the atmosphere after the pollen season is over. But the main conclusion is that high atmospheric pollen allergen levels in all the size fractions are observed only within the pollen season itself, and predominantly on days with a high pollen count.

In the observations presented here, no special attempt was made to collect atmospheric dust immediately after an episode of rain, under which condition other authors [7,8,11] found high levels of allergen under low pollen-concentration conditions. So, this potential controversy continues. The question of the nature and origin of the pauci-micronic allergen carrying particles, particularly during dry weather conditions, cannot be answered by the observations presented here. The three main hypotheses are: (i) allergen carriage by starch granules released in the atmosphere after osmotic rupture of pollen grains

[11]; (ii) airborne presence of non-pollen plant parts carrying small amounts of pollen allergen [3,12]; (iii) transfer of allergen from pollen grains to natural dust by some extraction and dispersion process [13]. The turbulent air in the lower layers of the atmosphere is always loaded with particles, mostly of pauci-micronic and sub-micronic sizes. Apparently, a portion of these particles is carrying pollen allergen particularly during the main pollen season. In the period after the main pollen season, when airborne pollen concentrations become low, this portion of allergen carrying pauci-micronic particles also becomes low, but remains present for some time. The fact that the allergen is apparently distributed over particles of several size fractions makes the third hypothesis of transfer to natural dust more likely than atmospheric carriage by more specific non-pollen plant parts or starch granules, which have a narrower range of pauci-micronic sizes.

Acknowledgements

The gift by Kabi Pharmacia Nederland bv of reagents for the RAST-inhibition procedure is gratefully acknowledged. The authors thank Dr P. S. Hiemstra for critical comments, Mr P. van Noort for providing the drawings, and Mrs C. C. Zuiderduin for the preparation of the manuscript.

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