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1. Introduction

Allergies are a major risk to human health. The World Health Organization (WHO) reported that 30–40% of the global population shows allergic sensitization (Pawankar et al., 2011). In Germany, 30% of the population are subject to allergies. Here, the most common allergy is the so-called hay fever that affects almost 15% of the population (Langen et al., 2013).

High altitude regions are believed to provide better conditions for people allergic to pollen (Charalampopoulos et al., 2013). Since temperature decreases with altitude phenological events and therefore the pollen season generally start later (Ziello et al., 2009). In addition, airborne pollen concentration is generally reduced with altitude (Markgraf, 1980; Clot et al., 1995; Gehrig and Peeters, 2000). However, local abundance of plant species can also cause higher concentrations at high altitude sites (Frei, 1997; Gehrig et al., 2011). Aguilera and Ruiz Valenzuela (2012) suggested that higher olive pollen amounts observed at elevated regions may be an intrinsic mechanism of these trees to compensate for limited pollination efficiency and a short growing period.

The degree to which allergic individuals react with symptoms does not only depend on pollen concentrations but also on the allergen content of the pollen (Buters et al., 2012). Whereas most studies, and also pollen forecasts, only evaluate the pollen concentration as daily averages of pollen per cubic meter of air (pollen grains/m^3), less attention has been paid to the pollen potency, the actual allergen release capacity of this pollen (allergen/pollen grain).

Regarding birch pollen, Schäppi et al. (1997) found an average allergen release of 6 pg Bet v 1/pollen grain in 1996 in Melbourne, Australia. Buters et al. (2012) reported a value of 3.2 pg Bet v 1/pollen grain which did not vary much between five European sites. Birch pollen of different years or regions was found to have an up to 5-fold difference in allergen release (Buters et al., 2008). In addition, 10-fold variations in Bet v 1 release were reported for different days within a single year. These variations were attributed to changes in the expression of Bet v 1 in the course of the pollen's maturation process reaching a maximum six days before pollination (Buters et al., 2010). Regarding grass pollen, Schäppi et al. (1999), for example, reported an allergen release of group 5 allergens in Melbourne of up to 14 ng/m^3.

Within this study we compared airborne pollen concentrations of birch (*Betula* ssp.) and grass (Poaceae) and their allergen content (Bet v 1 and Phl p 5) in 2009 and 2010 at Garmisch-Partenkirchen (GAP), located at 734 m a.s.l., and at the high alpine site Umweltforschungsstation Schneefernerhaus (UFS) at 2650 m a.s.l. A comparison of phenological and aerobiological data already revealed that the pollen season of birch and grass can be predicted by phenological observations and that temperature and wind conditions altered the characteristics of the pollen season at different altitudinal levels (Jochner et al., 2012). In order to assess the risk for people allergic to pollen, we now specifically identify days when severe hay fever symptoms can occur. To overcome limitations arising from forecasts based on pollen concentrations solely we evaluated pollen potency, its temporal change and its relation to meteorological data. Pollen data were analysed using back-trajectories in order to investigate the source regions that are associated with low/high pollen concentration/potency. In addition, we investigated the possible pollen origin for specific days which showed a sudden increase in pollen potency.

2. Material and methods

2.1. Monitoring sites

We sampled pollen at two sites in the Bavarian Alps – one located in the valley of GAP: 734 m a.s.l. (see Fig. 1). The pollen trap was outside of the city at a height of 2 m a.g.l. in a pasture, which was not cut during the pollen season. To the south of the city, mountainous slopes with mainly conifer forests stretch up to 1700 m a.s.l.

The second pollen monitoring site was located at UFS: 2650 m a.s.l. (see Fig. 1). Here, the pollen trap was placed on a terrace at 2 m a.g.l. The vertical distribution of birch is restricted to less than 1800 m a.s.l. in the Alps, allergenic grass species such as *Dactylis glomerata* L. (cocksfoot) and *Alopecurus pratensis* L. (meadow foxtail) grow up to an altitude of 1500–2000 m a.s.l. in the northern Alps (DWD, 1991). Thus, sampled birch and grass pollen at the UFS must originate from medium- (up to 100 km) and/or long-range (>100 km) transport.

2.2. Pollen monitoring

Pollen sampling was based on the standard equipment and methods proposed by the European Aeroallergen Network (EAN, Galán et al., 2014). Pollen was collected with Hirst-type seven-day recording volumetric pollen traps (Burkard Manufacturing Co. Ltd., UK) during the pollen seasons of 2009 and 2010. Pollen records had some gaps due to technical or organisational problems (see vertical lines in Fig. 2a–d and Fig. 3a–d).

Pollen counting was achieved with light microscopy along four longitudinal transects, representing ~10% of the slides according to the minimal requirements of EAN (Galán et al., 2014). Pollen were reported in the identical time frame as the allergen measurements, i.e., from midday of the current day to midday of the following day. The Pollen Index (PI) was defined as the total pollen detected during the sampling period of a given year. We focussed on grass pollen which is the major cause of pollinosis in many parts of the world and on birch pollen, the most important allergenic tree pollen in north, central, and eastern Europe (D'Amato et al., 2007). Symptom severity depends on pollen concentration (Durham et al., 2014). The threshold values which can cause hay fever symptoms (Table 1) are generally applied as a consensus but can vary regionally (de Weger et al. 2013) and might be influenced by other meteorological parameters. It is also important to note that these threshold values refer to the amount of pollen an individual is exposed to (e.g., while being outdoors).

2.3. Allergen sampling and analysis

We analysed the major allergen of birch (Bet v 1) and grass pollen (Phl p 5) in 2009 and 2010. Airborne allergen sampling was performed within 5 m of the pollen traps using a high-volume Chemvol® cascade impactor for the particulate matter (PM) sizes

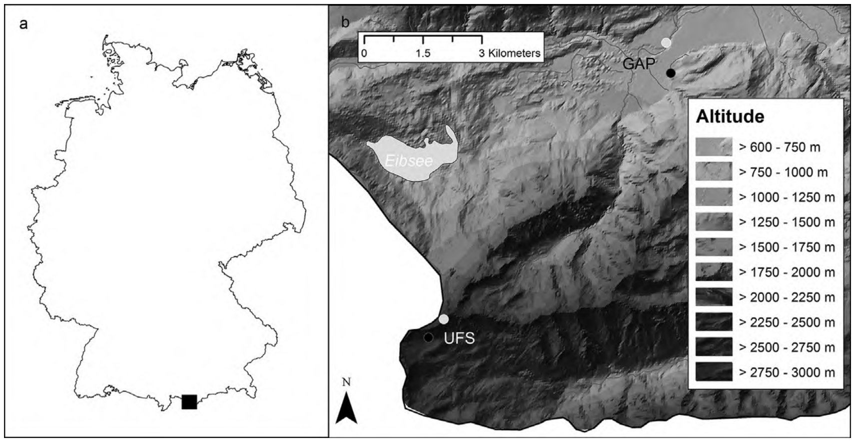

Fig. 1. Study area (a) located in southern Germany (black square), (b) Monitoring sites (black dots = pollen traps, grey dots = meteorological stations (DWD) related to UFS (Umweltforschungsstation Schneefernerhaus) and GAP (Garmisch-Partenkirchen), background: 50-m digital elevation model (Geobasisdaten © Bayerische Vermessungsverwaltung).

PM > 10 μm and 10 μm > PM > 2.5 μm (Butraco Inc., Son, The Netherlands) on polyurethane foam. The flow rate of 800 l/min was kept constant with a rotameter controlled high-volume pump (Digitel DHM-60, Austria).

Air was sampled from midday of the current day to midday of the following day. Since the impacting substrate was not exchanged on Saturdays, Sundays and holidays at UFS a mean value based on the accumulated allergen content was calculated for those days. Dates of delayed start or earlier completion of measurements, as well as missing data, are shown in Fig. 2a–d and Fig. 3a–d.

The impacting substrate was stored at temperatures <−20 °C. The substrate was further processed in a head-over-head rotator for 4 h in 0.1 M ammonium bicarbonate (pH 8.1). The extracts were aliquoted, frozen, lyophilized and stored at temperatures <−20 °C. Aliquots were reconstituted in 0.1 M phosphate buffered saline (pH 7.4) and serial diluted. Bet v 1 and Phl p 5 was measured using a two-site binding assay based on monoclonal antibodies (3B4F11D6 and 2E10G6G7; Allergopharma Joachim Ganzer KG, Germany) in an ELISA format. The ELISA had a day-to-day variability of 14% which is common for ELISAs (Buters et al., 2008).

Pollen counts recorded by Hirst-type volumetric traps become less reliable when pollen concentrations are low. Therefore, some studies (e.g., Buters et al., 2010) eliminated days <10 pollen grains/m^3 that probably affect pollen potency. We decided not to discard any data since at our vegetation-free monitoring site UFS pollen abundance was generally low.

Pollen potency was assessed on a daily basis by determining the average allergen release from one single pollen grain (pg Bet v 1 or Phl p 5/pollen grain). At UFS we obtained 40 (2009) and 51 (2010) daily mean values for Bet v 1 and 40 (2009) and 84 (2010) for Phl p

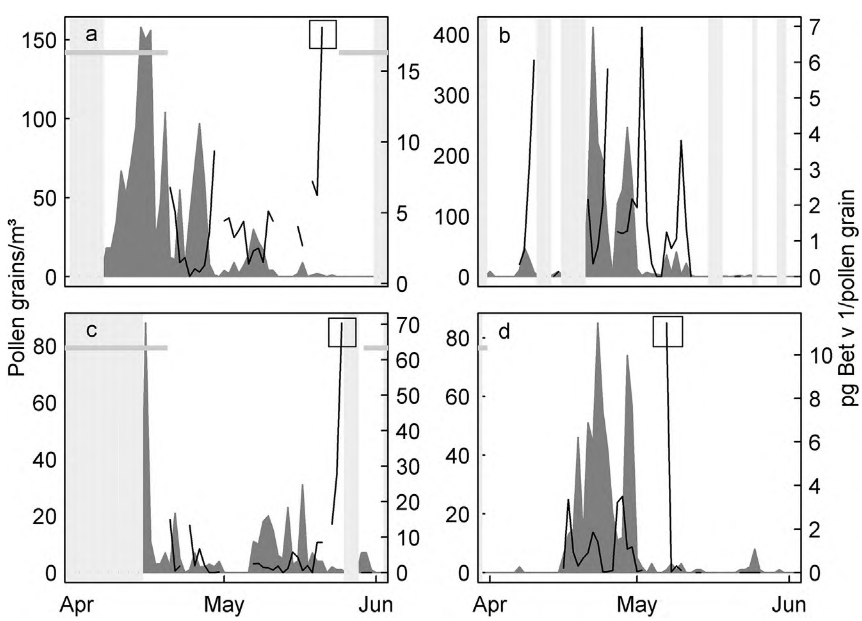

Fig. 2. Temporal course of birch (*Betula* ssp.) pollen concentration [pollen grains/m^3] (darkgrey) and pollen potency [pg Bet v 1/pollen grain] (black line) at Garmisch-Partenkirchen (GAP) in (a) 2009 and (b) 2010 and at Umweltforschungsstation Schneefernerhaus (UFS) in (c) 2009 and (d) 2010. Lightgrey vertical shading indicates missing pollen data, thick horizontal upper lines missing allergen data. Boxes indicate episodes which were analysed using back-trajectories (see Fig. 5a–c).

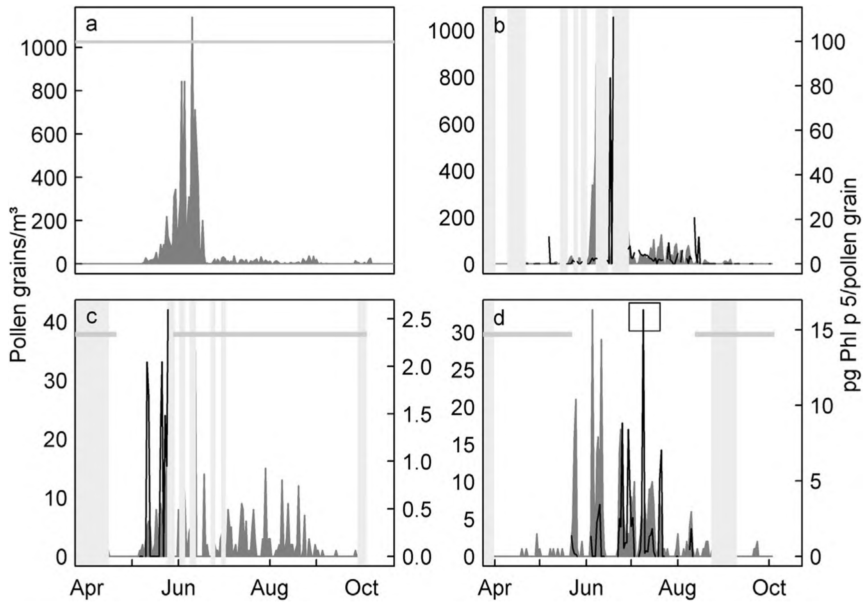

Fig. 3. Temporal course of grass (Poaceae) pollen concentration [pollen grains/m^3] (black) and pollen potency [pg Phl p 5/pollen grain] (black line) at Garmisch-Partenkirchen (GAP) in (a) 2009 and (b) 2010 and at Umweltforschungsstation Schneefernerhaus (UFS) in (c) 2009 and (d) 2010. Lightgrey vertical shading indicates missing pollen data,thick horizontal upper lines missing allergen data. Note, that no allergen data were available for GAP in 2009. The box indicates an episode which was analysed using back-trajectories (see Fig. 5d).

5. At GAP Bet v 1 daily means were obtained on 35 (2009) and 50 (2010) days, Phl p 5 was only sampled in 2010 on 137 days.

2.4. Meteorological data

Meteorological data of the German Meteorological Service (DWD) was derived for the climate stations at Garmisch-Partenkirchen (719 m a.s.l.) and at Zugspitze (2964 m a.s.l.) which is located 300 m above the UFS (Fig. 1). We analysed temperature, precipitation, relative humidity and sunshine duration based on hourly values from midday of the current day to midday of the following day. Data on precipitation at the Zugspitze station could not be used since daily values refer to the 12 am–12 am period in contrast to pollen data (12 pm–12 pm of the following day).

2.5. Statistical analyses

Following the recommendations of the EAN (ean.polleninfo.eu) the start and the end of the pollen season was defined as the date on which the cumulative sum of daily mean pollen concentration reaches 1% and 95% of the total annual sum, respectively.

Differences in pollen potency at GAP and UFS were investigated using the non-parametric Mann–Whitney U test. We calculated Spearman's correlation coefficients for the data available during the pollen seasons in order to detect probable relationships between pollen and allergen concentrations as well as meteorological data.

Analyses were conducted using IBM SPSS Statistics 22.0 and Figs. 2 and 3 were generated with R 3.1.2.

2.6. Backward trajectories

We calculated back-trajectories using HYSPLIT (Draxler and Hess, 1997) for each day in April–May 2009 and 2010. We selected the meteorological model data of GDAS with a resolution of 1°. The starting height of the 72-h back-trajectories was set to 1500 m a.g.l., the starting time to 6 pm.

We classified pollen loads in four groups of no, low, medium or high burden (Table 1). Pollen potency was also classified in four groups: 0: no pollen potency, 0.001–1 pg allergen/pollen grain: low pollen potency, 1.001–5 pg allergen/pollen grain: medium pollen potency, >5 pg allergen/pollen grain: high pollen potency. We only analysed data from the vegetation-free UFS since we suggest local influences at GAP are of higher relevance.

We further applied case studies (Fig. 5) for days with sudden increases in pollen potency (mostly > 10 pg Bet v 1 or Phl p 5/pollen grain). Here, we calculated four 72-h trajectories per day (starting times: 12, 18, 0, 6) for the three days before, the event day and the following day. The starting height was set to 1500 m a.g.l. for the respective coordinates of GAP and UFS. We analysed four specific episodes (squares in Figs. 2 and 3) but did not consider the events related to grass pollen at GAP in 2010 due to a high number of missing data. The corresponding Figs. 4a–d and 5a-d were generated using ArcGIS 10.2.1.

3. Results

3.1. Pollen concentrations

Pollen concentrations were drastically reduced at UFS compared to GAP. For birch, only 24.2% (PI: 343) of the pollen index in 2009 and 29.5% (PI: 583) in 2010 compared to GAP were measured (Table 2). For grass the reduction at UFS was even more pronounced: In 2009, 2010 only 4.2% (PI: 403) and 8.0% (PI: 372), respectively, were sampled.

Peak values at GAP were 158 (2009) and 412 (2010) pollen grains/m^3, but only 88 (2009) and 85 (2010) pollen grains/m^3 at UFS (Table 2, Fig. 2). Grass pollen were more abundant than birch pollen and peaked in the valley at 1139 (2009) and 1056 pollen grains/m^3 (2010), but only at 42 (2009) and 33 (2010) pollen grains/m^3 at UFS (Table 2, Fig. 3).

At UFS the start of the pollen season was 8 (2009) and 9 days (2010) later for birch, but 6 (2009) and 21 days (2010) earlier for grass compared to GAP. The end of the season at UFS was later (except for birch in 2010) and most pronouncedly delayed for grass in 2009 (25 days). The pollen season length at UFS was longer by 8 (2009) and shorter by 12 days (2010) for birch, but longer by 31

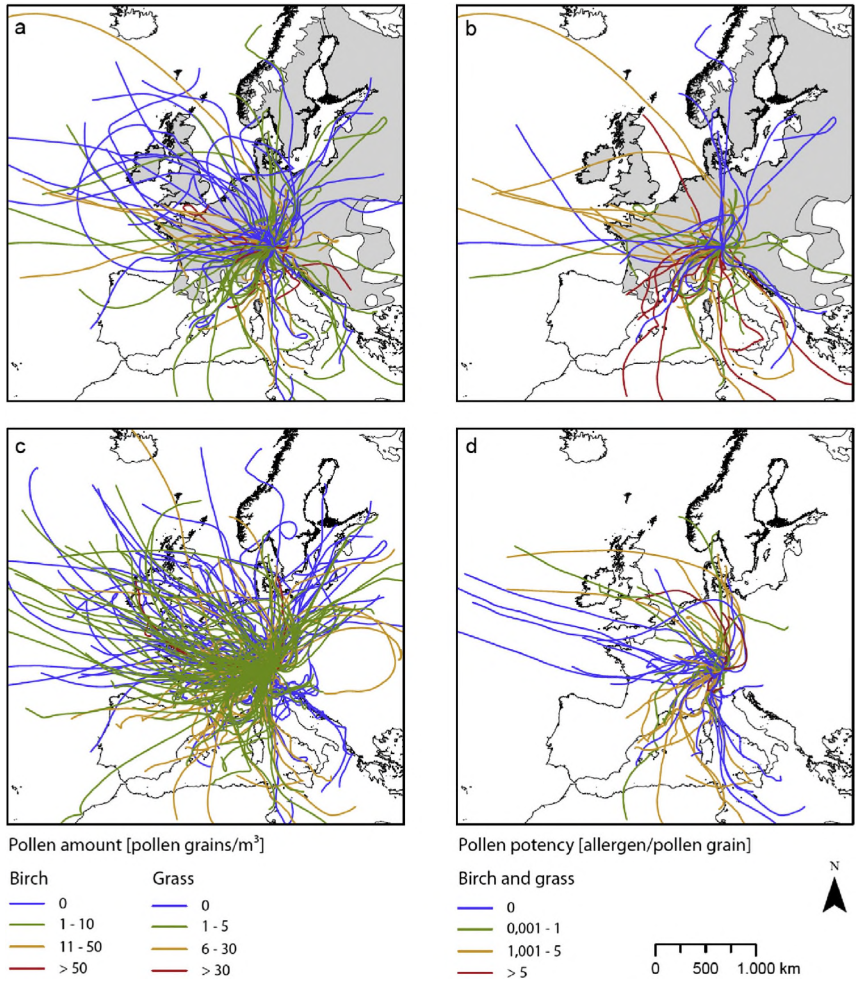

Fig. 4. 72-h back-trajectories of all available data in April and May 2009 and 2010 for *Betula* ssp. (a, b) and in May to August for Poaceae (c, d) calculated with the HYSPLIT model (NOAA) using meteorological model data of GDAS (NOAA). (a, c) Pollen amount, (b, d) pollen potency. Grey shading in (a) and (b) indicates the distribution of silver birch (*Betula pendula* Roth) (http://www.euforgen.org).

(2009) and 23 days (2010) for grass (Table 2).

Days with medically relevant pollen concentrations were frequently found at UFS (Table 2): For birch, there was a moderate burden on 8 (2009) and 9 days (2010) and even a high burden on 1 day (2009) and 5 days (2010). For grass pollen a lower threshold number leads to clinical symptoms and the years 2009 and 2010 were associated with 22 and 23 days of moderate burden and with 1 day of high burden in each study year.

3.2. Allergen release

In addition to lower peak allergen values, the total allergen content sampled per season at UFS was reduced compared to the lower station (only 55.7% and 18.0% for Bet v 1 in 2009 and 2010 and only 1.4% for Phl p 5 in 2010, Table 3). The majority of allergen was detected in the PM > 10 μm fraction (76–100%). The percentage of allergen deposited in PM < 10 μm was unrelated to airborne pollen concentrations. Only for birch pollen concentrations at GAP in 2009 we detected a strong negative correlation between the percentage in PM < 10 μm and pollen loads ($r_s = -0.701$, $p < 0.001$, analyses not shown).

Table 1
Threshold values for hay fever symptoms for birch (*Betula* spp.) and grass (Poaceae) pollen.

Burden	*Betula* ssp. [pollen grains/m^3]	Poaceae [pollen grains/m^3]
No	0	0
Low	1–10	1–5
Medium	11–50	6–30
High	>50	>30

There were also days on which pollen was found on the microscope slides but no allergen content could be detected with the cascade impactor (Table 3). Even more interestingly was the fact that 26 days were associated with no pollen found on the slides but allergen content deposited on the filters. This especially applied to birch (20 days). Mean pollen potency of the season was 3.3 pg Bet v 1/pollen grain at GAP in 2009 and 1.8 pg Bet v 1/pollen grain in 2010. A higher value (3.7 pg Bet v 1/pollen grain) was recorded in 2009 at UFS but in 2010 the value was lower (1.1 pg Bet v 1/pollen grain) compared to GAP. For grass this difference was even more pronounced and significant: 5.7 pg Phl p 5/pollen grain was found in 2010 at GAP; but only 1.5 pg Phl p 5/pollen grain at UFS. Pollen potency at UFS was especially low in 2009 (0.7 pg Phl p 5/pollen grain). We also observed pronounced daily variations in pollen potency (see Figs. 2 and 3).

In general, higher allergen concentrations were associated with higher pollen abundance (Table 4). All correlation coefficients were

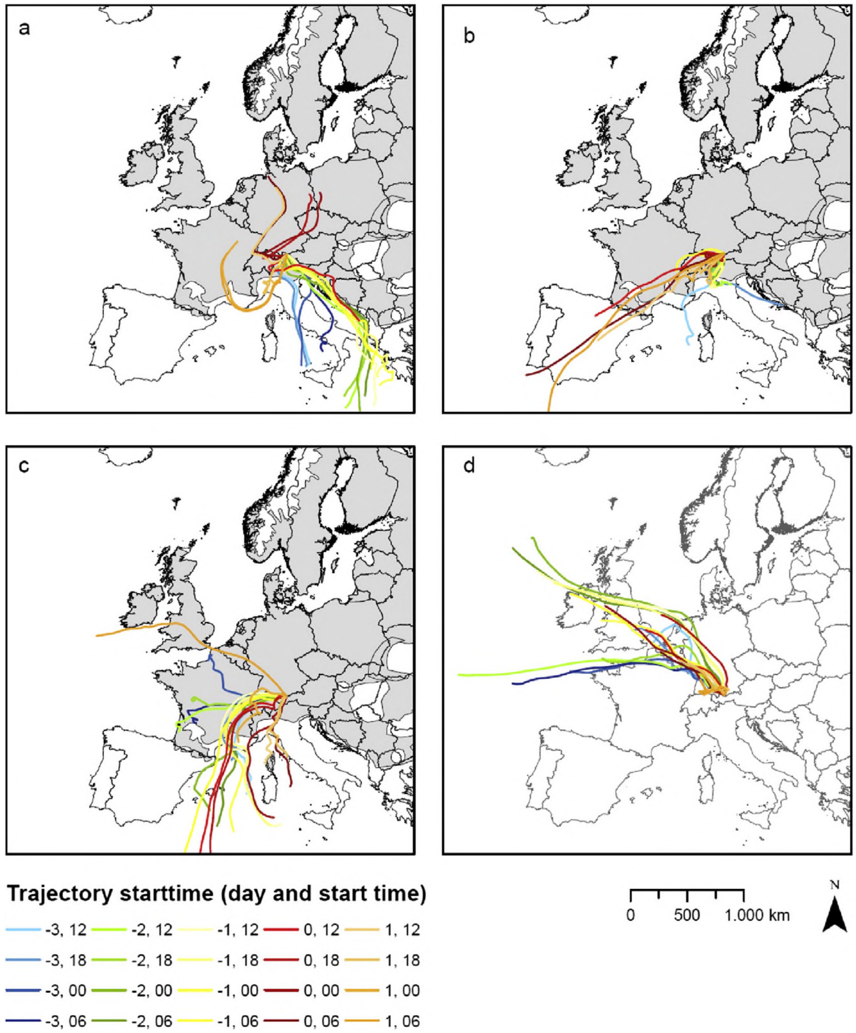

Fig. 5. Back-trajectories calculated with the HYSPLIT model (NOAA) using meteorological model data of GDAS (NOAA) for three days (−3, blue), two days (−2, green) and one day (−1, yellow) before a specific event (0, red) and for the day after (1, orange). Gradual colours show different starting times (12, 18, 0, 6) of trajectories. We analysed episodes with an increase in pollen potency on a) 7th May 2010 at UFS (*Betula* ssp.), b) 21st May 2009 at GAP (*Betula* ssp.), c) 26th May 2009 at UFS (*Betula* ssp.) and d) 9th July 2010 at UFS (Poaceae). Grey shading in (a), (b) and (c) indicates the distribution of silver birch (*Betula pendula* Roth) (http://www.euforgen.org).

significant, the statistical correlation was especially strong at GAP and the highest correlation was $r_s = 0.832$ ($p < 0.001$) for birch in 2009. Higher grass pollen concentrations were also linked to higher Phl p 5 values. For the analysed species a correlation coefficient below $r_s = 1$ is partly attributable to the fact that pollen can contain varying or even no allergen content (see Table 4, Figs. 2 and 3).

Table 2
Characteristics of pollen concentration, peak and season as well as days with burden at GAP (Garmisch-Partenkirchen) and UFS (Umweltforschungsstation Schneefernerhaus) for birch and grass pollen.

	Betula ssp.				Poaceae			
	GAP 2009	UFS 2009	GAP 2010	UFS 2010	GAP 2009	UFS 2009	GAP 2010	UFS 2010
Pollen								
Pollen Index (PI)	1481	343	1977	583	9535	403	4646	372
% of GAP	–	24.2	–	29.5	–	4.2	–	8.0
Peak [pollen grains/m^3]	158	88	412	85	1139	42	1056	33
Peak date	14.04.	15.04.	22.04.	23.04.	10.06.	12.06.	08.06.	05.06
Start of the pollen season	07.04.	15.04.	07.04.	16.04.	14.05.	08.05.	20.05.	29.04
End of the pollen season	07.05.	23.05.	07.05.	04.05.	26.07.	20.08.	08.08.	10.08
Length of the pollen season [days]	31	39	31	19	74	105	81	104
% of GAP	–	125.8	–	61.3	–	141.9	–	128.4
Days with burden (thresholds see Table 1)								
Low	17	27	19	18	56	61	40	46
Medium	12	8	7	9	55	22	32	23
High	12	1	9	5	36	1	33	1

Table 3
Characteristics of allergen content, pollen potency, days with pollen and allergen as well as meteorological conditions at GAP (Garmisch-Partenkirchen) and UFS (Umweltforschungsstation Schneefernerhaus) for birch pollen and its major allergen Bet v 1 as well as grass pollen and its major allergen Phl p 5. Meteorological data for the periods January to March (birch) and April to June (grass) are from the DWD stations Garmisch-Partenkirchen (GAP) and Zugspitze close to the UFS.

	Betula ssp.				Poaceae			
	GAP 2009	UFS 2009	GAP 2010	UFS 2010	GAP 2009	UFS 2009	GAP 2010	UFS 2010
Allergen content								
Sum [pg/m^3]	971.4	540.6	3618.1	650.8	No data	166.9	42989.1	596.8
% Of GAP	–	55.7	–	18.0	No data	No data	–	1.4
Peak [pg/m^3]	81.5	70.4	424.3	119.7	No data	67.5	14298.3	79.5
Peak date	10.04.	25.05.	25.04.	23.04.	No data	25.05.	10.06.	25.06.
Allergen [%] in > PM 10 μm	79.2	87.1	78.4	76.4	No data	100	84.1	78.6
Pollen potency								
Mean for the pollen season [allergen [pg]/pollen]	3.3	3.7	1.8	1.1	No data	0.7	5.7	1.5
Days with pollen and allergen								
Days with allergen but no pollen	8	5	0	7	No data	0	1	5
Days with pollen but no allergen	0	5	9	10	No data	12	51	27
Meteorological data								
Mean temperature [°C]	−1.2	−11.7	−0.8	−12.3	12.7	−1.5	11	−2.9
Sum of sunshine duration [hours]	250.4	384.4	316.4	458.8	552.4	519.7	452.3	479.3
Sum of precipitation [mm]	No data	690	111	335	381.1	442.4	449.3	592.7

Table 4
Spearman rank correlations between pollen concentration and allergen content/pollen potency for birch (*Betula* spp.) and grass (Poaceae) at GAP (Garmisch-Partenkirchen) and UFS (Umweltforschungsstation Schneefernerhaus) in 2009 and 2010. r_s = correlation coefficient, N = number of cases, significance level p: $^{***}p \leq 0.001$, $^{**}p \leq 0.01$, $^{*}p \leq 0.05$, ns: not significant ($p > 0.05$).

	Betula ssp.				Poaceae			
	GAP		UFS		GAP		UFS	
	2009	2010	2009	2010	2009	2010	2009	2010
	Pollen concentration c.f. allergen content							
r_s	0.832***	0.739***	0.440**	0.767***	no data	0.690***	0.533*	0.608***
N	18	23	34	19		64	19	81
	Pollen concentration c.f. pollen potency							
r_s	−0.764***	ns	−0.467*	ns	no data	ns	0.617**	0.386**
N	17		26				17	52

Higher pollen loads of birch were negatively correlated to pollen potency in 2009 at both monitoring stations (2010: no significant results). In contrast, higher grass pollen counts were associated with higher pollen potency (Table 4).

3.3. Influence of meteorology on pollen amount and potency

The months January–March were associated with lower temperatures and less sunshine hours at GAP in 2009 (compared to 2010), a year for which we recorded higher birch pollen potency (Table 3). Data of allergen release of grass in both years was only available for UFS. Here, higher pollen potency was linked to lower temperatures during April–June, comparable sunshine duration but higher rainfall amounts in 2010. We additionally examined the associations between meteorological variables and pollen concentration and potency during the course of the pollen season (Table 5): Higher temperatures were linked to increases in grass and birch pollen concentration at UFS (not significant in 2010) but to decreases in pollen concentration of birch at GAP (significant in 2009). Pollen potency was positively correlated with temperature at UFS for grass in both years. When significant, higher daily sunshine duration was associated with higher pollen loads and pollen potency. Higher values of precipitation and relative humidity were linked to lower pollen concentrations for all analysed cases. Precipitation was positively correlated to birch pollen potency at GAP in 2009. Higher levels of pollen potency were often significantly associated with reduced relative humidity (except for birch in 2009 at GAP).

3.4. Back-trajectory analyses

3.4.1. Possible pollen source regions

In general, high concentrations of birch pollen were transported from westerly directions (orange and red lines in Fig. 4a). There were some exceptions showing high concentrations from southerly, easterly or north-easterly tracks. Pollen potency (Fig. 4b) was especially high when air masses were directed from south-westerly to south-easterly regions. There was only one incidence when north–westerly air masses contributed to very high pollen potency. Low pollen potency (blue lines) was particularly recorded when the pollen was transported from northerly directions. For grass, 30 pollen grains/m^3 were exceeded at UFS in 2009 and 2010 only once and were associated with northerly and north–westerly air flows. Air masses transporting high loads (orange lines in Fig. 4c) were evenly distributed except for easterly directions which did not prevail during the measuring period. High pollen potency (red lines) was mostly related to northerly air flows (Fig. 4d).

3.4.2. Pollen transport during high pollen potency episodes

A rise in pollen potency from 0 to 11.5 pg Bet v 1/pollen grain in conjunction with only one birch pollen grain/m^3 at UFS was recorded on the 7th May 2010. On this date a low pressure area over the Alps moved to the south of Poland and strong warm air advection at high levels, along with cold air advection at low levels,

Table 5
Spearman rank correlations between daily (12–12) meteorological variables (mean air temperature, sunshine duration, precipitation sum, mean relative humidity) and daily (12–12) pollen concentration (N pollen) and potency for birch (*Betula* ssp.) and grass (Poaceae) at GAP (Garmisch-Partenkirchen) and UFS (Umweltforschungsstation Schneefernerhaus) in 2009 and 2010. r_s = correlation coefficient, N = number of cases, significance level p: ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05, ns: not significant (p > 0.05).

	Betula spp.								Poaceae							
	GAP 2009		UFS 2009		GAP 2010		UFS 2010		GAP 2009		UFS 2009		GAP 2010		UFS 2010	
	N pollen	Pollen potency	N pollen	Pollen potency	N pollen	Pollen potency	N pollen	Pollen potency	N pollen	Pollen potency	N pollen	Pollen potency	N pollen	Pollen potency	N pollen	Pollen potency
Mean air temperature																
r_s	−0.433*	ns	0.410**	ns	ns	ns	ns	ns	ns	no data	0.334***	0.572*	ns	ns	0.694***	0.342*
N	31		39								96	17			104	52
Sunshine duration																
r_s	0.416*	ns	ns	0.397*	0.732***	ns	0.510*	0.513*	0.464***	no data	0.220*	ns	0.582***	0.329*	0.712***	0.395**
N	31			26	23		19	18	74		96		64	56	104	52
Precipitation sum																
r_s	−0.644***	0.495*	no data	no data	−0.610**	ns	no data	no data	−0.373***	no data	no data	no data	−0.556***	ns	no data	no data
N	31	17			23				74				64			
Mean relative humidity																
r_s	−0.784***	0.646**	−0.344*	−0.414*	−0.680***	ns	ns	−0.536*	−0.626***	no data	−0.290**	ns	−0.560***	−0.437***	−0.718***	−0.542***
N	31	17	39	26	23			18	74		96		64	56	104	52

led to a strong uplift of air (Berliner Wetterkarte e 2010a). Back-trajectories for this event, the preceding three days and the day after are shown in Fig. 5a. The blue lines indicate a southerly origin of air masses three days before, followed by a more south-easterly direction two and one day before (green and yellow lines). On the 7th of May, air masses were associated with south-easterly and north-easterly routes (red lines) indicating a change of direction. After the event day, air masses were directed from Central France with a southerly track towards the Mediterranean Sea and headed north again (orange lines).

On the 21st May 2009, pollen potency was 18.1 Bet v 1/pollen grain and only one birch pollen grain/m^3 was recorded at GAP. On this day a trough over the Eastern Atlantic was associated with a south-westerly air flow in the upper level. An increase in the flow of the middle atmosphere led to an advection of warm air from the south (Berliner Wetterkarte e 2009a). Back-trajectories of the third day before this event (blue lines) show a south-westerly and south-easterly direction (Fig. 5b), then southerly (green, yellow) and later south-westerly routes passing France (red) dominated. The lengths of the trajectories were quite small shortly before and during the event (for two time points) indicating a regional source of pollen. After the event, air masses were again dominated by long-range transport from south-westerly directions (orange lines).

Fig. 5c shows the back-trajectories for the episode on the 26th May 2009 when only one birch pollen grain/m^3 was recorded at UFS but pollen potency soared to 70.4 pg Bet v 1/pollen grain. This day was associated with an advection of subtropical air masses (Berliner Wetterkarte e 2009b). Three days before, air was transported from southerly, north–westerly and westerly directions (blue lines) and was then, when pollen potency was comparably high, directed from more southerly routes along the Alps (green and yellow lines). On the event day, air masses passed the Alps in a south-north direction (red lines) indicating mountainous regions were the source of pollen with high allergen content. The day after was characterised by a change in air flow from southerly to a north–westerly route (orange lines).

The last analysed episode occurring on the 9th July 2010 at UFS was associated with three grass pollen grains/m^3 which implied a pollen potency of 16.3 Ph l p 5/pollen grain. On this day tropical air was transported in an easterly direction (Berliner Wetterkarte e 2010b), resulting in 9 °C measured at Zugspitze. On the days preceding this event, westerly and north–westerly air flows prevailed (blue, green and yellow lines, Fig. 5d). The trajectories of the event day were shorter (red lines) and air masses were directed from north–westerly regions. The subsequent day was characterised by a pronounced regional transport but only associated with a pollen potency of 0.21 Ph l p 5/pollen grain and 7 pollen grains/m^3. Thus, it might be likely that the high potency pollen originated from long-range transport passing the UK, Northern France, The Netherlands, Belgium or Eastern Germany.

4. Discussion

4.1. Pollen load and season

The PIs and pollen peaks were clearly reduced at the vegetation-free UFS site, especially for grass. This indicates more favourable conditions at mountainous regions for people allergic to grass pollen. However, pollen season length at the high altitude site was longer compared to the valley site for grass in all years and for birch in 2009. This may arise from the definition of the pollen season that includes between 1 and 95% of the respective total pollen sum and is therefore also influenced by the two distinct seasonal amounts at UFS and GAP. More interesting is the fact that several days with medically relevant pollen concentrations were found at UFS: Days with medium burden were especially associated with grass pollen since six or more pollen grains/m^3 may cause stronger symptoms (see Table 1). However, days with a high burden were found more frequently for birch, i.e., 50 pollen grains/m^3 were exceeded on six days at UFS. Thus, differences were detected with respect to the absolute number of airborne pollen, but also with respect to the different threshold values for birch and grass pollen concentrations causing hay fever symptoms. In addition, the daily peak pollen loads, probably associated with the highest burden and severest symptoms, ranged between 8.9 and 25.7% of the total PI. Thus, the PI cannot be ultimately linked to the severity of the pollen season.

4.2. Pollen potency

We also found a considerable reduction of the seasonal allergen at UFS (Table 3). In general, the sampled allergen increases as airborne pollen concentration increases: The correlation coefficients between pollen and allergen content ranged between $0.440 \leq r_s \leq 0.832$ (Table 4) indicating that airborne allergen originates mainly from pollen. Correlations between pollen concentration and potency showed species-specific relationships: Higher pollen loads were linked to higher pollen potency for grass, but lower pollen potency for birch in 2009. Therefore, actual pollen concentrations do not mirror the actual allergen content in a simple manner.

An important feature was that several days occurred when pollen but no corresponding allergen content was detected, particularly evident for grass pollen and its major allergen Phl p 5 (Table 3). In addition, there were also several days when Bet v 1 or Phl p 5 content was measured but no birch or grass pollen was detected. This was especially obvious at UFS and indicates that pollen presence/absence forecasts might be too simplistic since the actual allergen content can be tremendously under- or overestimated.

In our study, most of the allergen content (76–100%) was measured in the PM > 10 μm fraction (Table 3). This is also in accordance with Buters et al. (2010, 2012) who found circa 90% of Bet v 1 in this fraction, attributable to the diameter of birch and grass pollen (both > 20 μm). Allergen in the PM < 10 μm fraction, however, is an indication for allergen detached from pollen (Buters et al., 2015). It has already been shown that Bet v 1 or Phl p 5 can occur in the atmosphere as free allergen molecules or in association with PM even when no pollen can be detected (Schäppi et al., 1996, 1997, 1999). In general, Bet v 1 cannot be determined reliably in the small fraction of PM < 2.5 μm since the allergen is absorbed to diesel particles, also sampled in this fraction (Buters et al., 2010). However, owing to the effect of an imperfect particle separation in cascade impactors (Demokritou et al., 2002), intact pollen can still be detected, especially during episodes of low airborne pollen concentrations, in the aerodynamic smaller fraction (Riediker et al., 2000). In our study we only found a single significant but strong and negative correlation between the percentage of allergen found in the PM < 10 μm fraction and airborne concentration (birch at GAP in 2009). Site- and year-specific differences in pollen potency (Table 3) were most pronounced for Phl p 5: The average allergen content per grass pollen was about four times lower at UFS. Thus, people allergic to grass pollen may especially benefit from high altitudinal conditions.

It is well-known that the content of the major birch allergen Bet v 1 is highest at the start of pollination and lower both before and after (Buters et al., 2010; Beck et al., 2013). However, knowledge is lacking regarding the alteration of pollen potency after pollen is released in the air. If biological aging processes decrease allergen content during the time in which the pollen is transported, then pollen originating from long-range transport would be associated with a lower potency. However, Galán et al. (2013) found that episodes of high potency of olive pollen (*Olea europaea* L.) in Évora (Portugal) were associated with long-range transport of pollen originating from Córdoba (Spain).

Differences in allergen content might be linked to different birch genotypes (Schäppi et al., 1997) or environmental conditions. In our study, lower pollen potency occurred when higher temperatures of the preceding months of flowering were recorded (Table 3). However, analysing two years is not enough to be conclusive on the impacts of meteorological conditions prior to flowering on allergen content. Thus, we analysed the influence of actual meteorological conditions on pollen potency (daily data). When correlations were significant (Table 5), higher temperatures and sunshine duration as well as lower precipitation and relative humidity (exception for relative humidity: birch at GAP, 2009) were associated with higher pollen potency. More research is required to assess the relative importance of meteorological conditions during the pollen season compared to long-term conditions prior to flowering.

Other studies only addressed a small number of years or sites and showed non-uniform results on the influence of meteorology on allergen content of different species. Ahlholm et al. (1998) analysed Bet v 1 of mountain birches (Betula pubescens ssp. czerepanovii (Orl.) Hämet-Ahti) growing in northern Finland and reported that increased allergen was related to higher temperatures. Hjelmroos et al. (1995) showed that allergen content of birch (*Betula pendula* Roth) pollen was highest for catkins at the sunny southern side of the trees. Buters et al. (2008) analysed Bet v 1 from birches growing in two different regions in Germany for two consecutive years and found that the allergen release was higher when temperature and humidity were lower. In addition, a decrease in allergen content under higher temperatures was reported for common ragweed (*Ambrosia artemisiifolia* L., Ziska et al., 2003) and white goosefoot (*Chenopodium album* L., Guedes et al., 2009). A field study in Munich, Germany, showed that ozone was associated with an increase in the major birch allergen Bet v 1 (Beck et al., 2013). Experiments with grass species have shown that allergen content was enhanced subsequent to ozone fumigation (Masuch et al., 1997; Eckl-Dorna et al., 2010).

Differences in pollen potency might also be indicative of different pollen origins. We showed that high birch pollen loads were particularly transported from westerly directions, but pollen potency was especially high when air masses were directed from south-westerly to south-easterly regions (Fig. 4). At a first glance, this suggests that birch trees growing in warmer regions produce a higher allergen amount. However, for two reasons this is unlikely: Firstly, birch does not occur in the far south (see grey shading in Fig. 4), secondly, high pollen potency was mostly observed at the end of April and in May (see Fig. 2), very likely after the earlier flowering season in warmer/more southern regions. Therefore, atmospheric pollen at UFS in this period is more likely attributable to the flowering of birch trees in colder (i.e., high altitudinal) regions.

The analysed episodes associated with high birch pollen potency (Fig. 5a–c) occurred relatively late in the year (between 7th May to 26th May). Birch usually starts to flower from the beginning to mid-April at low level sites in Germany or adjacent eastern countries (D'Amato et al., 2007). Thus, pollen with high allergen levels more likely originated from mountainous regions of Germany or Austria. In addition, birches in mountainous areas flower somewhat later, around May (Jochner et al., 2012). Thus, we suggest that short- or medium-range transport of pollen from vegetated mountainous regions is the major source of high potency pollen. This finding may suggest that site conditions (e.g., lower temperature) or the maturation process of birch pollen (with higher allergen content at earlier flowering stages) could be a major explanation for differences in pollen potency.

It is difficult to distinguish whether the pollen was incorporated in the air masses at the uttermost end of the trajectories or in near proximity to the source location. Thus, we can only suggest that long-range transport was probably responsible for higher grass pollen potency on the 9th July 2010 at UFS. We assume that more allergenic pollen was transported from north—westerly regions. The explanation for differences in Phl p 5 levels, however, is even more complex: The grass family includes approximately 600 genera and over 10000 species with more than 400 species growing in Europe (Tutin et al., 2001). Since different grass species flower in different periods the grass pollen season in Europe lasts on average from April—September (Puc, 2011). Flowering times of the same species also differ spatially implying diverse possibilities of origin. Although grass pollen cannot be distinguished at the species level using light microscopy pollen potency varies across species (Schäppi et al., 1999; Puc, 2011). This implies that the occurrence and severity of allergic symptoms does not correspond to actual grass pollen counts and symptoms can be aggravated far from the pollen peak (Frenguelli et al., 2010). Detailed phenological observations of a diverse set of grass species would be needed to further investigate their allergenic properties, phenological behaviour and possible regions of origin.

5. Conclusions

The low potency, especially of grass pollen, in conjunction with the generally low airborne pollen concentration at the high altitude site suggests that allergic people might benefit in the Alps during the pollen season. Our findings indicate that a risk assessment relying solely on the actual pollen concentration does not necessarily reflect the actual conditions allergy suffers are exposed to: Days with no pollen might be related to days with detectable (and therefore also inhalable) allergen content, and days with pollen might be medically not relevant since their allergen content might be small or even zero. In order to unravel the influential factors on pollen potency we suggest that further studies should focus on long-term monitoring incorporating detailed analyses and measurements of airborne and plant-specific allergen content.

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References

Aguilera, F., Ruiz Valenzuela, L., 2012. Altitudinal fluctuations in the olive pollen emission: an approximation from the olive groves of the south-east Iberian Peninsula. Aerobiologia 28, 403—411.
Ahlholm, J.U., Helander, M.L., Savolainen, J., 1998. Genetic and environmental factors affecting the allergenicity of birch (*Betula pubescens ssp. czerepanovii* [Orl.] Hamet-ahti) pollen. Clin. Exp. Allergy 28, 1384—1388.
Beck, I., Jochner, S., Gilles, S., McIntyre, M., Buters, J.T.M., Schmidt-Weber, C., Behrendt, H., Ring, J., Menzel, A., Traidl-Hoffmann, C., 2013. High environmental ozone levels lead to enhanced allergenicity of birch pollen. PloS One. http://dx.doi.org/10.1371/journal.pone.0080147.
Berliner Wetterkarte e.V. (Eds.) (2009a) 21.05.2009. Berliner Wetterkt., 58(98), 1—8.
Berliner Wetterkarte e.V. (Eds.) (2009b) 26.05.2009. Berl. Wetterkt., 58(99), 1—8.
Berliner Wetterkarte e.V. (Eds.) (2010a) 07.05.2010. Berl. Wetterkarte, 59(92), 1—8.
Berliner Wetterkarte e.V. (Eds.) (2010b) 09.07.2010. Berl. Wetterkarte, 59(134), 1—8.
Buters, J.T.M., Kasche, A., Weichenmeier, I., Schober, W., Klaus, S., Traidl-Hoffmann, Menzel, A., Huss-Marp, J., Krämer, U., Behrendt, H., 2008. Year-to-year variation in release of Bet v 1 allergen from birch pollen: evidence for geographical differences between West and South Germany. Int. Archives Allergy Immunol. 145, 122—130.
Buters, J., Prank, M., Sofiev, M., Pusch, G., Albertini, R., Annesi-Maesano, I., Antunes, C., Behrendt, H., Berger, U., Brandao, R., Celenk, S., Galán, C., Grewling, Ł., Jackowiak, B., Kennedy, R., Rantio-Lehtimäki, A., Reese, G., Sauliene, I., Smith, M., Thibaudon, M., Weber, B., Cecchi, L., 2015. Variation of the group 5 grass pollen allergen content of airborne pollen in relation to geographical location and time in season. J. Allergy Clin. Immunol. 136 (1), 87—95.e6.
Buters, J., Thibaudon, M., Smith, M., Kennedy, R., Rantio-Lehtimäki, A., Albertini, R., Reese, G., Weber, B., Galán, C., Brandao, R., Antunes, C.M., Jäger, S., Berger, U., Celenk, S., Grewling, Ł., Jackowiak, B., Sauliene, I., Weichenmeier, I., Pusch, G., Sarioglu, H., Ueffing, M., Behrendt, H., Prank, M., Sofiev, M., Cecchi, L., 2012. Release of Bet v 1 from birch pollen from 5 European countries. Results from the HIALINE study. Atmos. Environ. 55, 496—505.
Buters, J.T.M., Weichenmeier, I., Ochs, S., Pusch, G., Kreyling, W., Boere, A.J.F., Schober, W., Behrendt, H., 2010. The allergen Bet v 1 in fractions of ambient air deviates from birch pollen counts. Allergy 65, 850—858.
Charalampopoulos, A., Damialis, A., Tsiripidis, I., Mavrommantis, Halley, J.M., Vokou, D., 2013. Pollen production and circulation patterns along an elevation gradient in Mt Olympos (Greece) National Park. Aerobiologia 29, 455—472.
Clot, B., Peeters, A.G., Fankhauser, A., Frei, T., 1995. Airborne Pollen in Switzerland 1994. Schweizerische Meteorologische Anstalt, Zürich.
D'Amato, G., Cecchi, L., Bonini, S., Nunes, C., Annesi-Maesano, I., Behrendt, H., Liccardi, G., Popov, T., van Cauwenberge, P., 2007. Allergenic pollen and pollen allergy in Europe. Allergy 62, 976—990.
Demokritou, P., Gupta, T., Ferguson, S., Koutrakis, P., 2002. Development and laboratory characterization of a prototype coarse particle concentrator for inhalation toxicological studies. J. Aerosol Sci. 33, 1111—1123.
de Weger, L.A., Bergmann, K., Rantio-Lehtimaaki, A., Dahl, Å., Buters, J., Déchamp, C., Belmonte, J., Thibaudon, M., Cecchi, L., Besancenot, J.-P., Galán, C., Waisel, Y., 2013. Impact of pollen. In: Bergman, C.K., Sofiev, M. (Eds.), Allergenic Pollen. A Review of the Production, Release, Distribution and Health Impacts. Springer, pp. 161—215.
Draxler, R.R., Hess, G.D., 1997. Description of the HYSPLIT4 Modeling System. NOAA Tech. Memo. ERL ARL-224. NOAA Air Resources Laboratory, Silver Spring, MD, p. 24.
Durham, S.R., Nelson, H.S., Nolte, H., Bernstein, D.I., Creticos, P.S., Li, Z., Andersen, J.S., 2014. Magnitude of efficacy measurements in grass allergy immunotherapy trials is highly dependent on pollen exposure. Allergy 69, 617—623.
DWD (Deutscher Wetterdienst), 1991. Anleitung für die phänologischen Beobachter des Deutschen Wetterdienstes. Deutscher Wetterdienst, Offenbach am Main.
Eckl-Dorna, J., Klein, B., Reichenauer, T.G., Niederberger, V., Valenta, R., 2010. Exposure of rye (*Secale cereale*) cultivars to elevated ozone levels increases the allergen content in pollen. J. Allergy Clin. Immunol. 126, 1315—1317.
Frei, T., 1997. Pollen distribution at high elevation in Switzerland: evidence for medium range transport. Grana 36, 34—38.
Frenguelli, G., Passalacqua, G., Bonini, S., Fiocchi, A., Incorvaia, C., Marcucci, F., Tedeschini, E., Canonica, G.W., Frati, F., 2010. Bridging allergologic and botanical knowledge in seasonal allergy: a role for phenology. Ann. Allergy & Immunol. 105, 223—227.
Galán, C., Antunes, C., Brandao, R., Torres, C., Garcia-Mozo, H., Caeiro, E., Ferro, R., Prank, M., Sofiev, M., Albertini, R., Berger, U., Cecchi, L., Celenk, S., Grewling, Ł., Jackowiak, B., Jäger, S., Kennedy, R., Rantio-Lehtimäki, A., Reese, G., Sauliene, I., Smith, M., Thibaudon, M., Weber, B., Weichenmeier, I., Pusch, G., Buters, J.T., 2013. Airborne olive pollen counts are not representative of exposure to the major olive allergen Ole e 1. Allergy 68, 809—812.
Galán, C., Smith, M., Thibaudon, M., Frenguelli, G., Oteros, J., Gehrig, R., Berger, U., Clot, B., Brandao, R., 2014. Pollen monitoring: minimum requirements and reproducibility of analysis. Aerobiologia 16, 339—346.
Gehrig, R., Jud, S., Schuepbach, E., Clot, B., 2011. Pollen measurements in an alpine environment — altitudinal gradients and transport. In: Clot, B., Camtois, P., Escamilla-Garcia, B. (Eds.), Aerobiological Monographs, towards a Comprehensive Vision. MeteoSwiss (CH) and University of Montreal (CA), Montreal, Canada, pp. 19—35.
Gehrig, R., Peeters, A., 2000. Pollen distribution at elevations above 1000 m in Switzerland. Aerobiologia 16, 69—74.
Guedes, A., Ribeiro, N., Ribeiro, H., Oliveira, M., Noronha, F., Abreu, I., 2009. Comparison between urban and rural pollen of *Chenopodium alba* and characterization of adhered pollutant aerosol particles. Aerosol Sci. 40, 81—86.
Hjelmroos, M., Schumacher, M.J., van Hage-Hamsten, M., 1995. Heterogeneity of pollen proteins within individual *Betula pendula* trees. Int. Archives Allergy Appl. Immunol. 108, 368—376.
Jochner, S., Ziello, C., Böck, A., Estrella, N., Buters, J., Weichenmeier, I., Behrendt, H., Menzel, A., 2012. Spatio-temporal investigation of flowering dates and pollen counts in the topographically complex Zugspitze area on the German-Austrian border. Aerobiologia 28, 541—556.

Langen, U., Schmitz, R., Steppuhn, H., 2013. Häufigkeit allergischer Erkrankungen in Deutschland. Ergebnisse der Studie zur Gesundheit Erwachsener in Deutschland (DEGS1). Bundesgesundheitsblätter 56, 698–706.
Markgraf, V., 1980. Pollen dispersal in a mountain area. Grana 19, 127–146.
Masuch, G., Franz, J.-T.H., Schöne, K., Musken, H., Bergmann, K.-C.H., 1997. Ozone increases group 5 allergen content of *Lolium perenne*. Allergy 52, 874–875.
Pawankar, R., Canonica, G.W., Holgate, S.T., Lockey, R.F. (Eds.), 2011. WAO White Book on Allergy 2011–2012: Executive Summary. World Allergy Organization, Milwaukee. www.worldallergy.org/publications/wao_white_book.pdf.
Puc, M., 2011. Threat of allergenic airborne grass pollen in Szczecin, NW Poland: the dynamics of pollen season, effect of meteorological variables and air pollution. Aerobiologia 27, 191–202.
Riediker, M., Koller, T., Monn, C., 2000. Differences in size selective aerosol sampling for pollen allergen detection using high-volume cascade impactors. Clin. Exp. Allergy 30, 867–873.
Schäppi, G.F., Monn, C., Wüthrich, B., Wanner, H.U., 1996. Direct determination of allergens in ambient aerosols: methodological aspects. Int. Archives Allergy Immunol. 110, 364–370.
Schäppi, G.F., Suphioglu, C., Taylor, P.E., Knox, B.R., 1997. Concentrations of the major birch tree allergen Bet v 1 in pollen and respirable fine particles in the atmosphere. J. Allergy & Clin. Immunol. 100, 656–661.
Schäppi, G.F., Taylor, P.E., Pain, M.C.F., Cameron, P.A., Dent, A.W., Staff, I.A., Suphioglu, C., 1999. 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. Clin. Exp. Allergy 29, 633–641.
Tutin, T.G., Heywood, V.H., Burges, N.A., Valentine, D.H., Walters, S.M., Webb, D.A. (Eds.), 2001. Flora Europaea, vol. 2. Cambridge University Press, Cambridge.
Ziello, C., Estrella, N., Kostova, M., Koch, E., Menzel, A., 2009. Influence of altitude on phenology of selected plant species in the Alpine region (1971–2000). Clim. Res. 39, 227–234.
Ziska, L.H., Gebhard, D.E., Frenz, D.A., Faulkner, S., Singer, B.D., Straka, J.G., 2003. Cities as harbingers of climate change: common ragweed, urbanization, and public health. J. Allergy Clin. Immunol. 111, 290–295.