

Year-to-Year Variation in Release of Bet v 1 Allergen from Birch Pollen: Evidence for Geographical Differences between West and South Germany

Jeroen T.M. Buters^a Anna Kasche^a Ingrid Weichenmeier^a Wolfgang Schober^a
Sabrina Klaus^a Claudia Traidl-Hoffmann^a Annette Menzel^b
Johannes Huss-Marp^a Ursula Krämer^c Heidrun Behrendt^a

^aDivision of Environmental Dermatology and Allergy GSF/TUM, ZAUM – Center for Allergy and Environment, Technical University Munich, Munich, ^bDepartment of Ecological Climatology, Technical University Munich, Freising, and ^cInstitut für Umweltmedizinische Forschung GmbH, Heinrich Heine Universität, Düsseldorf, Germany

Key Words

Birch · Pollen · Bet v 1 · Regional · Seasonal

Abstract

Background: The release of the aeroallergen Bet v 1 from pollen is a major determinant in the etiology of allergic airway disease due to birch pollen. **Objective:** We determined the release of the major birch pollen allergen Bet v 1 from pollen of birch trees growing in 2 different geographic regions in Germany for 2 consecutive years. **Methods:** Catkins were collected during pollination in 2002 and 2003 from 82 healthy trees in South (Munich) and West Germany (North Rhine-Westphalia). The release of Bet v 1 from pollen samples was determined by a Bet v 1-specific ELISA. **Results:** Pollen from South Germany released about 3 times more Bet v 1 than those from West Germany in both 2002 and 2003 ($p = 0.034$ and $p = 0.007$, respectively). This was independent of the number of pollen during the pollen flight season. In 2003, the release of Bet v 1 from pollen was more than 5 times higher than in 2002 in both regions (South Germany 6.1 times, $p < 0.001$; West Germany 5.4 times, $p = 0.003$). **Conclusions:** Despite large individual differences, there seem to be regional and year-to-year variations in Bet v 1 release

from birch pollen. Therefore, the combination of pollen count and release of Bet v 1 from this pollen must be assessed to estimate Bet v 1 exposure reliably.

Copyright © 2007 S. Karger AG, Basel

Introduction

Allergies are on the rise in the Western world, including allergies related to birch pollen [1–3]. About 20% of a study population of 1,159 adult Germans was sensitized to birch pollen [4]. The components of birch pollen (*Betula verrucosa* Ehrh. syn. *Betula pendula* Roth.) responsible for the observed allergic reactions are 7 unrelated proteins [5], available from <http://www.allergen.org> (accessed February 2007). In a European study [6], 98% of birch pollen-allergic individuals in Northern Europe were allergic to the protein Bet v 1 and a minority also to Bet v 2 (12–30%) or Bet v 4 (5–11%). In different climatic regions, like Switzerland and Italy, sensitization is also mainly to Bet v 1, but Bet v 2 or Bet v 4 sensitization make up 43% of the population.

The major birch allergen Bet v 1 is likely a plant steroid transporter that plays a role in plant defense and develop-

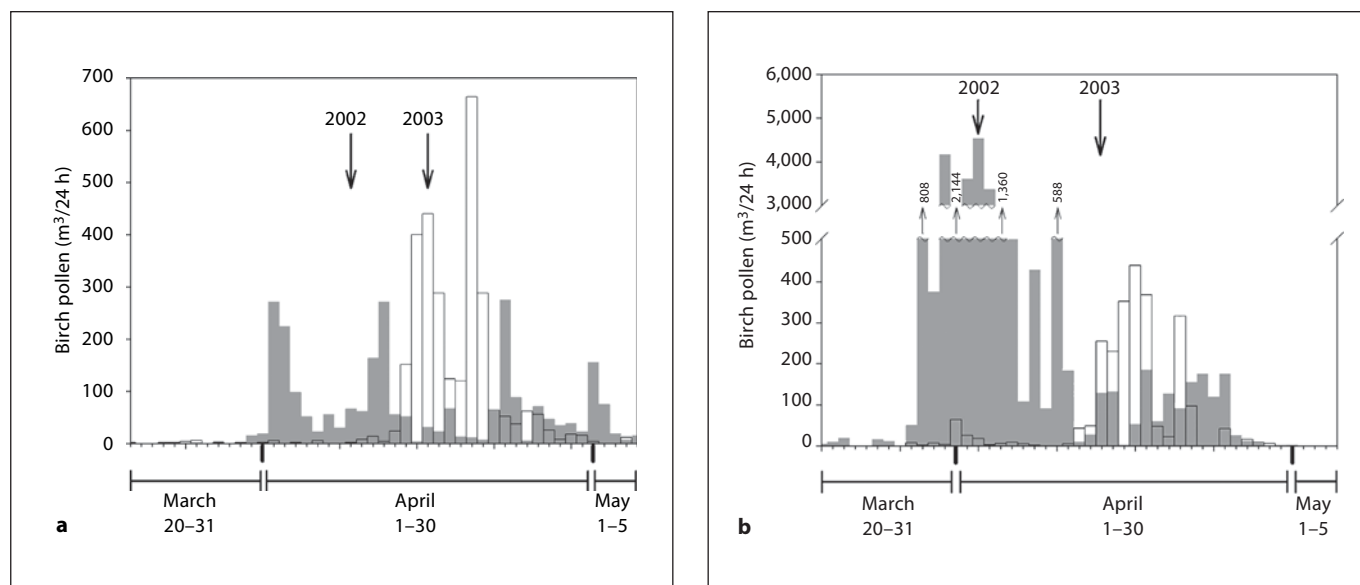


Fig. 1. Pollen flight in Munich (a) and North Rhine-Westphalia (b) in 2002 and 2003. Arrows indicate the time of sampling of birch catkins in each year and region. Shaded area is pollen flight in 2002.

ment [7]. It is a 17.4-kDa protein of which 42 isoforms are reported [8], but in extracts of pollen only about 9 isoforms were detected [7, 9, 10] with Bet v 1.0101 being the dominant allergenic isoform [11, 12]. Differences in allergenicity have been reported for some isomers of Bet v 1 [7, 13], including a lack of allergenicity for the Bet v 1.1001 (formerly Bet v 1l) isoform [14]. Bet v 2 is a profilin which cross-reacts with other profilins like Phl p 12 from grasses [15, 16] and Bet v 4 is a calcium-binding protein that cross-reacts with other polcalcins like Phl p 7 from timothy grass [17].

Although Bet v 1 is accepted as the major allergenic compound from birch pollen, additional compounds released from birch pollen, like pollen-associated lipid mediators, are also factors that may influence the allergic response to birch pollen [18–20].

Few studies have investigated the release of Bet v 1 from the pollen of individual birch trees [21]. Birch pollens are the exclusive source of Bet v 1 in ambient air [21, 22]. After release of pollen from the anthers, Bet v 1 can be redistributed, in part, from pollen into other components of ambient air, like starch granules or ambient particulate matter [23, 24]. The release of Bet v 1 at the source (pollen) is important to measure, as it will give an estimate of the actual exposure to Bet v 1 of people living near birch trees as is often the case in Central and Northern Europe [25, 26].

This study is part of the exposure assessment in the ongoing birth cohort study GINIplus (German Infant Nutrition Intervention study) [27]. As a part of this study we identified birch trees close to the children's dwellings in 2 geographic regions in Germany (40 trees in Munich and 42 trees in North Rhine-Westphalia), sampled catkins both in 2002 and 2003, and determined the Bet v 1 release of pollen of each individual tree.

Our objective was to investigate whether release of Bet v 1 from pollen is similar between trees growing in different geographical regions, and between years within these regions, in order to get additional information about exposure to Bet v 1 apart from birch pollen counts.

Methods

Birch Catkin Collection

Collection in 2002 was on 3 April in North Rhine-Westphalia, during the peak period of pollination in that region (pollen count from Bochum was considered as representative for that region; fig. 1a and b) and on 9 April in Munich, 3 days before a major birch pollen peak on 12 April. In 2003 we collected on 14 April in North Rhine-Westphalia, 3 days before the height of pollination, and on 16 April in Munich, during the peak of pollination.

About 50 catkins per tree were collected preferentially from the south side of a tree [28]. Each tree was characterized according to height, stem circumference at 1 m height, distance to high-traf-

fic roads, genus (*Betula pendula* or *Betula pubescens*), site of collection of the catkins (east, north, south or west), ripeness of the catkins and position to buildings (sunny, shaded). The same trees were sampled in both years. The catkins were put into a glass-house at 13% humidity, 28°C for 1 day. The spontaneously released pollen was then sifted through 100- and 70- μ m sieves, weighed, and stored in 5-ml glass HPLC vials sealed with rubber caps (Chromacol, Herts, UK) at -70°C until analysis. Samples were analyzed within 3 months.

Extraction and Bet v 1 Determination

Birch pollens were extracted at 5 mg pollen/ml in 0.1 M NH_4HCO_3 , pH 8.1, in 15-ml polypropylene tubes (Greiner, Frickenhausen, Germany), after 1 min vortexing and 4 h in an end-over-end rotator (Heidolph, Schabach, Germany) at 100 rpm at room temperature in the dark. The mixture was transferred to 2-ml Eppendorf vials (Hamburg, Germany), centrifuged for 5 min at 13,000 g, and the supernatant was collected. To 400 μ l extract, BSA was added to make 0.1% w/v and the samples were lyophilized and stored at 4°C until analysis.

This procedure of weighing and extracting pollen was performed with at least 3 separate samples of pollen from each tree. The Bet v 1 content of each extract was then measured 3 times on different days in duplicate (a total of 18 ELISA wells per tree).

Bet v 1 levels were determined using a sandwich ELISA with 2 Bet v 1-specific antibodies (MAK 3B4F11D6 and 2E10G6G7; Allergopharma Joachim Ganzer KG, Reinbek/Hamburg, Germany) in a 96-well Maxisorb microtiter plate (Nunc, Wiesbaden, Germany) as described previously [29] with 3,3',5,5'-tetramethylbenzidine (Sigma, Deisenhofen, Germany) as substrate. A standard curve with affinity-purified Bet v 1 (Allergopharma Joachim Ganzer KG) and a positive control, both in duplicate, were run with each assay. Only values within the linear part of the calibration curve were reported.

The antibody combination used for our sandwich ELISA recognizes 6 isoforms of Bet v 1, including Bet v 1.0101, detecting 95% of all isoforms present in a crude birch pollen extract [Dr. B. Weber, Allergopharma Joachim Ganzer KG, pers. commun.]. An aliquot of an extract of birch pollen was analyzed with each assay as a positive control to guarantee assay reproducibility. With this extract the intraday variability of the assay was 6.3% (n = 10). The day-to-day variability of the assay was 12% (n = 41).

Pollen Flight Data

Pollen flight data (birch pollen/ $\text{m}^3/24$ h) were obtained from 2 reference stations using Burkard pollen traps (Burkard Manufacturing Ltd., Hertfordshire, UK) of the Stiftung Deutscher Polleninformationsdienst, Bad Lippspringe, Germany. One trap was located in North Rhine-Westphalia in Bochum, Bergmannsheil (15 m above ground, 76 m above sea level). The other trap in the south of Germany in Munich was located at the Dermatology Clinic of the Ludwig Maximilians University (15 m above ground, 525 m above sea level). Yearly pollen data were the sum of all daily values during the birch pollen season in Germany.

Weather Data

Weather data were obtained from the Deutscher Wetterdienst, Offenbach (Main), Germany. Airport Greven (station No. 1766) was representative for North Rhine-Westphalia rural, Duisburg-Friemersheim (station No. 1084) for North Rhine-Westphalia ur-

ban, airport Erding-Moos (station No. 1262) for Bavaria rural and Munich city (station No. 3379) for Bavaria urban. Light intensity data for Duisburg-Friemersheim were not recorded and the values of Düsseldorf airport (station No. 10400, about 30 km from Duisburg-Friemersheim) were used. Ozone values for Munich (station No. BY037) and the airport Erding-Moos were obtained from Bayerisches Landesamt für Umweltschutz, Augsburg and for Greven (Ladbergen, station No. NW093) and Duisburg-Walsum (station No. NW034) from Umweltbundesamt, Berlin, Germany.

Skin Prick Testing

After obtaining informed consent, 10 birch pollen-allergic patients with allergic rhinoconjunctivitis (7 female, 3 male; age 35.8 ± 10.3 years; Bet v 1-specific IgE RAST class ≥ 3) and 3 non-atopic healthy subjects (1 female, 2 male; age 33.7 ± 12.0 years; RAST class 0) were included in the study. All individuals were subjected to skin prick testing with (1) a 1:10 dilution of an extract of 10 mg/ml pollen in PBS pH 7.4 without BSA, and (2) recombinant Bet v 1.0101 allergen (16 μ g/ml diluted in PBS; Biomay, Vienna, Austria). Skin prick testing of a saline solution, birch pollen skin prick solution (44 μ g Bet v 1/ml; Allergopharma) and a 1% w/v histamine solution served as negative and positive controls, respectively. Ten microliters of each solution was skin prick tested simultaneously with prick test lancets (Allergopharma) within a double-blind, intraindividually randomized trial in all subjects, once at both volar forearms of the subjects. After 20 min, each skin reaction was evaluated and wheal and erythema were measured manually and documented. In addition, planimetry was performed and skin reactions were photographed.

Statistical Analysis

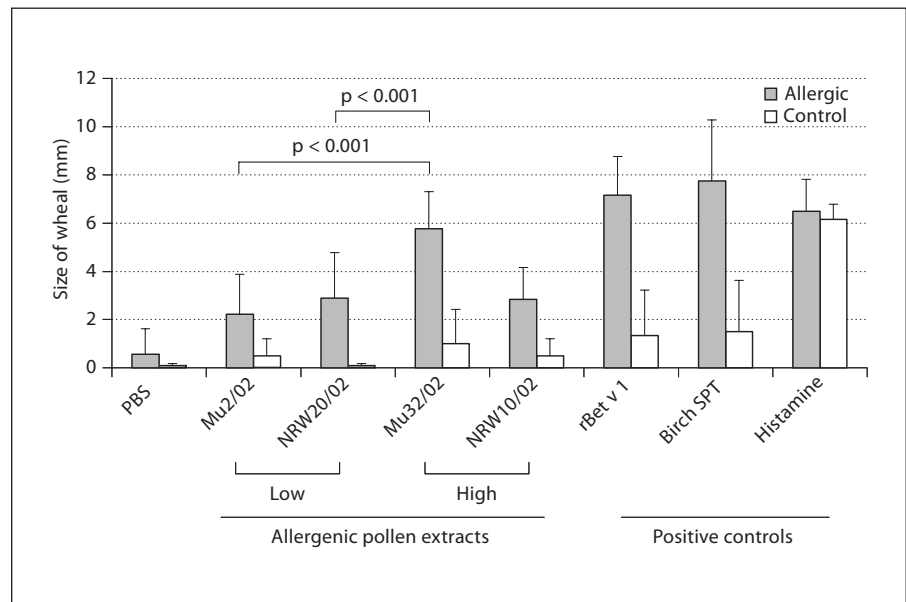
Differences between groups were analyzed by a paired t-test of the log-transformed data [30]. Values below the detection limit but with a discernable Bet v 1 content were treated as two thirds of the detection limit (that is 4 ng/ml Bet v 1). For the regional and year-to-year comparison, only those trees were evaluated that could be sampled in both years. Means were calculated as arithmetic means \pm SD, unless otherwise indicated. Then, geometric means with a 95% confidence interval were calculated instead, the log transformation correcting for skewed data.

Results

Extraction and Stability of Bet v 1 from Birch Pollen

Extraction with 0.1 M NH_4HCO_3 pH 8.1 at room temperature yielded about 20% more Bet v 1 than with PBS (data not shown). Also, Bet v 1 was more stable in NH_4HCO_3 than in PBS. Increasing the temperature of extraction to 37°C resulted in lower yields of Bet v 1. For these reasons we selected an extraction optimum of 4 h with 0.1 M ammonium bicarbonate pH 8.1 for all subsequent experiments. Ammonium bicarbonate has the additional advantage of evaporating upon lyophilization. Lyophilizing the samples reduced the Bet v 1 content by

Fig. 2. Wheal size after skin prick testing allergic subjects (n = 10) and controls (n = 3) with low Bet v 1 containing (Mu2 and NRW20/02) and high Bet v 1 containing (Mu32 and NRW10/02) birch pollen extracts. Data are presented as means \pm SD. Mu = Munich; NRW = North Rhine-Westphalia; PBS = phosphate buffered saline; rBet v 1 = recombinant Bet v 1.0101; SPT = skin prick test.



4.9% compared to prior to lyophilization (n = 41, p < 0.01). Aliquots of the same Bet v 1 extract were stable for at least 150 days. The procedure of determining the Bet v 1 content of pollen from 1 tree by repeatedly weighing, extracting and determining the Bet v 1 content of pollen of that sample limited the variability of Bet v 1 determination to 11% (n = 121).

Using these conditions, the extractable Bet v 1, divided by total extractable protein of pollen, was 0.95% in North Rhine-Westphalia in 2002 and 3.94% in 2003. In Munich the Bet v 1 content was 1.06% of extractable total protein in 2002 and 7.4% in 2003 (about 0.05% of Bet v 1 per total pollen weight, n = 30).

The release of Bet v 1 from birch pollen within each region varied from below detection (<6.2 ng Bet v 1/10 mg pollen) to 15,028 ng Bet v 1/10 mg pollen.

Skin Prick Testing

The variation in Bet v 1 content of extracts from birch pollen from different trees was also confirmed in skin prick test reactions with birch pollen-allergic individuals. In a trial with 10 allergic subjects and 3 nonallergic controls, a wheal similar to the histamine-positive control was obtained in most allergic subjects with a 1:10 dilution of 10 mg birch pollen/ml extract of pollen with a high release of Bet v 1 (fig. 2). Extracts with a low Bet v 1 content gave limited wheals (diameter 2.0 ± 1.7 and 2.6 ± 2.0 mm) and extracts with a high Bet v 1 content gave large wheals (diameter 5.2 ± 2.3 mm). The difference in wheal

size was significant between extracts of trees Mu2 and NRW20 (low allergenic) and tree Mu32 (high allergenic) from 2002 (both p < 0.001). From tree NRW 10, the predicted high allergenicity from the ELISA values was not confirmed in the skin prick test.

Weather Data

The climate was different in both regions of our study during the period from 1 January to 1 May, the period relevant for birch pollen development. Within each region an urban and a rural location were investigated as being representative for weather characteristics of that area (table 1). As expected, North Rhine-Westphalia was warmer in that period than Munich, had more days $\geq 5^\circ\text{C}$, and received more precipitation and generally less sunshine in both seasons. 2003 was colder in average temperature in the period from 1 January to 1 May, 2003 had fewer days $\geq 5^\circ\text{C}$, and received less precipitation but more sunshine (cumulative and average) than 2002 in both regions.

Regional and Year-to-Year Differences in Birch Pollen Flight

The pollen season (days >15 pollen/ $\text{m}^3/24$ h) was long in 2002, about 30 days in both regions, but brief in 2003 (about 17 days; fig. 1a and b). About 10 times more birch pollen was released in North Rhine-Westphalia in 2002 (Bochum, 23,961 pollen/ m^3 /season) than in 2003 (2,557 pollen/ m^3 /season). Four additional Burkard pollen traps spread over the region of North Rhine-Westphalia showed

Table 1. Climate characteristics for rural and urban North Rhine-Westphalia and Bavaria in 2002 and 2003

	North Rhine-Westphalia				Bavaria			
	rural ^a		Duisburg, urban		rural ^a		Munich, urban	
	2002	2003	2002	2003	2002	2003	2002	2003
Temperature, °C								
Average	6.59	4.97	7.65	6.42	4.65	2.30	5.61	3.49
Minimum	2.92	0.56	4.49	2.75	-0.92	-3.06	1.53	-6.64
Maximum	10.28	9.38	11.06	10.50	9.22	7.39	10.08	8.01
40 days before 1 April ^c	6.4	6.5	7.7	8.3	6.4	5.2	5.5	3.6
Days with average above 5°C ^b	84 (725)	59 (564)	89 (856)	72 (725)	75 (674)	43 (471)	69 (572)	59 (564)
Humidity, %								
Average	76.4	74.5	73.4	68.5	73.8	76.7	73.8	66.8
7:00 h	86.1	85.1	84.1	79.9	83.8	88.3	84.4	77.0
13:00 h	67.3	64.2	61.9	55.7	61.1	65.8	60.1	56.7
Sunshine								
Average, h/day	3.80	4.85	3.91 ^d	5.26 ^d	4.66	5.05	4.87	4.93
Cumulative, h ^e	460	586	473 ^d	637 ^d	563	611	588	595
Precipitation, mm/day ^f	20.7	15.7	24.6	18.8	14.04	9.18	14.50	12.08
Ozone, mg/m ³ /day	34.4 ^g	33.1 ^g	33.7	31.7	46.3	27.3	32.8	41.6

Data are presented as averages from 1 January to 1 May.

^a Values measured at airport Greven (North Rhine-Westphalia) and airport Erding-Moos (Bavaria).

^b The sum of temperature between 1 January and 1 May is given in parentheses.

^c Average temperature 40 days before 1 April [44].

^d Values of Düsseldorf airport (about 30 km distance).

^e Sum of sunshine hours between 1 January and 1 May.

^f Measured from 7:30 to 7:30 h the next day.

^g Values of Ladbergen.

similar birch pollen counts in 2002 (sampled in a shorter period from 4 to 25 April: Wesel 9,442 pollen/m³/season, Borken 6,132 pollen/m³/season, Bocholt 14,758 pollen/m³/season, Duisburg 9,015 pollen/m³/season; compared to Bochum 8,090 pollen/m³/season in the same period). The peak concentration of birch pollen in Bochum, North Rhine-Westphalia in 2002 was also about 10 times higher than in 2003 (4,544 birch pollen/m³/24 h in 2002 and 440 birch pollen/m³/24 h in 2003).

In Munich, the season for birch pollen was similar to North Rhine-Westphalia: about 32 days in 2002 and about 17 days in 2003. In contrast to North Rhine-Westphalia, the release of birch pollen in Munich over the whole season was comparable in both years (2,666 pollen/m³/season in 2002 and 2,968 pollen/m³/season in 2003). In Munich, pollen were released in 4 peaks in 2002, whereas in 2003 they were released as 2 somewhat higher peaks (4 peak pollen counts around 280 pollen/m³/24 h in 2002; a peak count of 440 pollen/m³/24 h and another one of 664 pollen/m³/24 h in 2003).

Regional Differences in Release of Bet v 1

A 3-fold regional difference in release of Bet v 1 from birch pollen was seen between the populations of trees that were sampled in Munich and North Rhine-Westphalia, both in 2002 and in 2003. Munich exhibited a higher release of Bet v 1 from pollen than North Rhine-Westphalia (Munich versus North Rhine-Westphalia: 2.9 times in 2002, $p = 0.034$; 3.3 times in 2003, $p = 0.007$; fig. 3). Our conclusions were not altered by removing trees of the genus *B. pubescens* from our analysis (1 in North Rhine-Westphalia and 4 in Munich).

Year-to-Year Differences in Release of Bet v 1

The release of Bet v 1 from birch pollen varied substantially in each season. In 2003 the geometric mean values for Bet v 1 in both regions were more than 5 times higher than in 2002 (5.4 times in North Rhine-Westphalia, $p = 0.003$; 6.1 times in Munich, $p < 0.001$; fig. 3).

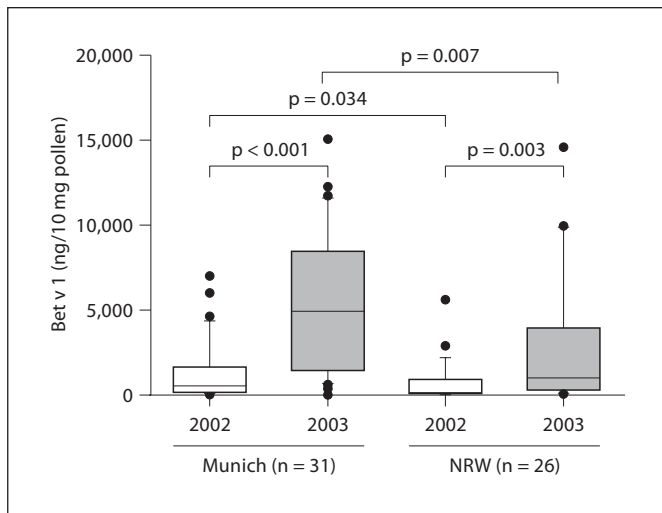


Fig. 3. Release of Bet v 1 from birch pollen from 2 regions of Germany in 2 successive years. NRW = North Rhine-Westphalia. 10, 25, 75 and 90% confidence intervals, geometric means and number of trees are given. Only those trees that were sampled in both years are depicted.

Discussion

In this paper we report the release of the major birch allergen Bet v 1 from pollen of individual trees, located at different geographical regions in the west and south of Germany in 2 consecutive years. The mean release of Bet v 1 from birch pollen differed significantly between years, with pollen from 2003 having a more than 5 times higher release of Bet v 1 than pollen from 2002, pointing to a year-to-year variability. This year-to-year difference was the same for both regions of Germany. A regional difference of 3 times in the release of Bet v 1 from birch pollen was observed, with Munich pollen liberating more allergen than pollen from North Rhine-Westphalia, indicating a higher Bet v 1 concentration in birch trees in the south. Both observations were independent of birch pollen counts.

We collected birch pollen by cutting catkins from birch trees, excluding cross-contamination with hazel (*Corylus avellana*) or alder (*Alnus glutinosa*) pollen. These pollen would have cross-reacted in our ELISA [29]. We do not know whether our method recognizes all Bet v 1 isoforms. Currently, 37 isoforms of Bet v 1 are described, but it is questionable how many of these are true naturally occurring isoforms. For Bet v 1.0101, which makes up at least 50% of birch pollen Bet v 1 allergens [12], it was shown that a skin prick test solution with this isoform is sufficient for

diagnosis of birch pollen allergy [31]. Purified Bet v 1 from birch pollen could be separated into 6 or 9 isoforms [10, 12]. We identified 9 isoforms of Bet v 1 in a purified birch pollen extract (data not shown), of which Bet v 1.0101 and 5 other isoforms were recognized in our sandwich ELISA. Also, a Bet v 1-depleted birch pollen extract was produced by using affinity purification with monoclonal antibodies 3B4 or 2E10, and immunoblotting with a birch pollen-allergic patients' serum pool did not reveal remaining Bet v 1. Therefore, we estimate that the assay is suitable for the quantification of at least 95% of Bet v 1 in our extracts.

The extracted amount of Bet v 1 of 0.05% of total pollen mass was in agreement with that of Schappi et al. [32], who observed levels of 0.07%. Our values for Bet v 1 per milligram of protein are between 1 and 7.4% of total extractable protein, which is about half of that reported by others (13.9%) [32]. The differences in release of Bet v 1 compared to Schappi et al. [32] can be explained by our longer extraction procedure which was aimed at maximum release of Bet v 1. This also yielded more total protein, resulting in a higher release of Bet v 1 per pollen, but a lower Bet v 1 content per milligram of protein. Other authors with other extraction buffers have reported a more rapid release from birch pollen [33]. However, because the Bet v 1 protein was stable in our extraction buffer we opted for complete Bet v 1 extraction. Additionally, our extraction buffer mimics the pH of the nasal fluids of allergic patients, which is also slightly alkaline [34].

Extracts of birch pollen were skin pricked and those with a low Bet v 1 content provoked limited reactions in the skin of birch-allergic subjects, whereas pollen with a high Bet v 1 content provoked a strong wheal reaction (see Results; fig. 2). Thus, the measured release of Bet v 1 correlated with the clinical signs and symptoms of pollen allergy. It cannot be excluded that allergic inhabitants of North Rhine-Westphalia react differently than those in Munich. The clinical effects for tree NRW10 did not discriminate this tree, which could be due to the presence of a hypoallergenic isoform of Bet v 1 in this particular tree [14].

The release of Bet v 1 from pollen is known to depend on the climatic conditions and side of the tree sampled [28, 35, 36]. The composition of the soil, the stage of the natural cycle the tree is in [37, 38], and the health status and genus of a tree are all likely to be important factors [39], as is well known for many commercially used plants [40]. Components of air pollution such as NO₂ can also influence the release of allergen from pollen [21, 41].

North Rhine-Westphalia was warmer and received more rain than Munich in both years (table 1). A region-

Table 2. Parameters of Bet v 1 exposure in 2 regions in Germany

	2002	2003	Ratio 2002/2003
North Rhine-Westphalia			
Peak pollen count, pollen/m ³ /24 h	4,544	440	0.10
Season pollen count, pollen/m ³ /season	23,961	2,557	0.11
Bet v 1 release, ng/10 mg pollen	176 (81–380) ^a	950 (499–1,810) ^a	5.40**
Peak Bet v 1 exposure, ng/m ³ /24 h ^b	0.63	0.33	0.52
Season Bet v 1 exposure, ng/m ³ /season	3.35	1.93	0.58
Munich			
Peak pollen count, pollen/m ³ /24 h	276	664	2.41
Season pollen count, pollen/m ³ /season	2,666	2,968	1.11
Bet v 1 release, ng/10 mg pollen	518 (273–982) ¹	3,135 (1,781–5,520) ¹	6.05***
Peak Bet v 1 exposure, ng/m ³ /24 h ^b	0.11	1.65	14.50
Season Bet v 1 exposure, ng/m ³ /season	1.10	7.39	6.74
South/west difference (Bet v 1 release)	2.94*	3.30**	

Exposure can be estimated either by birch pollen count, Bet v 1 release from pollen or, as proposed in this paper, as content of Bet v 1/m³ of air. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

^a Geometric means (with 95% confidence interval in parentheses) of trees sampled in both years.

^b Birch pollen grain weight according to Schappi et al. [23] (7.94 ng/pollen).

al difference in the release of Bet v 1 was observed between North Rhine-Westphalia in the west of Germany and Munich in the south during 2002 and 2003. The release of Bet v 1 from pollen was higher when temperature and humidity were lower (table 1). None of the observed weather parameters showed a significant correlation with release of Bet v 1 from birch pollen. Nevertheless, trends might be identified over longer periods of observation [42].

Bet v 1 is expressed late in pollen development [22, 43] and individual birch trees have their own pattern of pollen development. We therefore cannot exclude that differences in ripeness of the harvested birch pollen confounded our results. Indeed, the observed variation in Bet v 1 release from pollen from individual trees might be due to this effect. However, we analyzed 31 trees in Munich and 26 in North Rhine-Westphalia, each year close to the maximal peak of pollination in that area. The average of all trees in Bet v 1 release from pollen is taken as representative for that region for that year. In Munich in 2002 we sampled 3 days before maximal pollination for that area and in 2003 on the maximal peak of pollination (fig. 1a). For North Rhine-Westphalia this was exactly the opposite, we sampled on the peak of maximal pollination in that area in 2002 and 3 days before in 2003 (fig. 1b). In our results, however, in 2002 in both regions the release of Bet v 1 from pollen was at least 5 times lower than in 2003, and between regions in both years, pollen from

Munich released 3 times more allergen than those from North Rhine-Westphalia (fig. 3; table 2). These findings are more consistent with regional and year-to-year differences in Bet v 1 release from pollen than with differences in ripeness of the harvested pollen.

The large intratree variation within each region demonstrates that factors other than climatic ones can influence the release of Bet v 1 from pollen, as is also exemplified by the 2- to 3-year rhythm in birch pollen flight noticed by other authors [38, 44]. It is also possible that the populations in Munich and North Rhine-Westphalia are genetically different.

We sampled the same trees in 2 consecutive years and found that birch pollen in 2003 contained about 5 times more Bet v 1 than in 2002 in both regions of Germany (fig. 3). Pollen counts or the sum of pollen released during a season in Munich in 2002 (2,666 pollen/m³/season) was not very different from 2003 (2,968 pollen/m³/season), but the allergen release during each season was different. Thus, to estimate exposure of a population to the major birch allergen Bet v 1, both pollen counts and release of Bet v 1 of that pollen must be known. The exposure to pollen in 2003 in Munich according to the peak birch pollen count was 2.4 times higher than in 2002 (table 2). The actual release of Bet v 1 from pollen was 6.1 times higher in 2003 than in 2002 ($p < 0.001$). Thus, peak exposure to Bet v 1 in Munich in 2003 was 14.5 times higher than in 2002 (more pollen with more Bet v 1 in 2003). If we ac-

count for the whole season, then exposure to Bet v 1 in Munich was 6.7 times higher in 2003 than in 2002.

Using the same calculations for North Rhine-Westphalia in 2003 (table 2), Bet v 1 peak exposure was 0.52 times that of 2002 (10 times less pollen in 2003 with a 5 times higher release of Bet v 1), and for the whole season the exposure was 0.58 times lower in 2003, showing that Bet v 1 exposure in 2002 was higher than in 2003 in that region. The high release of pollen in Bochum, North Rhine-Westphalia in 2002 was not an aberration, as other traps in North Rhine-Westphalia showed similar high pollen counts in 2002.

Differences in exposure to Bet v 1 from birch pollen could influence allergic symptoms and sensitization of individuals. Thus, determining the release of Bet v 1 in addition to pollen count may be a valuable parameter to

environmental exposure studies [25, 26]. Our results also provide a rationale to evaluate whether differences in release of Bet v 1, independent of the number of pollen, show consequences in patients in both epidemiological and clinical settings.

Acknowledgments

We thank Dr. Bernhard Weber and Prof. Dr. Helmut Fiebig (Allergopharma Joachim Ganzer KG) for providing antibodies and standards for Bet v 1 and their helpful discussions in preparing the manuscript. The technical assistance of Antje Wallmuth and Christine Weil, and Elke Link's help in calculating the results are also greatly appreciated. Part of this study was funded by a grant from the German Ministry of Environment (FKZ: 20462296).

References

- Schäfer T, Ring J: Allergien. *Dtsch Med Wochenschr* 2002;127:781–783.
- Krämer U, Link E, Oppermann H, Ranft U, Schäfer T, Thriene B, Behrendt H, Ring J: Studying school beginners in western and eastern Germany: allergy trends and sensitizations 1991–2000. *Gesundheitswesen* 2002; 64:657–663.
- Stevens WJ, Ebo DG, Hagendorens MM, Bridts CH, De Clerck LS: Is the prevalence of specific IgE to classical inhalant aeroallergens among patients with respiratory allergy changing? Evidence from two surveys 15 years apart. *Acta Clin Belg* 2003;58:178–182.
- Nowak D, Heinrich J, Jörres R, Wassmer G, Berger J, Beck E, Boczor S, Claussen M, Wichmann HE, Magnussen H: Prevalence of respiratory symptoms, bronchial hyperresponsiveness and atopy among adults: West and East Germany. *Eur Respir J* 1996;9: 2541–2552.
- Vieths S, Scheurer S, Ballmer-Weber B: Current understanding of cross-reactivity of food allergens and pollen. *Ann NY Acad Sci* 2002;964:47–68.
- Movérare R, Westritschnig K, Svensson M, Hayek B, Bende M, Pauli G, Sorva R, Haah-tela T, Valenta R, Elfman L: Different IgE reactivity profiles in birch pollen-sensitive patients from six European populations revealed by recombinant allergens: an imprint of local sensitization. *Int Arch Allergy Immunol* 2002;128:325–335.
- Markovic-Housley Z, Degano M, Lamba D, von Roepenack-Lahaye E, Clemens S, Susani M, Ferreira F, Scheiner O, Breiteneder H: Crystal structure of a hypoallergenic isoform of the major birch pollen allergen Bet v 1 and its likely biological function as a plant steroid carrier. *J Mol Biol* 2003;325:123–133.
- Chapman MD, Pomés A, Breiteneder H, Ferreira F: Nomenclature and structural biology of allergens. *J Allergy Clin Immunol* 2007; 119:414–420.
- Friedl-Hajek R, Radauer C, O'Riordain G, Hoffmann-Sommergruber K, Leberl K, Scheiner O, Breiteneder H: New Bet v 1 isoforms including a naturally occurring truncated form of the protein derived from Austrian birch pollen. *Mol Immunol* 1999;36: 639–645.
- Batard T, Didierlaurent A, Chabre H, Mothes N, Bussières L, Bohle B, Couret MN, Ball T, Lemoine P, Focks Tejkl M, Chenal A, Clément G, Dupont F, Valent P, Krauth MT, André C, Valenta R, Moingeon P: Characterization of wild-type recombinant Bet v 1a as a candidate vaccine against birch pollen allergy. *Int Arch Allergy Immunol* 2005;136:239–249.
- Kraft D, Ferreira F, Vrtala S, Breiteneder H, Ebner C, Valenta R, Susani M, Breitenbach M, Scheiner O: The importance of recombinant allergens for diagnosis and therapy of IgE-mediated allergies. *Int Arch Allergy Immunol* 1999;118:171–176.
- Swoboda I, Jilek A, Ferreira F, Engel E, Hoffmann-Sommergruber K, Scheiner O, Kraft D, Breiteneder H, Pittenauer E, Schmid E, et al.: Isoforms of Bet v 1, the major birch pollen allergen, analyzed by liquid chromatography, mass spectrometry, and cDNA cloning. *J Biol Chem* 1995;270:2607–2613.
- Hartl A, Kiesslich J, Weiss R, Bernhaupt A, Mostböck S, Scheibhofer S, Flöckner H, Sippl M, Ebner C, Ferreira F, Thalhamer J: Isoforms of the major allergen of birch pollen induce different immune responses after genetic immunization. *Int Arch Allergy Immunol* 1999;120:17–29.
- Ferreira F, Hirtenlehner K, Jilek A, Godnik-Cvar J, Breiteneder H, Grimm R, Hoffmann-Sommergruber K, Scheiner O, Kraft D, Breitenbach M, Rheinberger HJ, Ebner C: Dissection of immunoglobulin E and T lymphocyte reactivity of isoforms of the major birch pollen allergen Bet v 1: potential use of hypoallergenic isoforms for immunotherapy. *J Exp Med* 1996;183:599–609.
- Andersson K, Marknell Dewitt A, Mattsson L, Ostling J, Lilja G, Nordlund M, Ballmer-Weber B, Scheurer S, Wangorsch A, Vieths S, Lidholm J: Critical assessment of interchangeability of cross-reactive food allergens in diagnostic tests for specific IgE. 26th Symposium of the Collegium Internationale Allergologicum, Malta, 2006, p 30.
- Ghunaim N, Grönlund H, Kronqvist M, Grönneberg R, Söderström L, Ahlstedt S, van Hage-Hamsten M: Antibody profiles and self-reported symptoms to pollen-related food allergens in grass pollen-allergic patients from northern Europe. *Allergy* 2005; 60:185–191.
- Neudecker P, Nerkamp J, Eisenmann A, Nourse A, Lauber T, Schweimer K, Lehmann K, Schwarzinger S, Ferreira F, Rösch P: Solution structure, dynamics, and hydrodynamics of the calcium-bound cross-reactive birch pollen allergen Bet v 4 reveal a canonical monomeric two EF-hand assembly with a regulatory function. *J Mol Biol* 2004;336:1141–1157.
- Traidl-Hoffmann C, Kasche A, Jakob T, Huger M, Plötz S, Feussner I, Ring J, Behrendt H: Lipid mediators from pollen act as chemoattractants and activators of polymorphonuclear granulocytes. *J Allergy Clin Immunol* 2002;109:831–838.

- 19 Plötz SG, Traidl-Hoffmann C, Feussner I, Kasche A, Feser A, Ring J, Jakob T, Behrendt H: Chemotaxis and activation of human peripheral blood eosinophils induced by pollen-associated lipid mediators. *J Allergy Clin Immunol* 2004;113:1152–1160.
- 20 Traidl-Hoffmann C, Mariani V, Hochrein H, Karg K, Wagner H, Ring J, Mueller MJ, Jakob T, Behrendt H: Pollen-associated phytoprostanoids inhibit dendritic cell interleukin-12 production and augment T helper type 2 cell polarization. *J Exp Med* 2005;201:627–636.
- 21 Jilek A, Swoboda I, Breiteneder H, Fogy I, Ferreira F, Schmid E, Heberle-Bors E, Scheiner O, Rumpold H, Koller HT, Breitenbach M: Biological function, isoforms, and environmental control in the Bet v 1 gene family; in Kraft D, Sehon A (eds): *Molecular Biology and Immunology of Allergens*. Boca Raton, CRC Press, 1993, pp 39–45.
- 22 Swoboda I, Dang TCH, Heberle-Bors E, Vicente O: Expression of Bet v 1, the major birch pollen allergen, during anther development. *Protoplasma* 1995;187:103–110.
- 23 Schäppi GF, Taylor PE, Staff IA, Suphioglu C, Knox RB: Source of Bet v 1 loaded inhalable particles from birch revealed. *Sex Plant Reprod* 1997;10:315–323.
- 24 Namork E, Johansen BV, Løvik M: Detection of allergens adsorbed to ambient air particles collected in four European cities. *Toxicol Lett* 2006;165:71–78.
- 25 Hesselmar B, Åberg B, Eriksson B, Åberg N: Allergic rhinoconjunctivitis, eczema, and sensitization in two areas with differing climates. *Pediatr Allergy Immunol* 2001;12:208–215.
- 26 Kihlström A, Lilja G, Pershagen G, Hedlin G: Exposure to birch pollen in infancy and development of atopic disease in childhood. *J Allergy Clin Immunol* 2002;110:78–84.
- 27 von Berg A, Koletzko S, Gröbl A, Filipiak-Pittroff B, Wichmann HE, Bauer CP, Reinhardt D, Berdel D: The effect of hydrolyzed cow's milk formula for allergy prevention in the first year of life: the German Infant Nutritional Intervention Study, a randomized double-blind trial. *J Allergy Clin Immunol* 2003;111:533–540.
- 28 Hjelmroos M, Schumacher MJ, Van Hage-Hamsten M: Heterogeneity of pollen proteins within individual *Betula pendula* trees. *Int Arch Allergy Immunol* 1995;108:368–376.
- 29 Schäppi GF, Monn C, Wüthrich B, Wanner HU: Direct determination of allergens in ambient aerosols: methodological aspects. *Int Arch Allergy Immunol* 1996;110:364–370.
- 30 Lorenz RJ: *Biometrie. Grundbegriffe der Biometrie*. Stuttgart, Gustav Fischer Verlag, 1989.
- 31 Pauli G, Oster JP, Deviller P, Heiss S, Bessot JC, Susani M, Ferreira F, Kraft D, Valenta R: Skin testing with recombinant allergens rBet v 1 and birch profilin, rBet v 2: diagnostic value for birch pollen and associated allergies. *J Allergy Clin Immunol* 1996;97:1100–1109.
- 32 Schäppi GF, Suphioglu C, Taylor PE, Knox RB: Concentrations of the major birch tree allergen Bet v 1 in pollen and respirable fine particles in the atmosphere. *J Allergy Clin Immunol* 1997;100:656–661.
- 33 Vrtala S, Grote M, Duchène M, van Ree R, Kraft D, Scheiner O, Valenta R: Properties of tree and grass pollen allergens: reinvestigation of the linkage between solubility and allergenicity. *Int Arch Allergy Immunol* 1993;102:160–169.
- 34 Podoshin L, Gertner R, Fradis M: Treatment of perennial allergic rhinitis with ascorbic acid solution. *Ear Nose Throat J* 1991;70:54–55.
- 35 Ahlholm JU, Helander ML, Savolainen J: Genetic and environmental factors affecting the allergenicity of birch (*Betula pubescens* ssp. *czerepanovii* [Orl.] Hämet-Ahti) pollen. *Clin Exp Allergy* 1998;28:1384–1388.
- 36 Tashpulatov AS, Clement P, Akimcheva SA, Belogradova KA, Barinova I, Rakhmawaty FD, Heberle-Bors E, Touraev A: A model system to study the environment-dependent expression of the Bet v 1a gene encoding the major birch pollen allergen. *Int Arch Allergy Immunol* 2004;134:1–9.
- 37 Jaeger S, Berger J: Phenology and human health: allergic disorders. WHO meeting, Rome, 16–17 January, 2003, p 13.
- 38 Hallsdóttir M: Birch pollen abundance in Reykjavik, Iceland. *Grana* 1999;38:368–373.
- 39 Helander ML, Savolainen J, Ahlholm J: Effects of air pollution and other environmental factors on birch pollen allergens. *Allergy* 1997;52:1207–1214.
- 40 Weber RW: Meteorologic variables in aerobiology. *Immunol Allergy Clin North Am* 2003;23:411–422.
- 41 Behrendt H, Tomczok J, Sliwa-Tomczok W, Kasche A, Ebner von Eschenbach C, Becker WM, Ring J: Timothy grass (*Phleum pratense* L.) pollen as allergen carriers and initiators of an allergic response. *Int Arch Allergy Immunol* 1999;118:414–418.
- 42 Estrella N, Menzel A, Krämer U, Behrendt H: Integration of flowering dates in phenology and pollen counts in aerobiology: analysis of their spatial and temporal coherence in Germany (1992–1999). *Int J Biometeorol* 2006;51:49–59.
- 43 Pettenburger K, Tejkl M, Valenta R, Hoffmann-Sommergruber K, Swoboda I, Heberle-Bors E, Breitenbach M, Rumpold H, Kraft D, Scheiner O: Distribution of allergens and allergen-coding mRNAs in various tissues of white birch. *Mol Immunol* 1992;29:1401–1406.
- 44 Spieksma FThM, Emberlin JC, Hjelmroos H, Jäger S, Leuschner RM: Atmospheric birch (*Betula*) pollen in Europe: trends and fluctuations in annual quantities and the starting dates of the seasons. *Grana* 1995;34:51–57.