

Concentrations of major grass group 5 allergens in pollen grains and atmospheric particles: implications for hay fever and allergic asthma sufferers sensitized to grass pollen allergens

G. F. SCHÄPPI*, P. E. TAYLOR*, M. C. F. PAIN†, P. A. CAMERON‡, A. W. DENT§, I. A. STAFF* and C. SUPHIOGLU¶

*Pollen and Allergen Research Group, School of Botany, The University of Melbourne; Departments of †Thoracic Medicine, and ‡Emergency, The Royal Melbourne Hospital; §Emergency Department, St Vincent's Hospital; and ¶Department of Allergy and Clinical Immunology, Monash University Medical School, Alfred Hospital, Melbourne, Australia

Summary

Background Grass pollen allergens are the most important cause of hay fever and allergic asthma during summer in cool temperate climates. Pollen counts provide a guide to hay fever sufferers. However, grass pollen, because of its size, has a low probability of entering the lower airways to trigger asthma. Yet, grass pollen allergens are known to be associated with atmospheric respirable particles.

Objective We aimed (1) to determine the concentration of group 5 major allergens in (a) pollen grains of clinically important grass species and (b) atmospheric particles (respirable and nonrespirable) and (2) to compare the atmospheric allergen load with clinical data to assess different risk factors for asthma and hay fever.

Methods We have performed a continuous 24 h sampling of atmospheric particles greater and lower than $7.2\ \mu\text{m}$ in diameter during the grass pollen season of 1996 and 1997 (17 October 1996–16 January 1997) by means of a high volume cascade impactor at a height of about 15 m above ground in Melbourne. Using Western analysis, we assessed the reactivity of major timothy grass allergen Phl p 5 specific monoclonal antibody (MoAb) against selected pollen extracts. A MoAb-based ELISA was then employed to quantify Phl p 5 and cross-reactive allergens in pollen extracts and atmospheric particles larger and smaller than $7.2\ \mu\text{m}$.

Results Phl p 5-specific MoAb detected group 5 allergens in tested grass pollen extracts, indicating that the ELISA employed here determines total group 5 allergen concentrations. On average, 0.05 ng of group 5 allergens were detectable per grass pollen grain. Atmospheric group 5 allergen concentrations in particles $>7.2\ \mu\text{m}$ were significantly correlated with grass pollen counts ($r_s = 0.842$, $P < 0.001$). On dry days, 37% of the total group 5 allergen load, whereas upon rainfall, 57% of the total load was detected in respirable particles. After rainfall, the number of starch granule equivalents increased up to 10-fold; starch granule equivalent is defined as a hypothetical potential number of airborne starch granules based on known pollen count data. This indicates that rainfall tended to wash out large particles and contributed to an increase in respirable particles containing group 5 allergens by bursting of pollen grains. Four day running means of group 5 allergens in respirable particles and of asthma attendances (delayed by 2 days) were shown to be significantly correlated ($P < 0.001$).

Correspondence: C. Suphioglu, Department of Allergy and Clinical Immunology, Monash University Medical School, Alfred Hospital, Commercial Road, Prahran, Victoria 3181, Australia.

Conclusion Here we present, for the first time, an estimation of the total group 5 allergen content in respirable and nonrespirable particles in the atmosphere of Melbourne. These results highlight the different environmental risk factors for hay fever and allergic asthma in patients, as on days of rainfall following high grass pollen count, the risk for asthma sufferers is far greater than on days of high pollen count with no associated rainfall. Moreover, rainfall may also contribute to the release of allergens from fungal spores and, along with the release of free allergen molecules from pollen grains, may be able to interact with other particles such as pollutants (i.e. diesel exhaust carbon particles) to trigger allergic asthma.

Keywords: allergen, allergic asthma, cross-reactivity, grass pollen, hay fever, Phl p 5, respirable particles, starch granules

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Introduction

Grass pollen is the major outdoor seasonal source of airborne allergens in cool temperate climates such as southern Australia and the allergenic role and importance of grass pollen in triggering hay fever and allergic asthma is well known and extensively documented [1,2]. Up to 95% of patients allergic to grass pollen possess IgE specific for group 1 allergens (glycoproteins with a molecular mass of 32–35 kDa) and 80% for group 5 allergens (28–32 kDa), these two groups forming the major allergens of grass pollen [3]. In the cool temperate climate of the Melbourne (Australia) region, rye grass (*Lolium perenne*) pollen is more abundant than pollen from other grass species [4], especially since rye grass is grown extensively as pasture grass in this area [5].

There are different risk factors that trigger asthma and hay fever among individuals sensitive to grass pollen allergens. Recently, we found that hay fever symptoms were significantly correlated with the grass pollen counts ($P < 0.001$ for nasal, $P < 0.005$ for eye symptoms) in Melbourne [6]. Grass pollen has a low probability of entering the lower airways to trigger asthma because of its aerodynamic diameter. Yet, grass pollen allergens have been recorded in atmospheric particles smaller than pollen grains [7,8]. Unlike grass pollen grains, these micronic particles are small enough to enter the human airways [9,10] and cause allergic reactions in the distal parts of the lungs. We have previously shown that rye grass pollen grains rupture in rainwater by osmotic shock, each grain releasing about 700 allergen-loaded starch granules in the size range of 0.6–2.5 μm . Isolated starch granules contain the major allergen Lol p 5 and are capable of triggering IgE-mediated reactions in grass pollen-sensitive patients [11]. The highly similar allergenic protein in timothy grass (*Phleum pratense*) Phl p 5, has also been shown to be present in starch granules [12].

To estimate the different risk factors that trigger asthma and hay fever among individuals sensitive to grass pollen allergens, it is necessary to determine the amount of allergens associated with respirable particles as a complementary approach to counting grass pollen. This is fundamental since pollen counts may include pollen grains devoid of the allergens and thus may not represent a true allergen load. In this study we present, for the first time, the actual amount of a major allergen in single grass pollen grains of various grass species and assess the extent to which grass pollen counts represent the true airborne allergen load in the Melbourne atmosphere.

Materials and methods

Preparation of grass pollen extracts

Pollen was obtained from Greer Laboratories (Lenoir NC, USA). Perennial rye grass (*Lolium perenne*), Italian rye grass (*L. multiflorum*), timothy grass (*Phleum pratense*), Orchard grass (*Dactylis glomerata*), velvet grass (*Holcus lanatus*), fescue (*Festuca elatior*) and cultivated rye (*Secale cereale*) pollen grains were counted by means of a haemocytometer after staining with Calberla's stain. The soluble grass pollen proteins were extracted in PBS as described elsewhere [13].

Gel electrophoresis of grass pollen extracts

Samples (20 μg of proteins/well) were denatured under reducing conditions and proteins were then separated by SDS-PAGE using 15% SDS gels following established protocols [13]. Gels were either stained with Coomassie brilliant blue R250 or left unstained for electrophoretic transfer to nitrocellulose.

Immunoblot analysis

Proteins were transferred from gels to nitrocellulose (BA 0.45 μm , Schleicher und Schuell, Dassel, Germany) and incubated with purified MoAb 1D11 (Allergopharma Joachim Ganzer KG, Reinbek/Hamburg, Germany) specific to Phl p 5 at 10 $\mu\text{g}/\text{mL}$ in PBS/BSA following established techniques [13]. Antibody binding was visualized by incubation with horseradish peroxidase-labelled antimouse Ig antibody (Dakopatts, Carpinteria CA, USA), followed by the addition of 4-chloro-1-naphthol (Sigma, Deisenhofen, Germany) as the enzyme substrate.

Quantification of Phl p 5 and cross-reactive allergens

A MoAb-based two-site binding assay (Enzyme-Linked ImmunoSorbent Assay, ELISA) was used to quantify the Phl p 5 concentrations, as described previously [8].

Concentration of soluble proteins, Phl p 5 and cross-reactive proteins in grass pollen grains

The grass pollen extracts were submitted to protein assay using bovine plasma gammaglobulin as standard, allowing sensitive and accurate detection of proteins of a wide molecular weight range and ELISA as described previously [8,13,14].

Concentrations of Phl p 5 and cross-reacting allergens in airborne particles of different aerodynamic diameters

Continuous 24-h sampling of particles of different aerodynamic diameters was performed during the grass pollen season of 1996 and 1997 (17 October 1996–16 January 1997) by means of a high volume cascade impactor (GMWS-2310, General Motor Works Inc., Cleves OH, USA) at a height of about 15 m above ground in Melbourne. An air stream (1130 L/min) was drawn through fibre filters (Pallflex Co., Putnam CT, USA). Two fractions of different-sized particles were deposited: the first stage collected particles with an aerodynamic diameter above 7.2 μm ; the following stage collected particles below 7.2 μm (particle size cut off at 50% collection efficiency for spherical particles with unity mass density according to the manufacturer's specifications). Every 24 h, the filters were replaced and samples frozen at -20°C until required. The concentrations of Phl p 5 and cross-reacting allergens of these filters was quantified by ELISA after extraction from particulate filters into phosphate-buffered saline (PBS) by shaking on ice for 3 h.

Transmission electron microscopic investigation of respirable particles

Particles with an aerodynamic diameter below 7.2 μm , collected on the 25 October and 6 December 1996, were

extracted from high-volume filters by agitation in acidified 2,2-dimethoxypropane (Sigma Chemical Co., St Luis MO, USA), infiltrated for 1 week in LR Gold resin (London Resin Co., UK) with continuous rotation, polymerized and sectioned as described previously [15]. Sections were observed using a Philips CM 120 Transmission Electron Microscope (TEM). Pollen grains of rye grass were fixed and embedded for TEM according to published methods [16].

Grass pollen count

Grass pollen count was performed during the grass pollen season of 1996 and 1997 using a Burkard volumetric trap (Burkard Manufacturing Co. Ltd, Rickmansworth, UK) according to the manufacturer's instructions over a continuous 24-h sampling period. The air flow was set to 10 L/min. Airborne pollen grains were trapped on an adhesive (10% Dow Corning A280 in xylene) on a microscope slide driven by a clockwork motor. The slides were mounted in Calberla's stain and grass pollen counted by light microscopy.

Meteorological data

Meteorological data (minimum and maximum temperatures, rainfall) were obtained from the National Climate Centre, Bureau of Meteorology, Melbourne.

Patient data

Daily records of attendances at the emergency departments of two Melbourne inner city teaching hospitals, The Royal Melbourne Hospital and St Vincent's Hospital in Melbourne, were examined during the grass pollen season of 1996 and 1997 and the number of patients in whom the diagnosis of asthma was considered the principal reason for attendance was extracted. Average number of attendance was two attendances/day, ranging from none to six. Diagnoses were coded on discharge or admission using a modified ICD 9CM coding set (Victorian Emergency Minimum Dataset; Department of Human Services, Melbourne). This allowed classification of obstructive airways disease as either asthma or chronic obstructive airways disease.

Statistical analysis

The data were compiled and analysed using Microsoft Excel 5.0 (Microsoft Corp., Redmond WA, USA) or Minitab 10 Xtra (Minitab Inc., State College PA, USA). Correlation analysis for nonparametric data used Spearman's Rank Correlation Coefficient. The level of significance was assessed between $P < 0.01$ and $P < 0.001$.

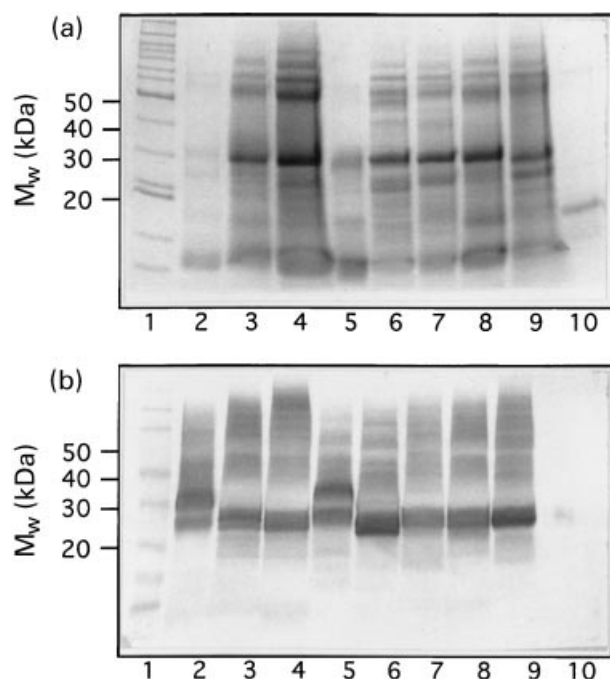


Fig. 1. SDS-PAGE of affinity purified Phl p 5 (lane 2), perennial rye grass (3), Italian rye grass (4), timothy (5), orchard grass (6), velvet grass (7), fescue (8), cultivated rye (9) and white birch (lane 10, control) pollen extracts, stained with Coomassie brilliant blue R250 (a), and electrophoretically transferred to nitrocellulose and incubated with purified MoAb 1D11 specific to Phl p 5 (b).

Results

Gel electrophoresis and immunoblot analysis of grass pollen extracts

MoAb 1D11 detects not only Phl p 5 but also other group 5 allergens in all of the tested grass pollen extracts (Fig. 1). We therefore, further in the text, refer to Phl p 5, and the cross-reacting allergens, as group 5 allergens collectively. Affinity purified Phl p 5 was used as reference and positive control, showing MoAb binding at 30 kDa; birch pollen extract, included as negative control, did not show any MoAb binding.

Concentration of group 5 allergens in grass pollen extracts and single pollen grains

Using the quantitative two-step ELISA, the soluble extract of all of the tested grass pollen extracts was shown to contain Phl p 5 cross-reactive allergen homologues, qualitatively confirming the qualitative Western analysis results, and again indicating that the ELISA employed here determines total group 5 allergen concentrations. The concentration of group 5 allergens in the tested grass pollen extracts was 393 $\mu\text{g/mL}$ on average, ranging from 192 $\mu\text{g/mL}$ (fescue) to 584 $\mu\text{g/mL}$ (Italian rye grass). A mean of 0.05 ng group 5 allergens was detected per pollen grain, which represents 15.7% of the soluble protein (Table 1).

Table 1. Soluble group 5 allergens in solution, mass, soluble protein and group 5 allergens per pollen grain, percentage of soluble protein and group 5 allergens per total grain mass and percentage of soluble group 5 allergens per total soluble protein in pollen extracts of a panel of clinically important grass species

	Soluble group 5 allergen content ($\mu\text{g/mL}$)	Mass per pollen grain (ng/grain)	Soluble protein per pollen grain (ng/grain)	Soluble group 5 allergens per pollen grain (ng/grain)	Soluble protein per total grain mass (%)	Soluble group 5 allergens per total grain mass (%)	Soluble group 5 allergens per total soluble protein (%)
Perennial rye grass	485	6.7	0.18	0.03	2.7	0.5	18.3
Italian rye grass	584	8.9	0.19	0.05	2.1	0.6	28.2
Timothy	327	9.4	0.24	0.03	2.6	0.3	12.7
Orchard grass	424	11.4	0.32	0.05	2.8	0.4	15.1
Velvet grass	240	5.9	0.17	0.02	2.9	0.2	8.4
Fescue	192	10.8	0.27	0.02	2.5	0.2	7.7
Cultivated rye	491	23.8	0.61	0.12	2.6	0.5	19.2
Average	392	11.0	0.28	0.05	2.6	0.4	15.7
White birch (negative control)	n.d.	18.8	0.28	0	1.5	0	0

n.d. = not detectable

These calculations were based on samples of 1 mg pollen, which contained amounts of pollen grains determined by means of a haemocytometer, so that results could be estimated on a per pollen grain basis.

Atmospheric group 5 allergen concentrations and their dependence on the amount of grass pollen

Group 5 allergens were identified on particles larger and smaller than $7.2\ \mu\text{m}$ by quantitative ELISA in extracts of filters from a high-volume sampler equipped with a single-stage cascade impactor. Figure 2 shows the concentrations of group 5 allergens in these two fractions during the grass pollen season in 1996/1997 in Melbourne. When ELISA data were compared with the corresponding pollen count, there was a direct correlation between the amount of group 5 allergens in large particles and the daily incidence of grass

pollen. Sampling periods with high numbers of grass pollen grains (23–25 October, 1–2, 8–9, 14–17, 24–29 November, 4–8, 14–15, 21–24, 27–28 December 1996, 3–6 January 1997) were generally characterized by elevated group 5 allergen concentrations in the large size particle fraction. A nonparametric test, Spearman's rank correlation, gave a coefficient $r_s = 0.842$ ($P < 0.001$, $n = 76$) for group 5 allergen concentrations in large particles vs the corresponding grass pollen counts. The correlation between total group 5 allergen concentrations and grass pollen counts ($r_s = 0.828$, $P < 0.001$, $n = 76$) was slightly lower and the correlation between group 5 allergens in respirable particles and grass pollen counts ($r_s = 0.357$, $P < 0.01$, $n = 76$) was significantly lower. There are some days during which group 5 allergens, but no grass pollen is recorded (e.g. on 2 December 1996 and 8–10 and 16 January 1997). These days have also been taken into consideration in the calculations.

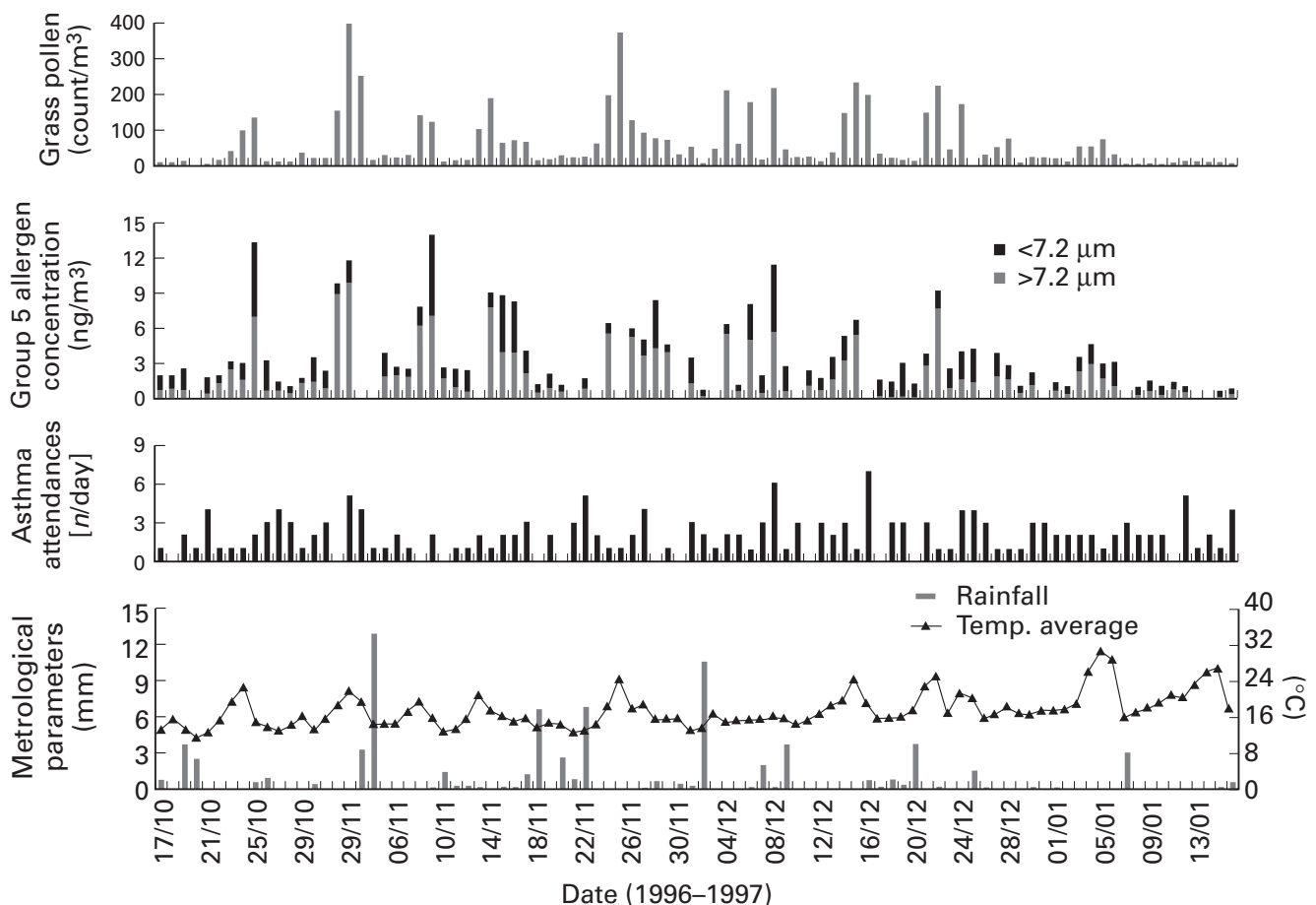


Fig. 2. Comparison of grass pollen count (counts/m³), group 5 allergen concentration (Phl p 5 and cross-reactive allergens; ng/m³) in two particle size fractions (above and below $7.2\ \mu\text{m}$) in atmospheric samples, asthma attendances (attendances/day) and meteorological parameters (rainfall [mm] and average temperature [$^{\circ}\text{C}$]) in Melbourne (17 Oct 1996–16 Jan 1997). Group 5 allergen data for 20 October, and 3, 4, 13, 21, 23, 25 and 30 November, and 3, 10, 16, 25, 26 and 31 December 1996, and 7, 13 and 14 January 1997 are not available.

Group 5 allergen concentrations in two different particle size fractions and their dependence on meteorological parameters

Using the high-volume sampler equipped with a single-stage cascade impactor, group 5 allergens were quantified from the resulting filters in terms of particles larger and smaller than $7.2\ \mu\text{m}$ and compared with the corresponding meteorological conditions (Fig. 2). Rainfall appeared to remove large allergenic particles from the atmosphere: upon rainfall both the number of grass pollen grains and the group 5 allergen concentrations in large particles generally decreased; at the same time, group 5 allergen concentrations in the small (respirable) particle size class increased. Rainfall was detected on 42 days of the study period. On these days, an average of 57% of the total atmospheric group 5 allergen load was detected in respirable particles, whereas on dry days, an average of only 37% of the group 5 allergens was found in this particle size fraction.

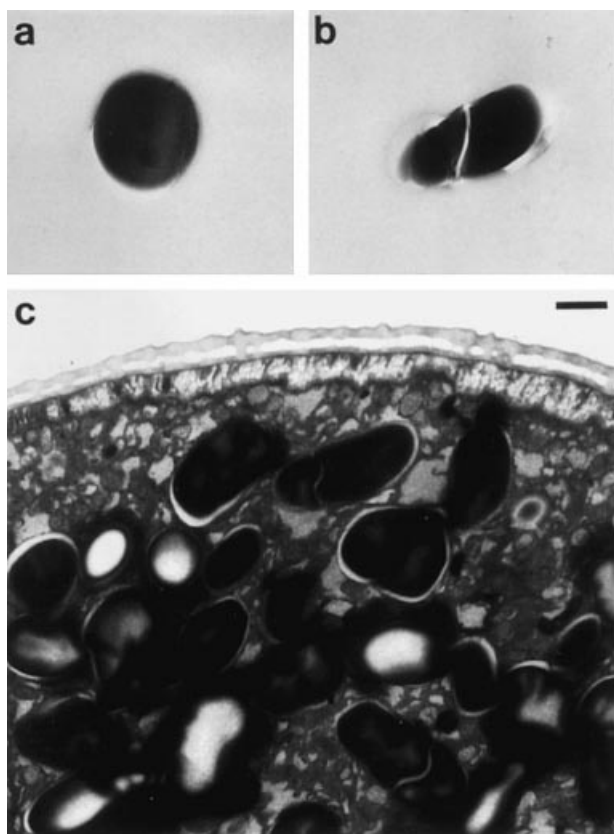


Fig. 3. Transmission electron micrographs of respirable particles, collected on the 25 October 1996 and extracted from air filters ($<7.2\ \mu\text{m}$) (a, b). Transmission electron micrograph of a sectioned rye grass pollen grain, containing starch granules morphologically similar to the respirable particles collected from the atmosphere (c); bar represents $1\ \mu\text{m}$.

Analysis of the pollen count slides in 3-h intervals shows a sudden decrease in the number of grass pollen grains upon rainfall: for example from 304 to 0 grains/ m^3 on the 25 Oct 1996, from 88 to 8 grains/ m^3 on the 28 Nov 1996 and from 232 to 0 grains/ m^3 on the 8 Dec 1996.

On both the 25 October and 6 December 1996, days with high concentrations of group 5 allergens in respirable particles (light rainfall after a period with high numbers of grass pollen), numerous starch granules with an aerodynamic diameter of $1\text{--}2.5\ \mu\text{m}$ were detected in extracts from high-volume filters collecting particles smaller than $7.2\ \mu\text{m}$ (Fig. 3a,b). These particles were morphologically similar to starch granules found in the cytoplasm of rye grass pollen grains (Fig. 3c). It should be noted here that only days with light rainfall, as opposed to dry days, were chosen as there is no starch granule concentration increase on dry days.

Pollen and starch granule equivalents to atmospheric group 5 allergen load

We propose here the concept of pollen and starch granule equivalents, that is the allergen load of atmospheric particles larger than $7.2\ \mu\text{m}$, can be represented by the number of pollen grain equivalents, and the allergen load of particles smaller than $7.2\ \mu\text{m}$ by the number of starch granule equivalents required to produce the particular load. For example, we know from the data presented here that the group 5 content of a typical grass pollen grain is 0.05 ng. The atmospheric group 5 load in particles larger than $7.2\ \mu\text{m}$, estimated from filters from a high-volume sampler fitted with a two-stage cascade impactor, can be divided by this figure, 0.05 ng, to provide the number of pollen equivalents (Fig. 4). On average, 700 starch granules, containing the group 5 allergens, can be found in a single grass pollen grain [11]. Thus, the group 5 content of a typical starch granule is 0.07 pg. The atmospheric group 5 load in particles smaller than $7.2\ \mu\text{m}$ can be divided by this figure, 0.07 pg, to provide the number of starch granule equivalents (Fig. 4), representing a hypothetical potential number of airborne starch granules from grass pollen. On dry days, the atmospheric group 5 allergen concentration in respirable particles is equivalent to 10 000 starch granules/ m^3 , while on days with rainfall, the starch granule equivalents may increase up to 10-fold (e.g. 25 October, 9, 15, 16, 28 November, 8 December). Pollen equivalents for group 5 allergen concentrations in particles $>7.2\ \mu\text{m}$ are directly correlated with the grass pollen count obtained using a Burkard spore trap. As for the group 5 allergen concentrations in particles larger and smaller than $7.2\ \mu\text{m}$, Spearman's Rank Correlation Coefficient for pollen equivalents and grass pollen count ($r_s = 0.842$, $P < 0.001$, $n = 76$) shows a more significant correlation with higher probability than starch granule equivalents and pollen counts ($r_s = 0.357$, $P < 0.01$, $n = 76$).

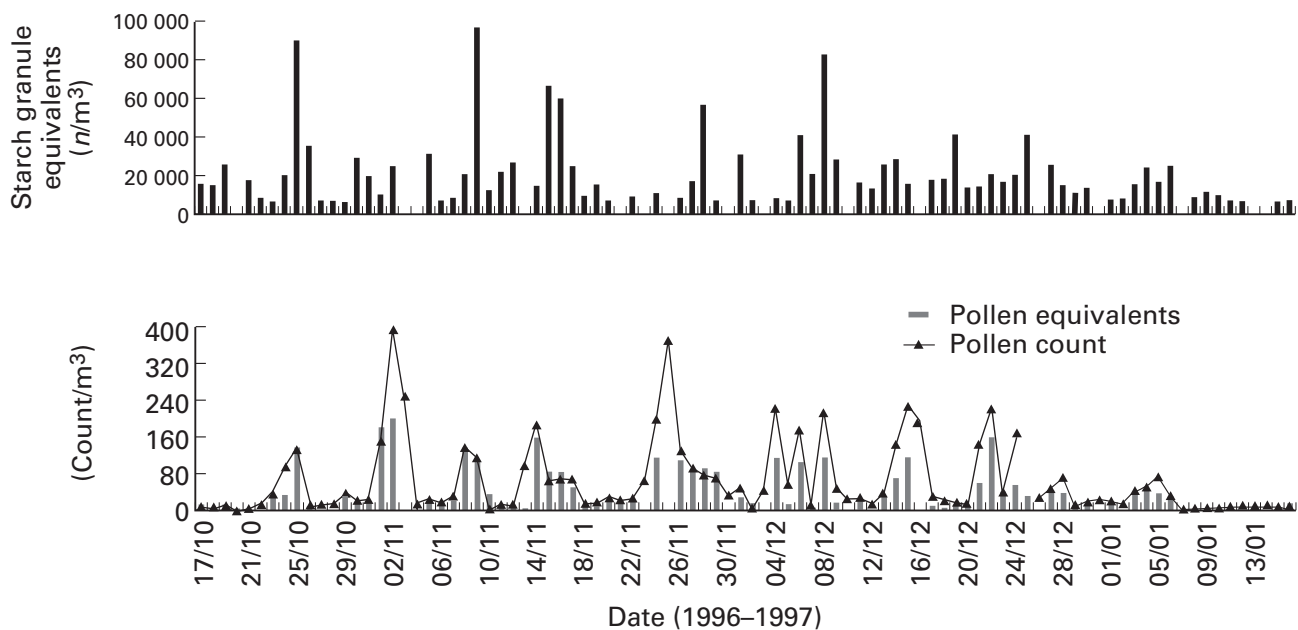


Fig. 4. Pollen counts (per m^3) compared with the calculated pollen equivalents (per m^3) to the group 5 allergen concentrations of particles $>7.2\ \mu\text{m}$ and the calculated starch granule equivalents (per m^3) to the group 5 allergen concentrations of particles $<7.2\ \mu\text{m}$ in Melbourne (17 Oct 1996–16 Jan 1997). Group 5 allergen equivalents data for 20 October and 3, 4, 13, 21, 23, 25 and 30 November, and 3, 10, 16, 25, 26 and 31 December 1996, and 7, 13 and 14 January 1997 are not available.

Asthma attendances and their dependence on grass pollen allergen load, grass pollen counts and meteorological factors

Patient data, along with group 5 allergen concentrations (in particles with an aerodynamic diameter above and below $7.2\ \mu\text{m}$), grass pollen counts and meteorological data (rainfall, average temperature) were compared on a daily basis and pooled for two, three and four day running means using Spearman's Rank Correlation Coefficient (Fig. 2). The daily and running mean values were compared with patient data delays of 0 to 4 days. There were no significant correlations for patient data compared with grass pollen counts, group 5 allergen concentrations in nonrespirable particles, total group 5 allergen concentrations and average temperature. The 4-day running mean of group 5 allergens in respirable particles was shown to be significantly correlated to the four day running mean of asthma attendances with a delay of two days ($r_s = 0.334$; $P < 0.001$, $n = 92$).

Discussion

In this study, we have for the first time made a direct comparison between the amount of the major grass group 5 allergens in daily atmospheric samples, detected by a quantitative ELISA of extracts from filters of a high-volume sampler, and the numbers of grass pollen grains in the

atmosphere, estimated using a Burkard volumetric trap. Total daily atmospheric group 5 concentrations in particles larger than $7.2\ \mu\text{m}$ correlated positively with the amount of detected grass pollen. Amounts of group 5 allergens ranged up to $14\ \text{ng}/\text{m}^3$ air. To understand the biological significance of these values, we have also calculated the group 5 allergen content of a panel of grass species on a per pollen grain basis. On average, $0.05\ \text{ng}$ of group 5 allergens per grass pollen grain was found, which represents 15.7% of the soluble protein in aqueous extracts. There have been no previous estimates of the amount of allergen in single pollen grains of various grass species.

Group 5 allergens have previously been recorded in atmospheric fine particles smaller than pollen grains, even when no grass pollen grains were present [8]. However, these studies were carried out over a prolonged sampling time period. Here, we have confirmed these findings in daily samples. We show here that group 5 concentrations in different particle size fractions are correlated with the amount of rainfall: particles of aerodynamic diameter $>7.2\ \mu\text{m}$ are predominant on dry days representing pollen-associated allergen, whereas on days with rainfall, particles $<7.2\ \mu\text{m}$ are predominant representing allergen-containing starch granules. Rye grass pollen grains have been shown to rupture in rainwater by osmotic shock, each grain releasing about 700 allergen-loaded starch granules in the size range of 0.6 – $2.5\ \mu\text{m}$ containing the major allergen Lol p 5 [11].

The highly similar allergenic protein in timothy grass Phl p 5, has also been shown to be present in starch granules [12], highlighting the significance of the findings presented here. In atmospheric samples, collected on a day with high concentration of group 5 allergen in respirable particles, high numbers of starch granules, morphologically similar to starch granules from rye grass could be detected, appearing to be the group 5 allergen-loaded respirable particles. Within minutes of grass pollen contacting rainfall, there is an increase of atmospheric starch granules as opposed to the recent findings of starch granule release from birch pollen: we found that airborne birch pollen grains settle on leaf surfaces and, following light rainfall, germinate. Later, pollen tubes burst, releasing inhalable particles carrying the birch major allergen Bet v 1 into the atmospheric aerosol [17], and causing an up to 10-fold increase in Bet v 1-loaded respirable particles with a delay of 24–48 h [18]. On the other hand, as a result of bursting grass and tree pollens, surface allergens would also be liberated into the atmosphere as free allergen molecules. Such free allergen molecules may be able to interact with other particles such as pollutants, plant fragments and hairs, etc., and therefore be able to gain access to the lower airways to trigger allergic asthma. Indeed, we have recently shown that the major rye grass pollen allergen Lol p 1 interacts with diesel exhaust carbon particles [19].

The effects of rainfall are dramatically reflected in the pollen and starch granule equivalents of the atmospheric allergen load. This concept represents a new way to view allergen loads in the atmosphere. On dry days, the atmospheric group 5 allergen concentration in respirable particles is equivalent to 10 000 starch granules/m³, while on days with rainfall, the starch granule equivalents may increase up to 10-fold, representing a high number of allergenic respirable particles and a high potential risk for allergic asthma sufferers. Grass pollen counts and pollen equivalents are significantly correlated. Inconsistencies are presumably due to the different collection efficiencies of the two different sampling devices used in this study, to the varying group 5 allergen content of grass pollen grains from different species and in the course of the pollen season and to pollen that has lost its allergenic contents.

There are different risk factors that trigger asthma and hay fever among individuals sensitive to grass pollen allergens. Recently, we found that hay fever symptoms were significantly correlated with the grass pollen counts ($P < 0.001$ for nasal symptoms, $P < 0.005$ for eye symptoms) in Melbourne [6]. To estimate the parameters that trigger asthma, data of patients in whom the diagnosis of asthma was considered the principal reason for attendance, group 5 allergen concentrations in particles with an aerodynamic diameter above and below 7.2 μm and grass pollen counts were compared. The 4-day running mean of group 5

allergens in respirable particles was shown to be significantly correlated to the 4-day running mean with a delay of 2 days of asthma attendances. The atopic status of the patients included in the emergency department data is unknown. Yet it has been shown for Melbourne that grass pollen allergens are the most important aggravating factor for spring and summer allergic asthma [20,21]. The diagnostic label 'asthma' may include some older patients with obstructive bronchitis and/or emphysema. Those uncertainties strengthen rather than weaken the clinical significance of the correlation described here.

The clinical implications of this work for grass pollen allergy are that the grass pollen count does not completely reflect the total allergen load in the atmosphere, yet our data show that both may be predictable from meteorological conditions (i.e. rainfall) during the grass pollen season. However, it should be stressed here that other factors, such as the time within the season, should also be considered. Group 5 allergen concentrations are correlated to grass pollen counts on both dry days (high concentrations) and days with rainfall (low concentrations). However, high concentrations of group 5 allergens in respirable particles occur on days with rainfall during the grass pollen season. These fine particles are potentially able to trigger allergic asthmatic reactions to group 5 allergens since the allergen occurs in respirable particles. Isolated starch granules have been shown to contain the major allergen Lol p 5 and to be capable of triggering IgE-mediated reactions in grass pollen-sensitive patients [11]. Our results confirm the previous findings that the group 5 allergen-loaded respirable particles are starch granules. These are released into the atmosphere upon rainfall by bursting of pollen grains.

Thunderstorms are known to be associated with epidemics of asthma [22]. In Melbourne, a five- to 10-fold increase in the number of patients reporting with asthma to emergency departments was reported during severe thunderstorms [21]. In London, a thunderstorm after the peak grass pollen concentration caused a sudden and extensive epidemic, affecting possibly several thousand individuals [23]. During the period analysed in this study, no thunderstorms occurred in the Melbourne area and correlation analysis showed a lag of 2 days between the occurrence of rainfall with increased amounts of allergen-loaded respirable particles and increases in asthma attendances at the emergency departments. This indicates that the data presented in this study describe symptomatic responses to average environmental conditions rather than peak events such as asthma epidemics associated with severe thunderstorms.

These findings will enable clinicians to advise and assist their patients who are allergic to grass pollen allergens in their allergen avoidance programmes; for example, on days with rainfall during the grass pollen season, asthma patients

might be advised to remain indoors or carry appropriate medication while being outdoors and use a fine particle-removing atmospheric filtration device.

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