

The allergen Bet v 1 in fractions of ambient air deviates from birch pollen counts

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Abstract

Background: Proof is lacking that pollen count is representative for allergen exposure, also because allergens were found in nonpollen-bearing fractions of ambient air.

Objective: We monitored simultaneously birch pollen and the major birch pollen allergen Bet v 1 in different size fractions of ambient air from 2004 till 2007 in Munich, Germany.

Methods: Air was sampled with a ChemVol® high-volume cascade impactor equipped with stages for particulate matter (PM) > 10 µm, 10 µm > PM > 2.5 µm, and 2.5 µm > PM > 0.12 µm. Allergen was determined with a Bet v 1-specific ELISA. Pollen count was assessed with a Burkard pollen trap. We also measured the development of allergen in pollen during ripening.

Results: About 93 ± 3% of Bet v 1 was found in the PM > 10 µm fraction, the fraction containing birch pollen. We did not measure any Bet v 1 in 2.5 µm > PM > 0.12 µm. Either in Munich no allergen was in this fraction or the allergen was absorbed to diesel soot particles that also deposit in this fraction. Pollen released 115% more Bet v 1 in 2007 than in 2004. Also within 1 year, the release of allergen from the same amount of pollen varied more than 10-fold between different days. This difference was explained by a rapidly increasing expression of Bet v 1 in pollen in the week just before pollination. Depending on the day the pollen is released during ripening, its potency varies.

Conclusion: In general, pollen count and allergen in ambient air follow the same temporal trends. However, because a 10-fold difference can exist in allergen potency of birch pollen, symptoms might be difficult to correlate with pollen counts, but perhaps better with allergen exposure.

Allergies are on the rise in the western world (1) but the incidence of sensitizations in children seems to have leveled off in some countries (2). It is calculated that the maximum incidence of sensitization will reach its peak at about 40%

sensitizations in the population around the middle of this century, when these children have grown up (3).

Birch pollen allergies are also increasing (4, 5). About 20% of the adult German population is sensitized to birch pollen, comparable to other Northern European countries (6), and half of sensitized individuals also show symptoms of allergic disease (7).

The proteins responsible for reactions to birch pollen belong to the Bet v family. There are currently seven members in the Bet v family (available from <http://www.allergome.com>, accessed May 2009). Over 90% of birch

Abbreviations

BSA, bovine serum albumin; ELISA, Enzyme-linked immunosorbent assay; PID, Pollen flight Information Service Germany (Stiftung Deutscher Polleninformationsdienst); PBS, phosphate buffered saline; PM, particulate matter.

pollen allergic individuals in Northern Europe are allergic to Bet v 1, a minority also to Bet v 2 or Bet v 4 (8). Bet v 1 is a 17-kDa protein with membrane binding and permeating capacities (9), perhaps needed in species recognition or fertilization. From Bet v 1, 37 isoforms are reported. However, commonly pollen expresses only a limited number of isoforms and patients generally recognize only about six of them (Dr. B. Weber, Allergopharma Joachim Ganzer KG, personal communication).

Birch pollen is the only source of Bet v 1 allergen (10). Other parts of birch trees, including other parts of the catkins, express low levels of Bet v 1 (11).

Exposure to allergen, also from birch, correlates with symptoms and indeed, allergen avoidance is the cornerstone of allergy treatment (12), although virtually impossible for airborne pollen. High exposure to birch pollen resulted 6 years later in more sensitization and allergic rhinitis in children compared to low exposure years (13). Northern European countries with higher birch pollen counts have higher rates of sensitization (6, 14), and climate changes increase the burden of pollen and may concomitantly increase allergic diseases (15). Very high exposure seems not to be protective against sensitizations to birch, but against development of symptoms (16).

All these studies used pollen counts as a measure of exposure. It is unknown whether allergen release of pollen is a better predictor of allergen exposure than counting pollen alone. Indeed, equal amounts of birch pollen from different years or different regions had up to fivefold difference in Bet v 1 release (17). In addition, strong symptoms in allergic individuals are reported on some days with very low or no birch pollen flight (18).

Currently, exposure to birch allergens in Germany is performed by counting birch pollen in ambient air with a network of about 46 Burkard pollen traps since 1983 (19). The aim of this study was to explore whether it is possible and delivers additional information to monitor Bet v 1 in different fractions of ambient air, compared to counting only birch pollen in assessing exposure to this ambient aeroallergen.

Methods

Ambient pollen count

Pollen was collected in a small park at 48°09'52.3" North, 11°35'35.4" East, 510 m above sea level and 1.80 m above ground in Munich in a Burkard pollen trap (Burkard Manufacturing Ltd., Hertfordshire, UK), 5 m away from the impactor (see below). This urban site was 260 m away from a 70.000 vehicles/day main road, separated by large trees and four-storied houses (Abt. Verkehrsplanung der Stadt München). Pollen count was compared to a reference pollen trap of the Stiftung Deutscher Polleninformationsdienst (PID), Bad Lippspringe, Germany, located about 4.4 km Southwest of our pollen trap. Pollen season was defined to start at 1% and end at 95% of seasonal pollen sum

(European Aerosol Network Database, <http://www.univie.ac.at>, accessed October 2008).

Ambient air sampling

Air was sampled at the same location and height as the Burkard pollen trap in a Rupprecht and Patashnick ChemVol® 2400 high-volume cascade impactor (Albany, NY, USA) from noon till noon next day. Flow was adjusted to 800 l/min with a Rupprecht and Patashnick flow measuring system (Albany) according the manufacturer's instructions. Prewashed polyurethane foam served as impacting substrate. Particulate matter (PM) in ambient air was fractioned into $>PM_{10}$, $10 \mu m > PM > 2.5 \mu m$, and $2.5 \mu m > PM > 0.12 \mu m$ (20). Each stage of a Chemvol® cascade impactor featured three identical, clearly separated collection slits. Thus the impacting substrates were cut in three identical parts and stored separately in polypropylene tubes at $-80^{\circ}C$ until extraction.

Extraction and analysis of Bet v 1

Bet v 1 was determined as described before (17). In brief: Impacting substrates were extracted with 0.1 M NH_4HCO_3 pH8.1 with 0.1% bovine serum albumin (BSA), lyophilized and redissolved in 1/10 of the original volume in phosphate buffered saline (PBS) (21). Bet v 1 was determined using a sandwich ELISA with the monoclonal antibodies 3B4F11D6 and 2E10G6G7 (Allergopharma Joachim Ganzer KG, Reinbek, Germany). All samples were determined at least in duplicate. Each assay was calibrated against purified natural Bet v 1 and included a positive control. Bet v 1 levels that were discernable but below our detection limit were treated as 2/3 of the detection level. Interassay variability was 14.0% ($n = 50$) in the higher range and 9.5% ($n = 44$) in the lower concentration range of the calibration curve. Limit of detection was 8.1 pg Bet v 1/m³.

All extracts were diluted such that the values were within the linear range of the calibration curve. Reported values were the mean of two filter segments.

Sequential birch pollen collection

About 15 catkins per sample were collected every other 2 days from all sides of the same *Betula pendula* trees from 3 weeks before pollination until all collected catkins were empty. Catkins were stored at room temperature for 1 day and sieved through 100 and 71 μm mesh sieves (Cisa Cedaccia Ltd., Barcelona, Spain). Pollen was stored at $-80^{\circ}C$ until analysis as described before (17).

Weather data

Weather data were obtained from the Deutscher Wetterdienst, Offenbach am Main, Germany (station 3379, Munich). Ozone data were from the Bayerisches Landesamt für Umweltschutz, Augsburg, Germany (station BY039, Lot-

hstrasse, Munich, Germany). Weather data were depicted as daily average at noon.

Immunogold labeling of Bet v 1

Samples from birch anthers were fixed anhydrously in saturated acrolein vapor. The specimens were embedded in Lowicryl K4M using the progressive lowering temperature technique.

Ultrathin sections were incubated for 30 min at 4°C with a specific monoclonal antibody to Bet v 1 (gift from Prof. Dr S. Vieths, Langen, Germany). Thereafter the grids were incubated for 2 h with goat anti-mouse IgG coupled with 10 nm colloid gold particles (British BioCell, Cardiff, UK). Finally, the grids were washed, fixed with 2% glutaraldehyde in PBS and analyzed by Scanning electronmicroscopy (SEM) (JEOL 1210, Tokyo, Japan). Unspecific binding was tested by either replacing the primary antibody with buffer or a nonspecific primary antibody of the same species.

Statistical analysis

Differences were analyzed with a paired Student's *t*-test unless stated otherwise. A *P*-value <0.05 was considered statistically significant.

Results

Birch pollen flight

The average seasonal birch pollen flight in Munich from 1994–2003 recorded by the official PID pollen trap lasted 27 ± 9 days with a cumulative count of 4431 ± 1946 pollen/m³/season and a birch pollen peak of 776 ± 621 pollen/m³ per 24 h that occurred on 19th of April ± 7 days (excluding the extreme year 1995 with 16 241 pollen/m³/season and a pollen peak of 4000 pollen/m³ per 24 h). The results from this trap were comparable to our pollen trap because in the years 2004–2007, when both traps were run simultaneously, no major difference in daily pollen count was discernable (n.s., *P* > 0.05). Our studied birch pollen seasons were all strong birch pollen flight years in Munich, Germany, except 2004 that was average. Pollen flight peak in 2005 was exceptionally strong and was only equaled by 1995. The days of maximal birch pollen peak occurred within the range reported for the preceding years (see Table 1 and Fig. 1), with 2007 peaking early on the 13th of April and 2006 peaking late on the 24th of April. The year 2007 was also exceptional because during the whole birch pollen season it did not rain.

Bet v 1 in ambient air

About 80 times more air was sampled with our impactor than with the Burkard pollen trap. This difference in volume was corrected for by standardization to cubic meter collected air. The seasonal sum of pollen and of allergen is given in Table 1.

In the >PM₁₀ fraction, $92.8 \pm 2.9\%$ of Bet v 1 (average of 4 years) was found, in the $10 \mu\text{m} > \text{PM} > 2.5 \mu\text{m}$, $6.9 \pm 2.9\%$, and in the $2.5 \mu\text{m} > \text{PM} > 0.12 \mu\text{m}$, only $0.5 \pm 0.2\%$. Intact pollen were detected in the $10 \mu\text{m} < \text{PM} < 2.5 \mu\text{m}$ stage, indicating blow-through or bounce-off. We tested standard diesel reference material NIST 2975 (Gaithersburg, MD, USA) and found that Bet v 1 disappeared from a solution dose dependently when diesel particles were added. One microgram of diesel particles were able to completely absorb about 150 pg of Bet v 1 (data not shown).

Bet v 1 in ambient air measured with the impactor correlated closely with pollen flight as determined with a Burkard pollen trap at the same location, see Fig. 1. Hazel pollen cross-reacted in our assay, as exemplified on March 27th, 2006. However, the birch pollen season in Munich is clearly separated from the hazel and alder pollen season and indeed no hazel or alder pollen were detected concomitant to birch pollen (see Fig. 1). Ash (*Fraxinus* species) pollen was concomitantly sampled with birch pollen but did not cross-react in our assay (Dr. B. Weber, personal communication). *Quercus* pollen, reported to contain a cross-reacting protein with Bet v 1 (22), flew in Munich after the birch pollen season.

Despite the same temporal trends in the concentration of Bet v 1 in ambient air and pollen flight, quantitatively pollen count did not correlate with Bet v 1 content of ambient air, see Figs 1 and 2. The peak in allergen content in air was before the main pollen peak in 2005. Later in the season of 2006, pollen flight was low but allergen content of air was high. More importantly, the average Bet v 1 release per pollen in 2007 was 115% more than the same amount in 2004. Within 1 year, if we exclude days <10 pollen/m³ per 24 h or <8.1 pg/Bet v 1/m³, the quotient of Bet v 1 in ambient air and pollen (i.e. Bet v 1 release per pollen) varied between 0.8 and 8.4 pg Bet v 1/pollen in 2007. A similar variation was observed in the other years (see Table 2).

The high value of 8.4 pg Bet v 1/pollen was similar to the Bet v 1 release from pollen collected directly from catkins of tree Antonien street (see Fig. 3), which at pollination contained 7.3 pg Bet v 1/pollen [assuming a pollen weight of 7.94 ng/pollen (21)].

Weather data

Of the four studied years, the weather during the prebirch pollen season from January 1st till March 31st, when anthers develop, was most different between 2006 and 2007, see Table 1. During this period, 2006 was the coldest, had the least sunshine and the fewest days above 2°C. The year 2007 was warmest, had the most sunshine and days above 2°C. Days above 2°C are birch flower growing days (23). Pollen peak was late in 2006 and early in 2007. However, these years were not the extremes in cumulative Bet v 1/m³ or Bet v 1 per pollen, see Table 1.

Weather briefly before pollination is depicted in Fig. 1 and in Table 3. In the week before pollination, 2007 was the warmest, the driest, and had the most sunshine of all years. The year 2004 was coldest, and had the least sunshine and had an average humidity compared to the other years. These

Table 1 Weather preceding birch pollen flight and birch pollen flight parameters for 2004 till 2007

	2004	2005	2006	2007
Pollen-flight				
Maximum birch pollen count (pollen/m ³ /24 h)	729	3522	1681	1080
Day of highest peak	16.04	16.04	24.04	13.04
Cumulative birch pollen count (pollen/m ³ /season)*	4766	10 839	7364	6617
Season length†	27	25	19	21
Bet v 1				
Maximum Bet v 1 (pg Bet v 1/m ³ /24 h)	282	2763	1593	1824
Cumulative Bet v 1 (pg Bet v 1/m ³ /season)*	4770	12 478	9643	14 235
Bet v 1 per pollen, average (pg/pollen)	1.001	1.151	1.304	2.151
Temperature (°C)‡				
Average	2.2 ± 4.9	1.2 ± 5.9	−0.5 ± 4.9	5.7 ± 3.7
Average daily min.	−0.9 ± 4.2	−2.2 ± 5.9	−3.7 ± 5.1	2.2 ± 3.3
Average daily max.	5.5 ± 6.1	4.8 ± 6.5	3.0 ± 5.0	9.5 ± 4.6
Days above 2°C§	40	37	21	79
Humidity (%)‡				
Average	72.5 ± 14.4	75.4 ± 10.5	79.5 ± 8.2	74.0 ± 9.3
Average at 7:30 h	79.2 ± 14.3	82.1 ± 11.6	85.8 ± 7.4	83.5 ± 10.0
Average at 14.30 h	64.5 ± 20.3	65.6 ± 15.7	71.1 ± 13.0	63.2 ± 15.7
Sunshine‡				
Average (h/day)	3.4 ± 3.9	3.7 ± 3.7	3.1 ± 3.7	3.8 ± 3.7
Cumulative (h)	304	359	280	339
Precipitation (mm/day)‡	2.4 ± 4.7	1.8 ± 3.3	1.9 ± 4.5	1.6 ± 2.5
Ozone (µg/m ³)‡	37.0 ± 16.2	33.2 ± 20.4	30.8 ± 24.8	35.8 ± 16.0

*Season for this parameter was from first to last day of sampling.

†Season for this parameter was defined as days between 1% till 95% of all pollen for that season.

‡Days from January 1st till March 31st.

§Days above 2°C are birch flower growing days, after van Vliet 2002.

2 years showed the biggest difference in cumulative Bet v 1/m³ and Bet v 1 release per pollen.

release any Bet v 1 until just before pollination (data not shown).

Sequential sampling of birch pollen

We collected birch pollen that was spontaneously released from anthers from about 10 days before pollination and also with low yield after pollination. The closer to pollination, the higher the yield of pollen (data not shown). From that pollen no Bet v 1 was released until about six or less days before pollination, see Fig. 3A. This was because of absent expression and not because of hindered release of Bet v 1 as immunogold staining showed very little Bet v 1 inside pollen grains > 6 days before pollination, see Fig. 3B. From 6 days before pollination, Bet v 1 release increased rapidly from zero to up to 9137 pg/10 mg pollen (7.3 pg Bet v 1/pollen). Pollen collected after pollination of that tree released little Bet v 1.

In 2005, we sampled pollen from the same trees and isolated pollen from catkins after freeze-drying the catkins. We freeze-dried the catkins to obtain even more unripe pollen that were not spontaneously released from catkins. Again, and also more distant from pollination, unripe pollen did not

Discussion

We found that Bet v 1 was congruent with birch pollen flight, supporting the notion that the only source of Bet v 1 is birch pollen. However, between 2004 and 2007, a 115% difference in allergen release from pollen existed. Moreover, within each year the potency of the pollen to release Bet v 1 varied 10-fold between days. The difference is probably because of the ripening process of Bet v 1 as allergen increased in the 6 days before pollination from zero to maximum. Thus, low potency or high-potency pollen can arise from the same catkin, depending on the day the pollen are emitted, which depends on local weather close to pollination.

In a previous study where we collected pollen directly from birch trees, pollen from 2003 released five times more allergen than those from the same trees in 2002 (17), and again pollen from 2004 from the same trees were stronger than those from 2003 (J. T. M. Buters, unpublished data). In the current study, 2004 was the year with the least potent pollen. These

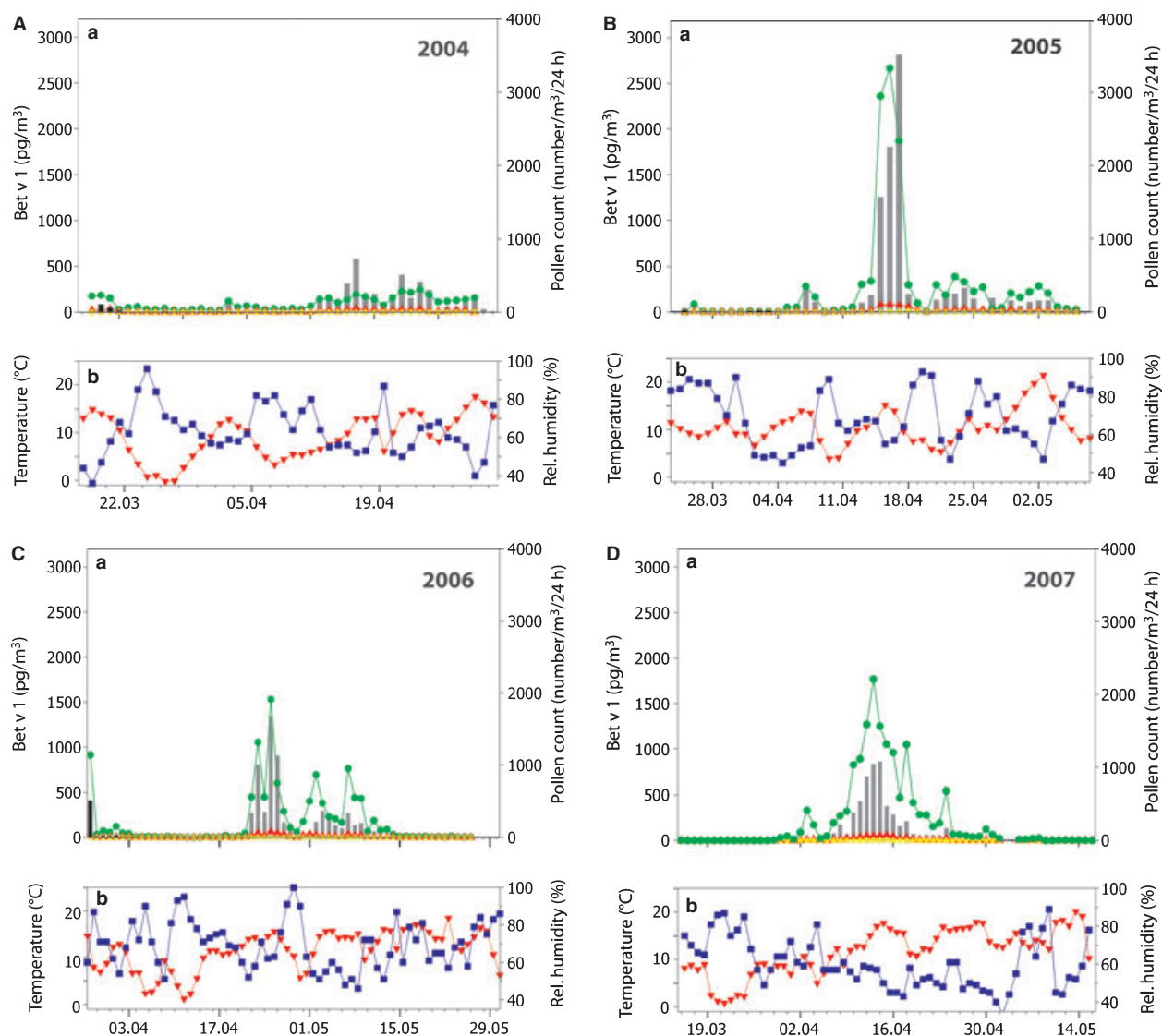


Figure 1 Bet v 1 content of ambient air in Munich, Germany from 2004 (A) till 2007 (D). (a) Bet v 1 content in the fractions particulate matter (PM) > 10 µm (—●—), 10 µm > PM > 2.5 µm (—▲—), and 2.5 µm > PM > 0.12 µm (—★—) on different days. Birch pollen flight is

depicted as shaded bars. Dark bars depict hazel pollen flight (only visible in 2004 and 2006) (b) Temperature (—▼—) and humidity (—■—) averages on each day.

lines of evidence point to even larger differences in Bet v 1 release from pollen from different years than the differences presented in this article.

Our ELISA detected >95% of the Bet v 1 proteins in a natural birch pollen extract. Natural birch pollen extract also contains nonallergic or low allergic isoforms (24), which we did not detect (data not shown). However, Bet v 1.0101 represents at least 50% of natural Bet v 1 (25), the low allergenic forms make up only a minority of the proteins. Pooled serum from ten birch pollen allergic individuals recognized the same isoforms as our ELISA antibodies did (data not shown). Thus we expect that our ELISA represents the allergenic content of birch pollen, also because skin prick reactions correlated with our measured Bet v 1 content (17).

More than 93% of the allergen was found in the PM > 10 µm fraction of ambient air, similar to what other authors reported (26–28). About 7% was also found in the 10 µm > PM > 2.5 µm fraction. We think this is because of the incomplete separation of pollen in the PM > 10 µm stage, a common feature of impactors (29). Birch pollen is 22 µm in diameter but their aerodynamic diameter is not defined (30). This implies that some intact pollen probably has deposited in the 10 µm > PM > 2.5 µm stage. Indeed, incomplete separation of pollen by high-volume sampling was reported (28).

Studies monitoring Bet v 1 in ambient air demonstrated that Bet v 1 is also present in fractions of ambient air that do not contain pollen, especially in the respirable PM_{2.5} (PM < 2.5 µm) fractions (26, 27, 31–35). Despite ample

Table 2 Bet v 1 content of pollen

		Unit	2004	2005	2006	2007
Bet v 1	Mean of days*	pg Bet v 1/pollen†	1.48 ± 1.30	1.73 ± 1.25	1.86 ± 1.41	2.97 ± 12.13
	Mean of season	pg Bet v 1/pollen‡	1.00	1.15	1.30	2.15
	Mean 1 week before pollen peak*	pg Bet v 1/pollen	0.68 ± 0.29	1.44 ± 0.57	1.00 ± 0.33	1.93 ± 0.92
	Maximum*	pg Bet v 1/pollen	5.76	6.45	6.67	8.44
	Minimum*	pg Bet v 1/pollen	0.33	0.25	0.46	0.81

*Calculation only used days >10 pollen/m³ and days >8.1 pg Bet v 1/m³ (detection limit of the ELISA).

†Calculated as average of allergen/m³ per day divided by pollen/m³ of that day.

‡Calculated as seasonal sum of allergen/m³ divided by seasonal sum of pollen/m³.

Table 3 Weather preceding pollination 1 week and birch pollen flight parameters for 2004 till 2007

	2004	2005	2006	2007
Pollen-flight				
Maximum birch pollen count (pollen/m ³ /24 h)	729	3522	1681	1080
Day of highest peak	16.04	16.04	24.04	13.04
Cumulative birch pollen count (pollen/m ³ /season)*	4766	10 839	7364	6617
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Cumulative Bet v 1 (pg Bet v 1/m ³ /season)*	4770	12 478	9643	14 235
Bet v 1 per pollen (pg/pollen)	1.001	1.151	1.304	2.151
Temperature (°C)				
Average	8.1 ± 2.6	10.4 ± 3.9	13.0 ± 1.4	13.0 ± 2.5
Average daily min.	3.8 ± 1.6	5.5 ± 3.3	7.6 ± 1.3	6.3 ± 1.9
Average daily max.	12.6 ± 3.9	15.0 ± 5.0	18.9 ± 2.6	19.5 ± 3.2
Days above 2°C‡	7	7	7	7
Humidity (%)				
Average	62.4 ± 10.7	63.0 ± 5.2	62.9 ± 6.8	57.1 ± 2.8
Average at 7 : 30 h	78.6 ± 11.8	81.9 ± 4.8	80.6 ± 9.0	74.3 ± 6.0
Average at 14 : 30 h	47.9 ± 14.6	49.0 ± 6.5	50.9 ± 11.9	40.7 ± 5.5
Sunshine				
Average (h/day)	4.7 ± 3.9	6.8 ± 3.5	7.8 ± 4.0	11.3 ± 2.0
Cumulative (h)	33.1	47.3	54.6	79.0
Precipitation (mm/day)	0.04 ± 0.08	0.03 ± 0.08	0.60 ± 0.11	0
Ozone (µg/m ³)	59.6 ± 10.0	54.1 ± 13.2	32.5 ± 65.4	59.4 ± 5.3

*From first to last day of sampling.

†Season was defined as days between 1% till 95% of all pollen for that season.

‡Days above 2°C are birch flower growing days, after van Vliet 2002.

evidence in the literature, in our fraction 2.5 µm > PM > 0.12 that contained the respirable particles, no allergen in none of the years was detected. We found that Bet v 1 was readily scavenged by other particles present in this fraction, i.e. adsorption to diesel exhaust particles collected in the same fraction (on-filter effect).

Simultaneous monitoring of birch pollen and the major allergen from these pollen showed several discrepancies, see Fig. 1 and the summary of results in Fig. 2 and Table 2. In general, allergen content in ambient air followed pollen counts (see Fig. 2). This points to birch pollen being the only

source of the allergen Bet v 1, in agreement with other authors who showed that other parts of birch trees contain little Bet v 1 (10, 11, 36, 37). However, there were major discrepancies between pollen count and allergen content of ambient air. Pollen in 2007 released 115% of the amount of Bet v 1 released in 2004. In 2005, the peak of allergen was 1 day ahead of the peak in pollen. Both in 2006 and 2007, two peaks of Bet v 1 occurred later in the season when pollen counts were low. Within 1 year, the allergen content per pollen varied day-to-day, and the minimum and maximum were at least 10-fold different, see Table 2.

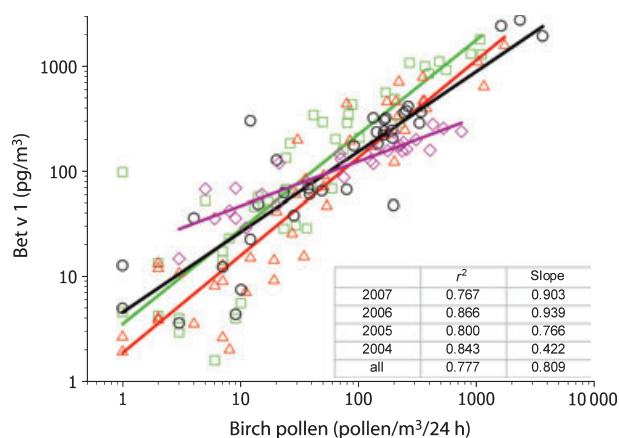


Figure 2 Correlation between pollen and allergen concentration in ambient air. Pollen count was compared to allergen concentration in ambient air 2007 (□), 2006 (△), 2005 (○), 2004 (◇). Coefficients of correlation (linear regression) and slopes are given.

Other authors also monitored Bet v 1 in ambient air and also described discrepancies between allergen content of ambient air and pollen flight (21, 26, 27, 38, 39). To our knowledge, no report on yearly variation in Bet v 1 release from ambient pollen is published. Taken together, pollen from different trees, from different regions (17), from different years, and from different days vary substantial in Bet v 1 release of their pollen. It is safe to assume a possible 10-fold difference between pollen.

The difference in allergen release from pollen is certainly related to weather conditions as other factors that influence allergen release like tree genetics, pollution and soil conditions are unlikely to change so rapidly. Weather conditions are given in Table 1. Anthers and pollen are generated in the season preceding pollination, but stay dormant until after a cold period temperature starts rising in late winter (40). Weather differences from late winter till pollination ('long term' weather) were most extreme between 2007 and 2006, but extremes in allergen release from pollen were not between these years.

Allergen release from birch pollen was zero in immature pollen. Only briefly before pollination, Bet v 1 release from pollen was detectable (see Fig. 3A). The lack of Bet v 1 release from pollen was not because of obstructed release of allergen, as immunogold labeling of Bet v 1 inside pollen also showed little Bet v 1 in immature pollen but ample Bet v 1 in mature pollen (see Fig. 3B). Thus, Bet v 1 is expressed only in the week before pollination. Other authors (10, 37) also suspected late expression of Bet v 1. After pollination few pollen were collected from overripe anthers, which released little Bet v 1. This shows that Bet v 1 is present in pollen only for a short period, and development of Bet v 1 is probably dependent on 'short term' weather conditions. Indeed, when we compared the temperature and sunshine in the week before pollination, then the years most contrasting in Bet v 1 release (2004 versus 2007) also showed the largest differences in 'short-term' weather (see Table 3). Number of pollen generated and released from anthers might be predicted using 'long-term' weather (41, 42); allergen release from pollen seems to depend on 'short term' weather.

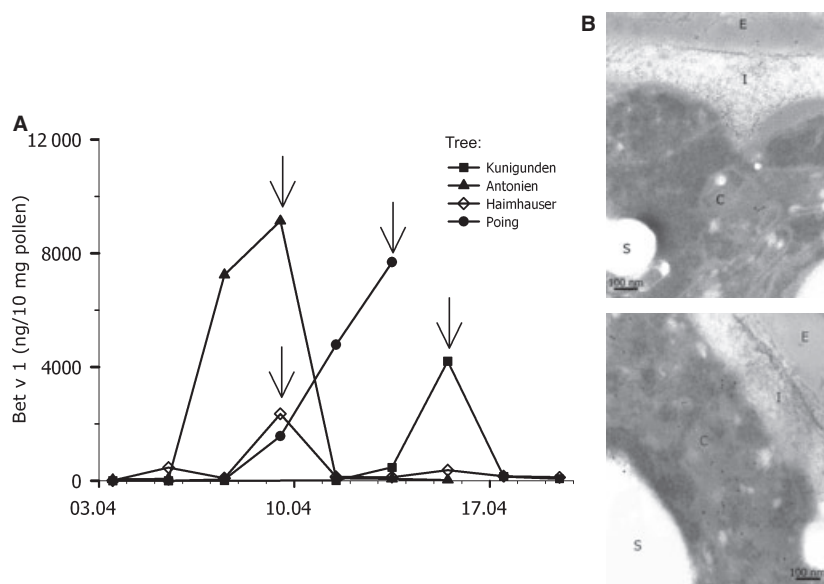


Figure 3 Development of Bet v 1 release from birch pollen. (A) Bet v 1 release from sequentially sampled birch pollen from the same trees in 2007. Arrows indicated pollination of that tree. (B) Example of Bet v 1 expression in birch pollen after immunogold

staining and transmission electron microscopic imaging of pollen collected from tree Antonien street on 29.3 and 12.4.2005. E, exine; I, intine; C, cytoplasm; S, starch granule. Bars indicate 100 nm.

Conclusions

In general, pollen count and allergen overlapped. Bet v 1 was almost exclusively found in the $PM > 10 \mu m$ fraction of ambient air, the fraction where birch pollen is collected in cascade impactors. The difference in estimation of allergen exposure between pollen counts and ELISA measurements can however be 10-fold or more. That pollen differs in allergen release is explained by a rapidly increasing expression of Bet v 1 in pollen in the week just before pollination. Depending on the day the pollen is released during ripening, its potency varies.

Whether allergen monitoring in ambient air is a better parameter than counting pollen needs to be proven by studies comparing symptoms with exposure.

Clinical implications

Exposure to Bet v 1 is not congruent with birch pollen count. Symptoms and efficacy of treatment might be difficult to

correlate with pollen counts, but perhaps better with allergen exposure.

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