

Comparison between outdoor and indoor airborne allergenic activity

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Background: Allergenic pollens are usually detected in outdoor air by using volumetric spore traps, which allow measurement of atmospheric concentration as pollen grains per m³ of air. The results of the pollen count are useful primarily for outdoor environments while most people spend most of the day indoors.

Objective: The purpose of our study was to compare outdoor pollen levels with allergenic activity measured both outdoors and indoors.

Methods: We used a Lanzoni spore trap to measure airborne Urticaceae pollen and filters collecting particles simultaneously indoors and outdoors and assayed each filter for *Parietaria judaica* allergenic activity. Samples were collected at the Allergological Service of the "A. Cardarelli" Hospital in Naples with the balcony open on some days and closed on others. Allergenic activity (ng/m³) was measured using the immunocapture RAST.

Results: With the balcony open there was no great difference between outdoor and indoor allergenic activity, but with the balcony closed there was a reduction of indoor allergenic activity of about one-third in comparison with outdoor allergenic activity. Statistical analysis (Pearson correlation test) indicated a significant correlation between outdoor allergen levels and indoor allergen levels with the balcony open ($r = .4415$, $P < .05$), but not with the balcony closed ($r = .3160$, $P > .05$); a significant correlation between outdoor pollen count and indoor allergen levels with the balcony open ($r = .4809$, $P < .05$), but not with the balcony closed ($r = .3858$, $P > .05$); and a highly significant correlation ($r = .5225$, $P < .001$) between outdoor pollen count and outdoor allergen levels.

Conclusions: These data provide scientific evidence for the recommendation to hay fever patients to remain indoors during seasons with high levels of outdoor pollens.

Ann Allergy Asthma Immunol 1996;77:147-52.

INTRODUCTION

It is widely recognized that changes in airborne allergenic pollen concentrations and allergic symptoms in sensitized subjects are related. This relationship is not always straightforward since it can be influenced by many factors, such as the type of allergenic pollen, phase of the pollen season, and the "priming effect," which apparently

results in lower pollen levels eliciting symptoms in allergic patients later in the season as compared with earlier seasonal symptoms. Furthermore, allergenic pollen grains are usually counted microscopically in samples from outdoor air collected using volumetric spore traps, which in themselves involve certain variables, particularly their height off the ground and distance from the patient's house. These instruments (frequently Hirst-like devices such as Burkard or Lanzoni in Europe) do offer information about variations in outdoor airborne pollen concentrations. Most people, however, spend a majority of their days indoors where the environment is often physically separated from out-

doors. In fact we usually recommend that patients with hay fever stay indoors during pollen season, but we do not know whether this recommendation is really useful. Consequently the behavior of indoor pollen allergens is also important.¹⁻⁴ Allergists are interested, then, in knowing whether keeping the indoor environments physically separated from outdoors (by keeping doors and windows closed) is an effective means to control penetration of outdoor allergens.

Some studies have shown that indoor pollen concentrations can be quite high during the pollen production season.³⁻⁶ For the most part, these studies have been carried out with windows open and without simultaneous measurement of outdoor allergen activity. In this study we compared the outdoor and indoor airborne concentrations of *Parietaria judaica* allergenic activity. *Parietaria judaica* is the most important cause of hay fever in the Naples area and one of the most important in the Mediterranean area.⁷⁻¹²

MATERIAL AND METHODS

High-Volume Sampling

Airborne allergens were captured on glass-fiber filters mounted on a modified air sampler (CF/20 Gelaire, Flow Labs, Milan, Italy) (Fig 1). The apparatus consists of a double-head membrane pump connected by two flow control flow-meters and a volumetric counter that measures the volume of air captured. This sampler operates at a constant air flow rate (20 L/min), and particulate material is harvested onto diameter borosilicate microfiber glass filters with acrylin resin binder (AP20 Millipore, Vimodrone, Milan, Italy). Usually this type of sampler utilizes only one filter mounted. We modified the apparatus, connecting a double

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Supported in part by a grant by Regione Campania

Received for publication April 19, 1995.

Accepted for publication February 1, 1996.



Figure 1. High volume air sampler (CF/20 Gelaire, Flow Labs, Milan, Italy) with the modified double tube, each with one filter, so that one tube and one filter are outside the room (in this figure with open balcony) and the other tube and filter are inside. Both operate at 10 L/min.

tube, each with one filter, so that one tube and one filter were outside the room and the other tube and filter were inside. Both operated at 10 L/min, measured by a flowmeter (Lanzoni, Bologna, Italy). The filters have an efficiency of at least 84% for trapping all anemophilous particles more than 3 μm in diameter.

Air was sampled for four hours daily from March 10 to June 16, 1994 (from 8:30 to 12:30 AM, which are the hours with the highest atmospheric concentration of *Parietaria judaica* pollen in the central Mediterranean area in the months studied)^{10,13,14} for a total of 51 days (27 days with balcony open and 24 with balcony closed).

Room Characteristics

The sampler was placed in a room of the Allergy Department at the "A. Cardarelli" Hospital, situated on the Vomero hill in Naples, about 140 m above sea level.

The 120-m³ room has a central heating but no air conditioning, a balcony, and a door, which was always closed during the sampling periods. Each morning, almost two hours before starting the sampling, the room was washed.

Usually, only two persons were working at the computer or at optical microscope inside the room during sampling and indoor air was disturbed little by their movements.

Pollen Count

Air samples for pollen counts were collected outdoors using a 7-day recording volumetric spore trap (VPPS 2000 Lanzoni, Bologna, Italy). This pollen trap has the same geometry and technical characteristics as the Hirst trap. The Lanzoni trap was placed on the flat roof of the "A. Cardarelli" Hospital in Naples, about 25 m above the ground. Pollen collection was carried out without interruption all year long. Only Urticaceae pollen grains were considered in this study. The genus includes both *Parietaria judaica* and *Urtica* since it is not possible to differentiate these taxa under the optical microscope.

The atmospheric concentration of Urticaceae pollen grains was expressed as 24 hour means concentration per cubic meter of air.

Parietaria judaica Pollen Extract

Parietaria judaica pollen was purchased from Allergon AB (Valinge, Sweden). Extraction was done (5% wt/vol) in 0.15 M phosphate buffer containing 0.05% sodium azide, pH 7.4 (PBS), for 24 hours at 4 °C with stirring. Particulate material was removed by centrifugation (18000 rpm for 40 minutes). The supernatant was dialyzed against 0.015 M PBS in visking tubes (Spectra/Por, Oscar Mires, Milan, Italy) with nominal cut-off 3500 Da, lyophilized and stored at -20 °C. Protein content was measured according to the Bio-Rad procedure. The allergenic potency of *Parietaria judaica* extract was determined by the radioallergosorbent test (RAST)-inhibition technique according to Yman et al,¹⁵ using an in-house standard (Laboratorio Farmaceutico Lofarma, Milan, Italy). The lyophilized *Parietaria judaica* extract was dissolved in water at a concentration of 4 mg/mL as protein content and analysed by sodium dodecyl sulphate-polyacrylamide gel (SDS-PAGE) (12% separation gel and 4% stocking gel) according to Laemmli¹⁶ in the presence of 2-mercaptoethanol. Gel was stained with Coomassie Brilliant Blue G-250 (Sigma, Milan, Italy). Resolved bands were approximately in

the molecular weight range of 10 to 80 kDa as determined by a comparison with standard proteins of known molecular weight.¹⁷ The band with a molecular weight of 10 to 15 kDa was particularly intense, known to correspond to the major allergen of *Parietaria judaica*, Par j I.¹⁸ This extract was used to immunize animals and to prepare immunoadsorbancy materials.

Specific Antiserum to *Parietaria judaica* Pollen Extract

Antiserum to *Parietaria judaica* extract was raised in rabbits by subcutaneous injection of extract (3 mg as protein content measured according to the Bio-Rad procedure), emulsified in Freund's complete adjuvant. Subsequent challenges every 3 weeks for 6 months were performed using incomplete Freund's adjuvant. One week after the last immunization, blood was taken and the immunoglobulin fraction of serum was obtained by precipitation with ammonium sulphate (40% saturated solution). Specific anti-*Parietaria judaica* antibodies were purified by affinity chromatography as previously described.¹⁹ Briefly, 50 mL of *Parietaria judaica* extract containing 100 mg of protein was covalently bound to 3.5 g of cyanogen bromide-activated Sepharose 4B (Pharmacia, Milan, Italy) according to the manufacturer's instructions. The immunoadsorbent was then transferred to a col-

umn and washed with PBS until its optical density at 280 nm was close to zero.

The ammonium sulphate-precipitated IgG fraction from hyperimmune rabbit sera was applied to the immunoadsorbent and specific anti-*Parietaria judaica* antibodies were then eluted with 0.1 M glycine-HCl and 0.5 M NaCl, pH 2.5, immediately neutralized by Tris 0.2 M, dialyzed against PBS and stored in aliquots at -20°C . The protein concentration was estimated on the basis of absorbance at 280 nm, assuming $E\ 1\%/1\text{ cm} = 14.0$.

Specific antiserum tested in cross-immunoelectrophoresis gave at least eight different precipitating arcs, including the arc of Par j I allergen.²⁰

Serum Pools

We used a pool of sera from patients allergic to *Parietaria judaica* (class 3 and 4 as RAST). None of them had been undergoing immunotherapy. The reactivity of pooled sera against single allergens of *Parietaria judaica* was evaluated by SDS-PAGE and immunoblotting²¹; positive bands for the major allergen (10 to 15 kDa) and for other allergens (25, 45, and 66 kDa) were observed. No band appeared using normal human serum.

Immunocapture RAST

Polystyrene beads (Precision Plastic Balls, Chicago, Illinois, USA) were

sensitized with an optimal concentration ($10\ \mu\text{g}/150\ \mu\text{L}$) of affinity-purified anti-*Parietaria judaica* antibodies by overnight incubation at room temperature. After saturation with PBS, pH 7.2, 0.15 M 4% BSA (bovine serum albumine) (PBS-BSA), beads were placed in contact for six hours with a series of twofold dilutions of known amounts of *Parietaria judaica* extract (reference curve) or with extracts from the filters used to collect pollen grains from air.

Beads were then washed with PBS-BSA and incubated overnight with the RAST-positive human serum pool. Bound IgE was detected after further six hours incubation of the beads with $50\ \mu\text{L}$ of ^{125}I -anti IgE (Sferikit, Laboratorio Farmaceutico Lofarma, Milan, Italy). The residual radioactivity was measured in a gamma-scintillation counter and data are expressed as the percentage of bound radioactivity. The amount of *Parietaria judaica* allergens on the filters was interpolated from a reference curve and expressed as ng of allergenic activity/ m^3 of air.

Treatment of the Filters Used to Collect Pollen Grains in the Air

Five milliliters of buffer (NH_4HCO_3 , 0.125 M + 0.01% BSA) was added to tubes containing the glass fiber filters (AP 20, Millipore, Vimodrone, Milan, Italy) used to collect particles present in the air. After overnight incubation at 4°C under stirring, the extraction solution was filtered on $0.22\text{-}\mu\text{m}$ membranes and lyophilized. The lyophilized extract was then dissolved in $250\ \mu\text{L}$ of water and the solution assayed for allergenic activity by immunocapture RAST.

Control Extracts

Gramineae and Fagaceae extracts were kindly provided by the Production Department of Laboratorio Farmaceutico Lofarma, Milan, Italy.

Statistical Analysis

Correlations between pollen count and immunochemical quantification of airborne allergenic activity outdoor and indoor were evaluated using the Pearson correlation test (r).

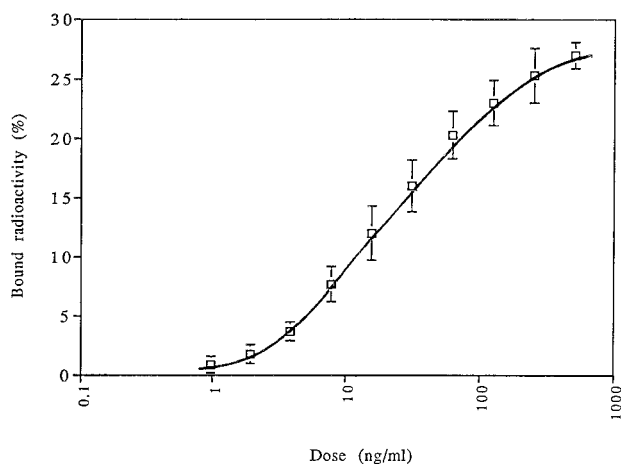


Figure 2. Reference curve obtained using twofold dilution of *Parietaria judaica* pollen extract in immunocapture RAST.

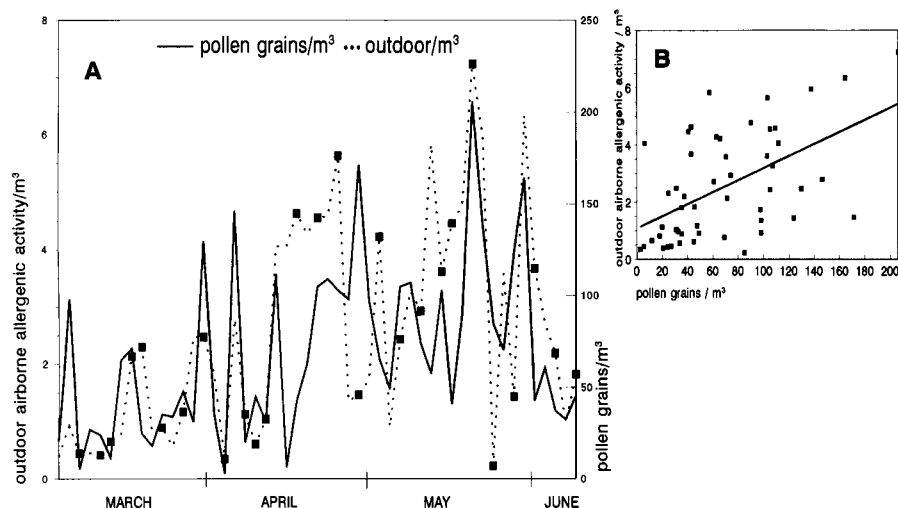


Figure 3. (A) Comparison between Urticaceae pollen count (grains/m³ of air as daily mean value using a Lanzoni volumetric device), *Parietaria* aeroallergen activity outdoor (ng protein/m³ of air as value obtained in 4 hours of daily sampling using the high-volume air sampler in pointed days) from March 10 to June 16, 1994. Data of the outdoor airborne allergenic activity/m³ are on the Y1 line [(—■—), ■ indicates the days of sampling with balcony open] and on the Y2, the pollen/m³ (—) are reported. (B) Linear regression between pollen count (abscissa) and outdoor airborne allergenic activity (ordinate).

RESULTS

A satisfactory dose-response curve was obtained in a wide range of concentrations of *Parietaria judaica* pollen extract. *Parietaria judaica* airborne allergenic activity trapped on glass fiber filters was quantitated by extrapolation from the reference curve shown in Figure 2. The results are expressed as ng protein/mL from which derives the value ng protein/filter divided by 4, considering that the volume of water used to dissolve the lyophilized sample was 250 μ L.

The outdoor *Parietaria judaica* allergenic activity was compared with the indoor activity. The days with the balcony open were examined separately from the days with balconies closed (Table 1). Considering all days sampled, the results of high volume sampling for indoor and outdoor *Parietaria judaica* allergen levels are significantly related (Figs 3 and 4).

Statistical analysis indicated a significant correlation between outdoor allergen levels and indoor allergen levels with the balcony open ($r = .4415$, $P < .05$), but not with the balcony closed ($r = .3160$, $P > .05$); a significant correlation between outdoor pol-

len count and indoor allergen levels with the balcony open ($r = .4809$, $P <$

.05), but not with the balcony closed ($r = .3858$, $P > .05$); and a highly significant correlation ($r = .5225$, $P < .001$) between outdoor pollen count and outdoor allergen levels.

DISCUSSION

Pollen allergy is of great clinical importance, considering that in Europe from 5% to 10% of the population suffer allergies to pollen from grasses, trees, or weeds. Consequently the allergenic components of pollen have been the subject of much biochemical and immunochemical research over the last three decades.

Accurate diagnosis and treatment of pollinosis requires both clinical examination of the patient and allergologic testing in vivo and possibly also in vitro and exposure assessment. Usually the onset, duration, and intensity of clinical symptoms in patients with respiratory allergy to pollen are correlated with counts of atmospheric pollen grains, although this relation is not always simple.^{22,23}

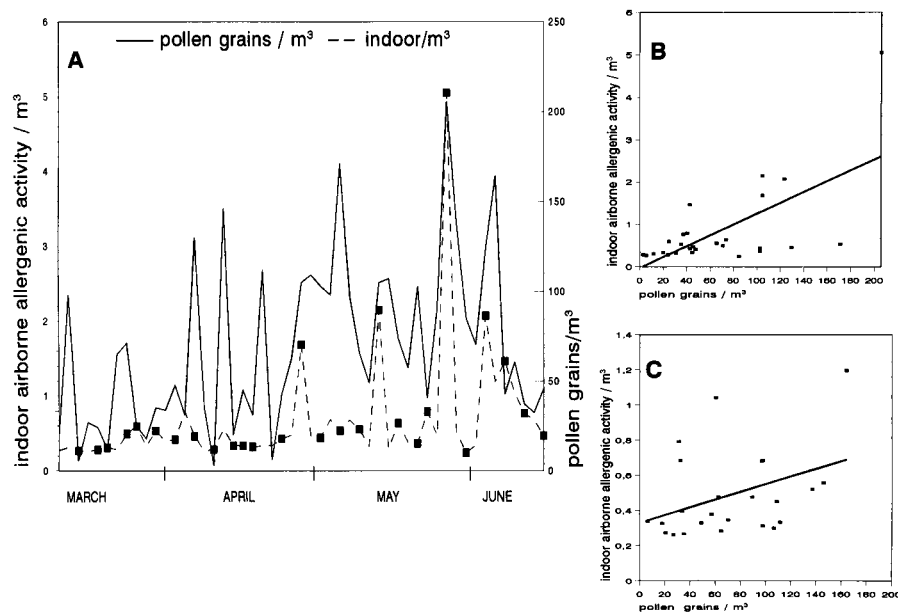


Figure 4. (A) Comparison between Urticaceae pollen count (grains/m³ of air as daily mean value), *Parietaria* aeroallergen activity indoor (ng protein/m³ of air as value obtained in 4 hours daily of sampling by high-volume air sampler in pointed days) from March 10 to June 16, 1994. Data of indoor airborne allergenic activity/m³ are on the Y1 line [(—■—), ■ indicates the days of sampling with balcony open], on the Y2, the pollen/m³ (—) are reported. (B) Linear regression between pollen count and indoor airborne allergenic activity with balcony open. (C) Linear regression between pollen count (abscissa) and indoor airborne allergenic activity with balcony closed (ordinate).

Table 1. Allergenic Activity (ng/m³) of Indoor and Outdoor Air (as mean value during four hours of sampling for each day)

Day of Sampling†	Weather Condition‡	Indoor, ng/m ³	Outdoor, ng/m ³	Pollen Grains/m ³ *
10/3/94	S	0.27	0.39	20.7
11/3/94	S	0.31	0.92	97.98
14/3/94†	C	0.27	0.44	5.52
15/3/94	R	0.26	0.45	26.91
16/3/94†	R	0.28	0.42	24.15
18/3/94†	S	0.31	0.65	11.73
21/3/94	C	0.28	0.77	64.86
22/3/94†	C	0.49	2.13	71.07
23/3/94†	C	0.59	2.3	24.84
24/3/94	C	0.32	0.82	17.94
25/3/94†	PC	0.53	0.89	35.19
28/3/94	W	0.39	0.57	33.81
29/3/94†	S	0.42	1.17	47.61
30/3/94	S	0.79	2.48	31.05
31/3/94†	W	0.46	2.47	129.72
6/4/94	S	0.27	1.81	35.19
8/4/94†	R	0.28	0.34	2.76
11/4/94	C	0.56	2.79	146.28
12/4/94†	C	0.34	1.12	20.01
13/4/94†	C	0.33	0.61	44.85
14/4/94†	PC	0.32	1.04	31.05
15/4/94	PC	0.33	4.06	111.78
18/4/94	C	0.34	4.05	6.21
19/4/94†	S	0.43	4.63	42.78
20/4/94	C	0.47	4.27	62.79
21/4/94†	C	1.69	4.56	104.88
22/4/94	PC	0.45	4.58	109.02
26/4/94†	PC	0.44	5.64	102.81
27/4/94	S	0.68	1.36	97.98
28/4/94†	S	0.54	1.47	171.12
29/4/94	S	0.68	1.74	97.29
2/5/94†	C	0.55	4.22	65.55
3/5/94	W	0.33	0.91	48.99
4/5/94†	S	2.15	2.44	104.88
6/5/94	C	0.3	3.27	106.95
7/5/94†	S	0.64	2.93	73.83
9/5/94	C	0.38	5.83	57.27
10/5/94†	PC	0.37	3.62	102.81
11/5/94†	S	0.79	4.46	40.71
12/5/94	PC	0.45	4.77	89.7
16/5/94†	S	5.05	7.24	205.62
17/5/94	PC	0.52	5.95	137.31
18/5/94†	R	0.24	0.23	84.87
19/5/94	C	0.34	3.58	70.38
20/5/94†	PC	2.07	1.44	123.51
23/5/94	S	1.19	6.35	164.22
1/6/94†	S	1.47	3.67	42.78
6/6/94	S	1.04	2.71	60.72
7/6/94†	S	0.77	2.19	37.26
8/6/94	S	0.68	0.97	32.43
16/6/94†	S	0.47	1.83	45.54

* Daily mean value of sampled days from March 10 to June 16, 1994.

† Days with open balcony.

‡ S = Serene, C = cloudy, R = rain, W = wind, and PC = partially cloudy.

Differences arise especially with regard to the distance of the pollen trap from the ground. These traps, which are usually at roof level, detect changes in pollen flight usually later and to a lesser extent than traps installed at ground level because of daily variations in convection. This means that a ground level pollen trap records pollen release earlier than a high-level pollen trap. This may partly explain why patients may report severe symptoms during periods when traps on a roof record low pollen counts, caused by the large amounts of undetected pollen close to head height. Further, outdoor pollen counts may not reflect the total airborne allergen exposure, since pollen allergens may be carried in microaerosol suspensions. In fact we sometimes observe allergic manifestations in our patients before our samplers detect atmospheric pollen grains and also after the end of the pollen season. Consequently, it may be more useful to quantitate the allergenic activity present in the atmosphere, independently of the carrier particles (pollen or other plant debris in micro-particles).²²⁻³¹

Many people spend most of the day indoors, at home, or at work and indoor pollen patterns may differ considerably from outdoor ones. Previous studies have shown that indoor pollen concentrations can be quite high during the pollen production season, especially in rooms with the windows open. Our study confirms these observations.

We selected *Parietaria judaica* because of its clinical importance in southern Italy and the whole of the Mediterranean area. This plant grows spontaneously on walls and ruins, especially on tufaceous stone, and is not found inside homes. Its pollen reaches very high concentrations in the atmosphere in Naples over long periods, particularly during the spring and early summer. Urticaceae pollen counts include both *Parietaria judaica* and *Urtica dioica*. However, *Urtica* pollen is not common in Naples and its pollen is likely to be present in very small quantities compared with *Parietaria ju-*

daica. *Urtica dioica* does not appear to have any allergenic relevance in Naples and there is no allergenic cross-reactivity between these two species.^{7,32} We consequently do not consider that the *Urtica* pollen confounds our data significantly.

It is interesting to note that with the balcony open there was little difference between indoor and outdoor allergenic activity, but closing the balcony greatly reduced the indoor allergenic activity, which was then about one-third of that outdoors. In other words allergenic activity outside closed houses is on average three times the indoor allergenic activity.

As a result, staying indoors with the windows and doors closed could be a good way of limiting the symptoms of pollinosis for patients allergic to *Parietaria judaica* pollen.

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