

Frequency and distribution of insect pollinated pollen in air samples of Hirst-type volumetric pollen and spore traps in Austria from 2008 to 2017

KATHARINA BASTL ¹, MAXIMILIAN BASTL ¹, LUKAS DIRR¹, MARKUS BERGER^{1,2} & UWE BERGER ¹

¹Research Group Aerobiology and Pollen Information, Department of Oto-Rhino-Laryngology, Medical University of Vienna, Vienna, Austria, ²Paracelsus Medizinische Privatuniversität, Salzburg, Austria

Abstract

Standard Hirst-type automated pollen and spore traps are used in aerobiology to assess ambient pollen concentrations with a focus on allergenic taxa, which are wind-pollinated. The relationship of insect to wind pollinated pollen concentrations has not been examined. We analysed the pollen data from the pollen seasons (1 April–31 August) 2008–2017 of seven pollen traps distributed across Austria located at different elevations. The included pollen monitoring stations cover the diversity of the countries' landscape: from large cities to alpine areas. The data was grouped into taxa known as insect (39 taxa) and taxa known as wind pollinated (43 taxa). We found a low occurrence of insect pollinated pollen in comparison to wind pollinated pollen in all stations. The mean pollen concentration of insect pollinated pollen was low and ranged from 0 to 22 pollen/m³ air. Correlation coefficients showed the dependence of the results on the parameters year, station and year × station, thus explaining the variability of the data. However, differences in abundances of insect and wind pollinated pollen concern also the time of the year with later peaks (May–June) of insect pollinated pollen in comparison to wind pollinated pollen (April). Insect pollinated pollen shows a different timing and quantitative aspect in comparison to wind pollinated pollen in air samples of Hirst-type pollen traps and thus a presumed dependence on local factors. These results should be taken into account when interpreting ancient pollen spectra to reconstruct palaeoenvironments and when monitoring recent climate change using aerobiological archives.

Keywords: *insect pollinated pollen, pollen concentrations, Hirst-type volumetric traps, air samples, outdoor air*

Plants have flowers that are adapted to different types of pollination. While angiosperm phylogeny shows a development from insect pollination (entomophily) to wind pollination (anemophily) (Faegri & van der Pijl 1979; Reeves & Olmstead 1998; Blattner & Kadereit 1999; Li et al. 1999; Linder 2000) the reverse is true in gymnosperms (Culley et al. 2002). Flowers with a morphology adapted to anemophily are commonly characterised by feathery stigmas, long stamen filaments and the absence or reduction of the perianth, nectaries and fragrance. In contrast, entomophilous flowers mostly develop simple stigmas, stigma filaments of variable length and a showy perianth that is often equipped with

nectaries and/or fragrance (Friedman & Barrett 2009). In addition to these macroscopic floral traits also palynological traits vary between these two pollination types (Vansell & Griggs 1952).

Pollen grains of wind pollinated (anemophilous) plants are relatively small, occur in large quantities (high pollen to ovule ratio), rarely show prominent ornamentation and have no or only little amounts of pollenkitt. This eases their release from the anthers and dispersal over long distances. Pollen of insect pollinated (entomophilous) plants is highly variable in size, has a lower pollen to ovule ratio, usually a more complex pollen wall ornamentation and is covered by sticky pollenkitt. All these properties serve for a better adher-

Correspondence: Maximilian Bastl, Research Group Aerobiology and Pollen Information, Department of Oto-Rhino-Laryngology, Medical University of Vienna, Währinger Gürtel 18–20, A-1090 Wien, Austria. E-mail: maximilian.bastl@meduniwien.ac.at

(Received 1 August 2019; accepted 15 October 2019)

ence to insects but limit the ability of the pollen to be distributed by wind (Hesse 1981; Culley et al. 2002).

Modern aerobiology relies on the evaluation of air samples mostly assessed with Hirst-type pollen and spore traps (Hirst 1952) besides new tools such as modelling and integration of symptom data. Other techniques range from gravimetric methods such as the Rotorod sampler (Frenz 1999) to devices with optical recognition (Oteros et al. 2015) or laser scattering with the use of fluorescence spectra (Crouzy et al. 2016). To date, only Hirst-type pollen and spore traps are considered as a standardised method including the provision of minimum recommendations concerning handling, preparation and evaluation of samples (Galán et al. 2014) and including the archiving of samples relevant for clinical or climate related studies. The results of these evaluations in routine aerobiological work are the fundament for pollen forecasts, pollen calendars and aerobiological studies. Analysts in Austria determine and count every pollen grain that is identifiable. Most of the evaluated taxa belong to well-known sources of pollen allergy and wind pollinated plants, such as trees (*Alnus*, *Corylus*, *Betula*), grasses (Poaceae) and weeds (*Artemisia*, *Ambrosia*). Allergen avoidance and thus pollen forecasts are crucial for pollen allergy sufferers, since pollen allergies are a global health problem affecting the quality of life (WAO 2013).

Beyond that, pollen archives from daily pollen concentrations kept in the European Pollen database (<https://www.ean-net.org/en/the-ean-database/more-about-ean.html>) can also be used for actuopalaentological studies. Since dispersed pollen are among the most frequent and abundant fossils in Cenozoic (the past 66 million years) and older strata, they are one of the most complete archives of vegetation and environmental (climate) history. In order to be able to decipher the fossil pollen and spore record, it is important to understand how the actual vegetation of an area is reflected in the pollen rain. Examples for such studies range from modern tree bark samples of India (Quamar & Bera 2019), cotton-wool based traps in Africa (Julier et al. 2018) to a comparison of pollen influx rates in pollen traps, peats and lake sediments from northern Fennoscandia (Hicks & Hyvärinen 1999). Results from recent pollen spectra and different sample types help understanding fossil samples without precise knowledge of the surrounding vegetation. It has long been noticed that wind-pollinated taxa are overrepresented in pollen diagrams whereas insect-pollinated taxa are underrepresented (e.g. Prentice 1988).

In this article we explore the relative contributions of wind-pollinated and insect-pollinated taxa to the pollen rain across Austria. The aims of our study are to reveal the (1) frequency, (2) variability during the

year and (3) the variability in different biogeographic regions of insect pollinated pollen in air samples of Hirst-type volumetric pollen and spore traps. To achieve this, data from seven Austrian pollen monitoring stations were re-evaluated during this study.

A low proportion of insect pollinated pollen compared to wind pollinated pollen was assumed a priori due to the characteristics described earlier, whereas the variability across different biogeographic regions in Austria was expected to be relatively low. This first in-depth analysis of the frequency of insect pollinated pollen in the air was performed in order to extract further environmental information from the composition of the pollen spectrum in routine aerobiological work.

Material and methods

Seven pollen monitoring stations were selected in Austria to cover a wide range of different biogeographic regions, vegetation types and altitudes (Table I). Pollen data from ten years was analysed (2008–2017). Only for one station data was not available for the entire period, namely for Allentsteig (operation started only in 2011). In the following, we briefly describe the natural conditions, vegetation types and climatic conditions in the vicinity of the seven included stations (Figure 1).

Allentsteig is a city in the region of Krems within the Waldviertel (Forest Quarter) in Lower Austria. The pollen monitoring station is situated on the military training area Allentsteig representing the montane vegetation belt. The surrounding area is characterised by woods. The Waldviertel is characterised by the vegetation types of silicate pine woods, riparian forests and ravine forests with bog forests, dry heaths and mat grasslands as peculiarities (Rícek 1982). The climate for the Bohemian Massif shows

Table I. Selected pollen monitoring stations and relevant information including altitude above sea level and biogeographic region within Austria.

Location	Altitude above sea level (m)	Biogeographic region
Allentsteig	596	Bohemian Massif
Linz	266	Danube valleys and pre-alpine areas
Wien	198	Pannonian Lowlands
Klagenfurt	446	Illyrian Basins
Zell am See	764	Alpine valleys central-south
Obergurgl	1930	Northern limestone Alps
Innsbruck	620	Alpine valleys Austria-west

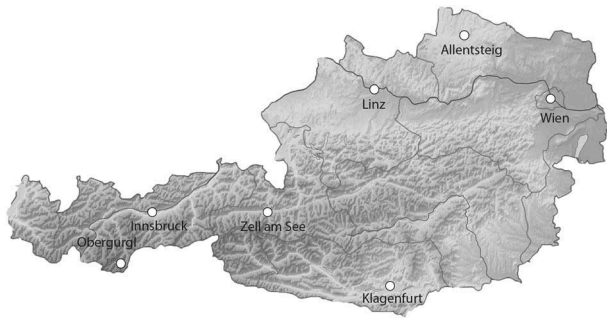


Figure 1. Map of Austria showing the topography (in colour: green = lowlands to dark brown = highest elevations; in greyscale: middle grey = lowlands to dark grey = highest elevations), country and federal states borders and the distribution of selected pollen traps (circles).

a continental and oceanic influence and is either warm temperate fully humid with warm summers or boreal fully humid with warm summers depending on the elevation (Cfb or Dfb; Rubel et al. 2017).

Linz is the capital of Upper Austria and the third largest city in Austria. The region around Linz is characterised by riparian forests, wetland meadows, but also dry habitats, terraced landscape, hill country and arable lands. The Danube valley and the pre-alpine areas in Austria show several vegetation types depending on the elevation. The lowlands are characterised by riparian forests and meadows, mixed deciduous forests and terrace landscapes. The hills and pre-alpine areas are dominated by farming landscapes such as hay meadows, dry grasslands and beech mixed woodland with an increasing proportion of spruce with increasing altitude (Essl 2003). The climate for the Danube valleys and pre-alpine areas is warm temperate fully humid with warm summers, boreal fully humid with warm summers and boreal fully humid with cool summers with boreal climate in the montane zones and oceanic influence (Cfb, Dfb and Dfc; Rubel et al. 2017).

Vienna is the capital of Austria and its largest city. It shows the lowest elevation of the selected pollen monitoring stations. The surrounding region is characterised by the Vienna basin and the lowlands including forests, fields and steppe. The Pannonian Lowlands are characterised by the vegetation type of forest steppe consisting of closed oak forests, grasslands with dry rocky grasslands and grass steppe as peculiarities (Willner 2013). The climate for the Pannonian Lowlands shows a continental influence and is warm temperate fully humid with warm summers (Cfb; Rubel et al. 2017).

Klagenfurt is the capital of Carinthia and is situated in the basin of Klagenfurt. The Illyrian basins are characterised by mixed forests of Scots pine,

beech, sweet chestnut and oak, sessile oak forests, oak-grove-hornbeam forests and alluvial forests (Kilian et al. 1994). The climate for the Illyrian basins shows a transition from Mediterranean to continental influence and is either continental warm temperate fully humid with warm summers or boreal fully humid with warm summers depending on the elevation (Cfb or Dfb; Rubel et al. 2017).

Zell am See is a city in the federal state of Salzburg. The surrounding region is characterised by mountains and the valleys themselves by mixed forests such as oak and Scots pine forest fragments, spruce and fir forest and partly alluvial forests (Kilian et al. 1994). The climate for the alpine valleys central-south shows an alpine influence and is warm temperate fully humid with warm summers and boreal fully humid with cool summers depending on the altitude and alpine influence (Cfb and Dfc; Rubel et al. 2017).

Obergurgl is a village in the Ötztal Alps in Tyrol. This pollen monitoring station is the one with the highest elevation in Austria and surrounded by mountains including several glaciers. The northern limestone Alps are characterised by mountain pines, rocky grounds and alpine grass meadows (Greimler 1997). The climate for the northern limestone Alps shows an alpine influence and is boreal fully humid with cool summers and polar tundra depending on the altitude and alpine influence (Dfc and ET; Rubel et al. 2017).

Innsbruck is the capital of Tyrol in the west of Austria. The region is known for a weather phenomenon called foehn, the Inn valley, inner alpine dry valleys, mountains and grazing areas. The alpine valleys Austria-west are characterised by the vegetation types of oak mixed forest, spruce and fir forest, montane spruce forest, Scots pine forests and alluvial forests on wet slopes (Kilian et al. 1994). The climate for the alpine valleys Austria-west shows an alpine influence and is warm temperate fully humid with warm summers with influence of boreal fully humid with warm summers depending on the elevation (Cfb and Dfb; Rubel et al. 2017).

Pollen types evaluated and defined as insect pollinated are (39 in total): *Aesculus*, *Allium*, Apiaceae, Asteraceae (*Centaurea*, *Senecio*), Boraginaceae (*Phacelia*), Brassicaceae, Campanulaceae, Caryophyllaceae, *Cornus*, Ericaceae (*Calluna*), Fabaceae (*Robinia*, *Styphnolobium*), *Forsythia*, *Fraxinus ornus*, *Garrya*, *Geranium*, *Hedera*, *Helianthemum*, *Hippophae*, Hypericaceae, *Ilex*, *Impatiens*, Juncaceae (*Luzula*), *Ligustrum*, Lamiaceae, Liliaceae, *Lotus*, Malvaceae (*Tilia*), *Philadelphus*, Polygonaceae, Ranunculaceae (*Thalictrum*), *Rhamnus*, Rosaceae (*Filipendula*, *Potentilla*, *Spiraea*), Rubiaceae, *Sambucus*, Scrophulariaceae, *Tamarix*,

Triglochin, *Viscum* and *Vitis*. The taxa included in brackets are evaluated separately in addition to their family.

These pollen types belong to trees (e.g. *Styphnolobium*, *Aesculus*, *Tilia*), shrubs (e.g. *Philadelphus*, *Tamarix*, *Sambucus*), herbaceous plants (*Phacelia*, *Senecio*) or to taxa that include more than one life form (e.g. Malvaceae, Rosaceae, Rubiaceae) as well as to evergreen (e.g. *Viscum*) and deciduous plants (e.g. *Fraxinus ornus*). *Fraxinus ornus* (insect pollinated) can be determined and separated from *Fraxinus excelsior* (wind pollinated) and was therefore included in our investigation: the morphology of its reticulum is different, the grain shows pori and is in general smaller. Some of the taxa can be wind pollinated as well, but predominantly are insect pollinated (e.g. *Luzula*, Brassicaceae). *Acer* was excluded completely from the analysis because of *Acer negundo* – a wind pollinated species that occurs frequently in Austria and cannot be separated well enough from insect pollinated species of *Acer* in the aerobiological routine by the means of light microscopy. Indeterminate pollen was excluded as well from the analysis.

Wind pollinated taxa (43 in total) comprise: *Abies*, *Ailanthus*, *Alnus*, *Ambrosia*, *Artemisia*, *Betula*, *Buxus*, Cannabaceae, *Carpinus*, *Castanea*, *Cedrus*, Chenopodiaceae, *Corylus*, Cupressaceae (*Juniperus*, *Taxus*), Cyperaceae, *Fagus*, *Fraxinus* (except *Fraxinus ornus*), *Ginkgo*, *Humulus*, *Iva*, *Juglans*, *Larix*, *Mercurialis*, *Morus*, *Olea*, *Ostrya*, *Picea*, *Pinus*, *Plantago*, *Platanus*, Poaceae, *Populus*, *Quercus*, *Rumex*, *Salix*, *Secale*, *Triticum*, Typhaceae, *Ulmus*, Urticaceae, *Xanthium* and *Zea mays*.

All pollen monitoring stations included use pollen and spore traps of the Hirst design (Hirst 1952). Devices operate conforming to the recommendations of the aerobiological community and thus are usually positioned on a flat roof with no obstacle in close vicinity of the device to ensure catchment from all wind directions. The aerobiological samples were evaluated following the standards of the European Aerobiological Society and the EAN Database (Galán et al. 2014). The beginning/end of the year was excluded from the analysis since pollen occurs rarely in late autumn/winter time and not all pollen monitoring stations are active during this time period. Thus, all analyses were performed for a time frame encompassing April to end of August.

Statistics and visualisation

The R statistical software 3.6.0 (R Core Team 2019) was used for the performed computations and R package ggplot2 (Wickham 2016) and Rioja was used to

generate the graphs (Figures 2–5). We used descriptive statistics to explore the behaviour of insect pollinated pollen in comparison to wind pollinated pollen. Percentages and the mean daily pollen concentration were calculated for each year and station (Tables II, III). In addition, graphs (Figure 2) were computed as line plots to show the progress of insect and wind pollinated pollen for each year (as a mean of all stations). The square root of the sum over all stations of insect or wind pollinated pollen was divided by the maximum of these values. This approach was used to represent the changes of the series over the year more appropriately, whereas the division serves the purpose of transforming the two series to a comparable scale. A seven-day moving average to smoothen the curve was applied as well. Pollen diagrams (Figures 3–5) were computed for April to June 2015 for three selected stations. The square root of the pollen concentrations was used as mentioned earlier for an improved visualisation with the inclusion only of taxa > 0 for the respective station and time period.

Correlation coefficients on the differenced square root of the sum of insect or wind pollinated pollen were computed for each year and station to examine a possible connection between the two series and its variability (Table IV). It is important to analyse the change from day to day instead of value of the day itself in order to remove a trend in the series, because both datasets (insect and wind pollinated pollen) depend on a seasonal course. Therefore, the time series were differenced to observe a possible, underlying relationship independent from the seasonal course. Pearson correlation was chosen and the coefficients themselves were calculated.

Furthermore, we performed an analysis of variance (ANOVA), which is shortly discussed, but not presented herein as a separate table, because the results were highly significant. The ANOVA was performed on the square root of the values to test the influence of the parameter year, station and the interaction between year and station. The transformation of the values was applied to produce a closer approximation to the required normal distribution. We chose this method because ANOVA is the standard method to test the (linear) influence of parameters on an observed variable.

Results

The concentrations of pollen from insect and wind pollinated plants were assessed in a standardised way (same method and time period). Pollen grains were assigned to insect or wind pollinated taxa before the analysis which is not a standard aerobiological routine (see Materials and methods section).

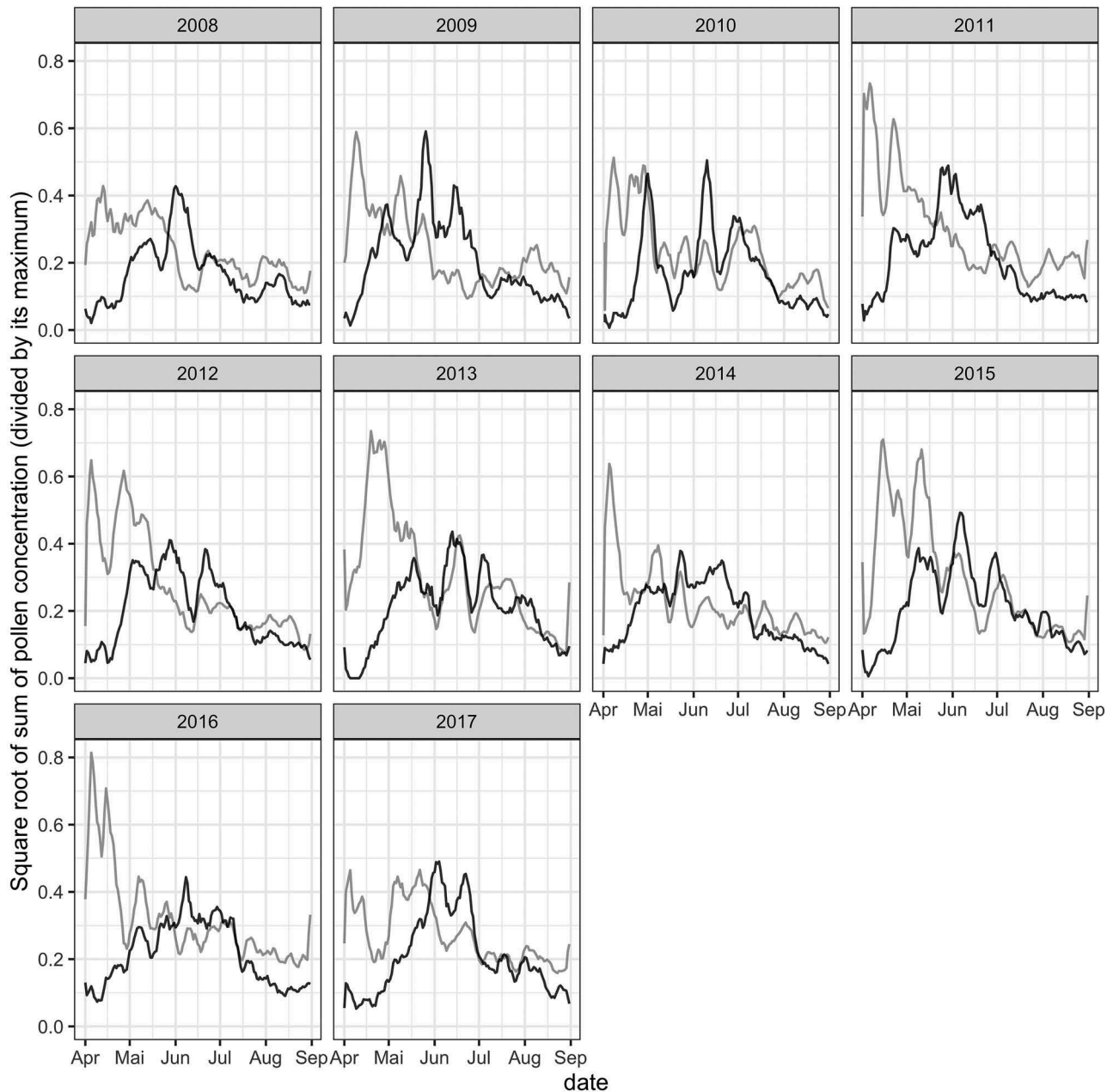


Figure 2. Line plots of all selected Austrian stations for 2008 to 2017 with insect pollinated pollen (black) and wind pollinated pollen (dark grey) as seven-day sliding mean of the square root of the sum of the pollen concentration divided by its maximum over the observed pollen season. Note the peaks during the middle of the season and the variation in the first third versus the stable relationship in the last third of the time period.

The progress of the insect pollinated pollen concentrations over the season was more stable than the progress of the wind pollinated pollen concentrations (Figure 2). The peak of insect pollinated pollen usually occurs in the months May and June, while there is variation in April apart from a general increase during this month. The months July and August show a decrease and the most stable pattern of all observed months during the years, which are characterised by a general decrease. In addition, the

graphs evidence variation in the yearly amount as described later, not only in the progress.

The wind pollinated pollen concentrations show a higher variation in both amount and progress. The months April and May are characterised by high variation. They include the peak, which occurs earlier than in insect pollinated pollen. The trend is overall decreasing for the following months except for a small peak during end of June/beginning of July and an increase towards September. In sum, the

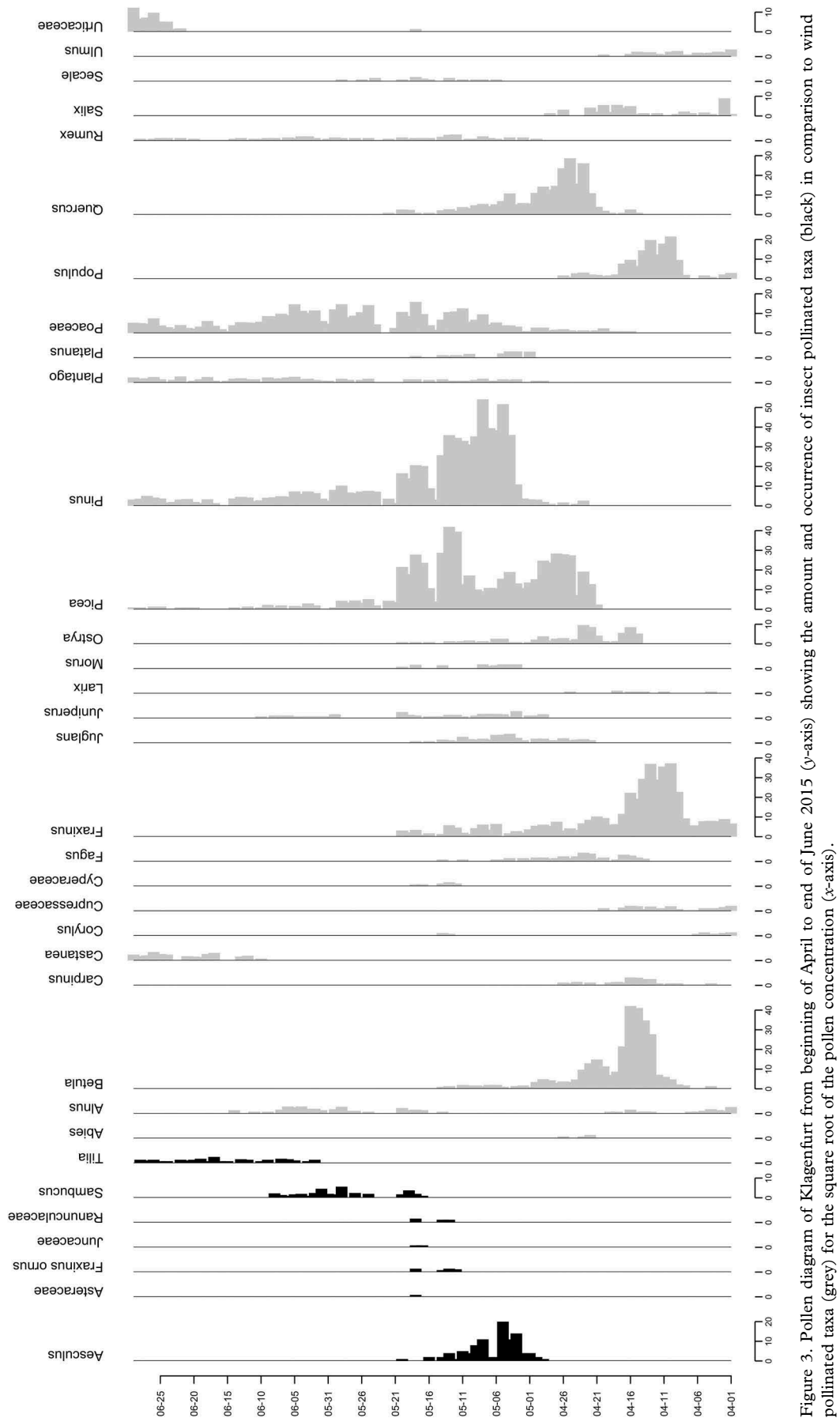


Figure 3. Pollen diagram of Klagenfurt from beginning of April to end of June 2015 (y-axis) showing the amount and occurrence of insect pollinated taxa (black) in comparison to wind pollinated taxa (grey) for the square root of the pollen concentration (x-axis).

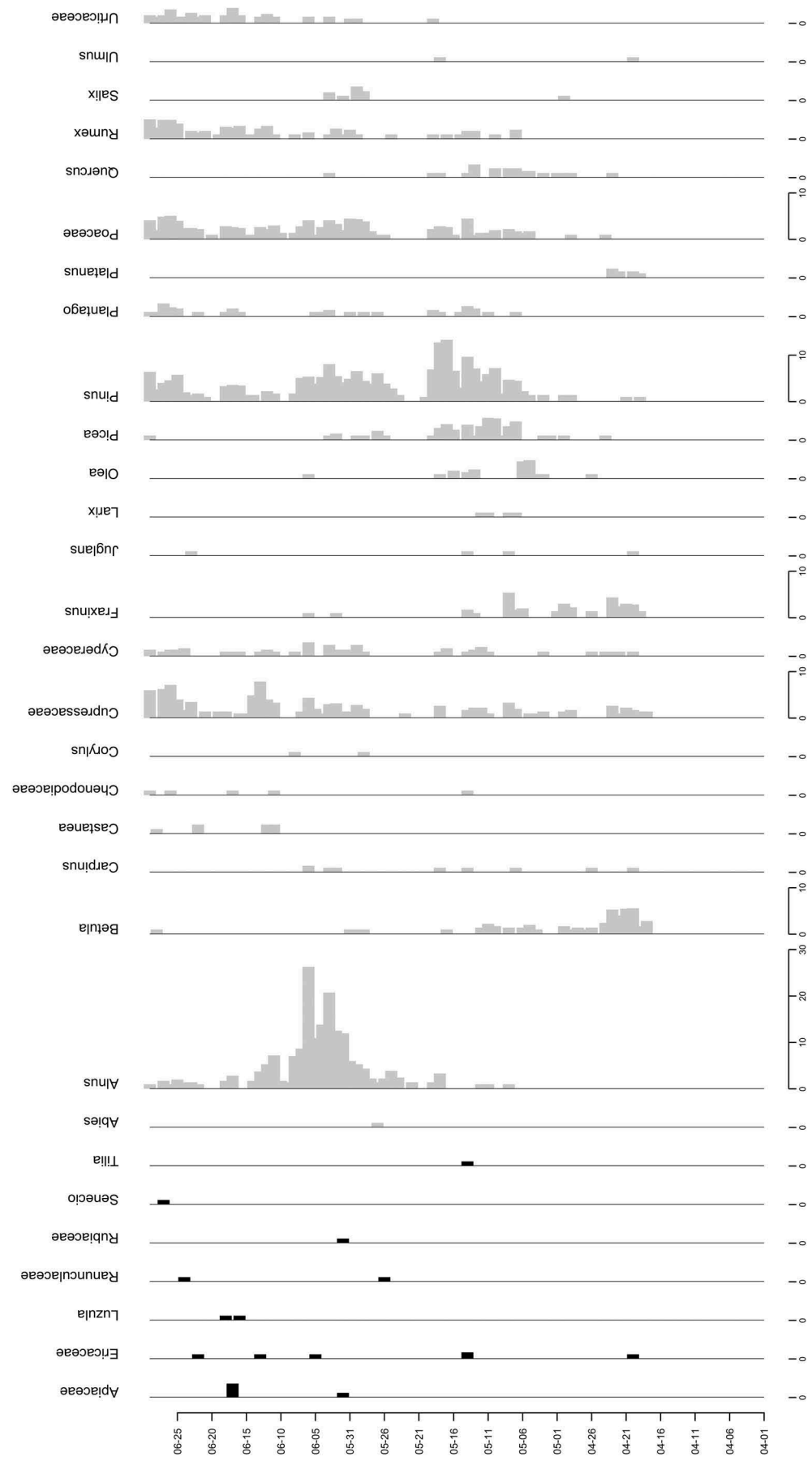


Figure 4. Pollen diagram of Obergurgl from beginning of April to end of June 2015 (y-axis) showing the amount and occurrence of insect pollinated taxa (black) in comparison to wind pollinated taxa (grey) for the square root of the pollen concentration (x-axis).

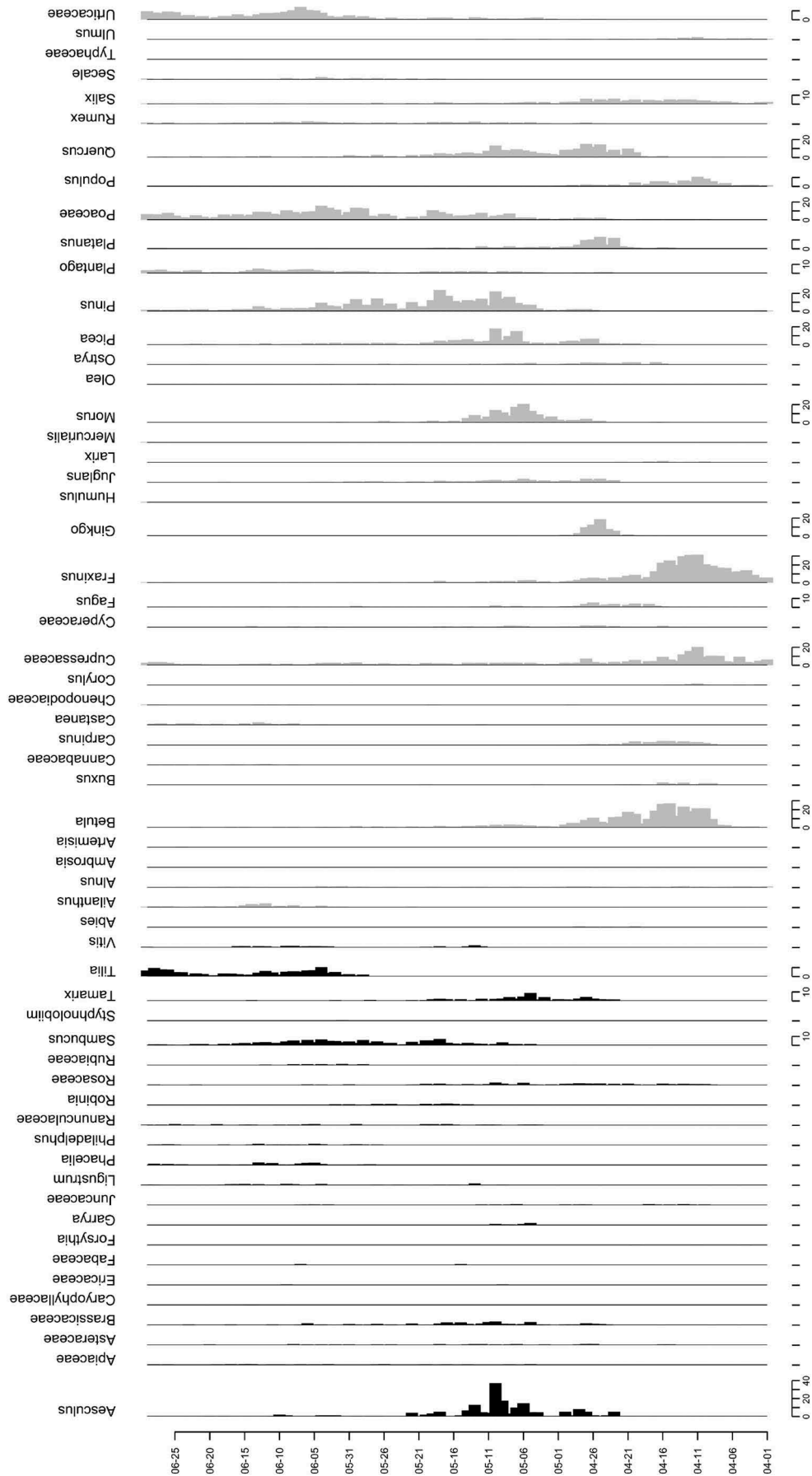


Figure 5. Pollen diagram of Vienna from beginning of April to end of June 2015 (y-axis) showing the amount and occurrence of insect pollinated taxa (black) in comparison to wind pollinated taxa (grey) for the square root of the pollen concentration (x-axis).

Table II. Percentages of insect pollinated pollen per year 2008–2017 (1 April–31 August) and station. Note the consistency over the years and certain variations per station.

	Allentsteig	Linz	Vienna	Klagenfurt	Zell am See	Obergurgl	Innsbruck
2008		1.9	6.4	3.2	2.9	2.2	2.8
2009		3.5	12.6	1.9	3.7	2.5	3.9
2010		1.7	5.9	2.6	3.0	3.5	3.3
2011	2.1	4.7	10.2	2.9	1.4	2.4	5.3
2012	4.5	3.4	10.2	1.5	3.0	2.2	5.4
2013	3.4	3.0	11.6	2.1	1.0	2.5	2.3
2014	3.8	2.9	12.1	2.6	1.7	2.5	5.8
2015	2.7	2.5	9.4	1.9	1.5	1.7	3.1
2016	2.9	1.6	7.7	1.4	4.9	1.4	4.2
2017	1.9	2.2	8.3	0.6	3.5	1.9	4.0

Table III. Mean daily pollen concentrations of insect/wind pollinated pollen rounded to whole numbers per year 2008–2017 (1 April–31 August) and station.

	Allentsteig	Linz	Vienna	Klagenfurt	Zell am See	Obergurgl	Innsbruck
2008		0/52	12/277	3/226	1/35	0/41	2/110
2009		3/135	22/284	2/169	1/41	1/68	1/127
2010		1/140	12/258	2/162	1/39	1/50	6/185
2011	6/287	1/92	15/219	2/410	0/42	1/49	4/164
2012	8/335	1/122	11/135	2/314	1/41	1/51	2/157
2013	7/321	1/98	15/297	2/285	0/40	1/81	2/270
2014	6/202	1/69	11/122	2/155	0/19	0/37	3/164
2015	5/197	1/135	18/246	2/406	0/32	1/68	2/279
2016	4/205	1/158	14/275	2/440	3/84	1/89	3/204
2017	2/149	2/117	17/200	1/240	3/61	1/46	2/178

Table IV. Correlation coefficients for each station and year 2008–2017 (1 April–31 August). The coefficients were calculated as differenced square root of the sum of insect or wind pollinated pollen. All correlations are positive and all > 0 are significant.

	Allentsteig	Linz	Vienna	Klagenfurt	Zell am See	Obergurgl	Innsbruck
2008		54.0	25.3	7.9	24.6	25.3	55.6
2009		27.3	36.2	13.5	33.6	26.7	47.8
2010		32.6	34.2	17.0	29.2	25.2	30.2
2011	28.1	34.1	38.0	7.9	34.8	21.3	50.0
2012	19.2	12.8	37.0	0.0	32.8	34.2	38.9
2013	35.3	15.2	26.1	13.9	23.1	22.0	45.0
2014	34.6	43.2	45.1	10.7	28.0	33.7	21.6
2015	31.7	40.8	47.4	26.9	25.4	16.5	3.0
2016	32.3	14.9	44.2	21.0	41.0	21.9	22.7
2017	25.0	27.3	25.3	7.3	66.3	37.4	31.5

progress of the insect and the wind pollinated pollen concentrations is different (Figure 2).

Three pollen diagrams were produced as an exemplary case to show the variability of insect pollinated pollen taxa in different locations and the dominance of wind pollinated pollen in general. Vienna was selected as lowland station, Obergurgl as station with the highest elevation and Klagenfurt as a comparison in between those extremes and intermediate also in a geographic sense (Figure 1). The domination of wind pollinated pollen taxa is obvious for all three examples (Figures 3–5). Klagenfurt shows the

domination of *Aesculus*, *Sambucus* and *Tilia* among the insect pollinated pollen (Figure 3). Obergurgl (Figure 4) recorded the lowest values in general and is not dominated by specific insect pollinated pollen types, but includes taxa like *Luzula* and *Senecio* not recorded in the other comparison stations. Vienna (Figure 5) shows the highest diversity of taxa, both insect and wind pollinated and the importance of *Aesculus*, *Sambucus*, *Tamarix* and *Tilia* among the insect pollinated pollen.

The percentages of insect pollinated pollen out of all evaluated pollen range for all selected stations from

0.6 (2017 Klagenfurt) up to 12.1 (2014 Vienna), whereas they range per station from 1.9–4.5 (Allentsteig), 1.6–4.7 (Linz), 5.9–12.1 (Vienna), 0.6–3.2 (Klagenfurt), 1.0–4.9 (Zell am See), 1.4–3.5 (Oberurgl) and 2.3–5.8 (Innsbruck; Table II). A rather low percentage up to around 6% was found to be the general pattern for all stations except for Vienna. The station Vienna starts at a rather high percentage and reaches up to around 12%. The lowest range and percentages are observed for Klagenfurt. In addition, a yearly variation can be observed. This variation exhibits no clear pattern for all stations, e.g. the peak year per station and the increase/decrease trend do not occur in parallel for all stations. There seems to be no trend/relationship concerning the sea level and the percentage of insect pollinated pollen, since the percentages, e.g. from a high elevated site like Oberurgl (1930 m) are well comparable to those of a low elevated site like Linz (266 m).

The mean daily pollen concentrations of insect versus wind pollinated pollen (Table III) illustrates the quantitative aspect: Insect pollinated pollen occurs in low daily amounts ranging from zero to a maximum mean daily pollen concentration of 22 in the samples examined herein. The highest values occur again for Vienna (11–22), followed by Allentsteig (2–8), Innsbruck (1–6), Klagenfurt (1–3), Linz as well as Zell am See (0–3) and Oberurgl (0–1). It should be noted, that these values are a mean and that there is a seasonal progress in the increase/decrease of insect pollinated pollen concentrations (Figure 2).

The correlation coefficients were calculated per year and station separately and result in significant positive values for nearly all instances (Table IV). Variation is observed between different years of the same station and between stations as illustrated by the respective ranges. Highest correlation coefficients were found for Zell am See (23.1–66.3), followed by Innsbruck (3.0–55.6), Linz (12.8–54.0), Vienna (25.3–47.4), Oberurgl (16.5–37.4), Allentsteig (19.2–35.3) and Klagenfurt (0.0–26.9). There seems to be no common trend or pattern for all stations or years in contrast to the results from the percentages (Table II). This means there is no parallel increase/decrease pattern for certain years or a station specific behaviour. However, the coefficients do indicate a significant dependence on the station and the year.

The results of the ANOVA gave highly significant *p*-values for all three parameters (values below 0.001). The ANOVA thus indicates that there is a strong influence of the year, station and year $\times$ station on the amount of insect pollinated pollen and adds to the results of the variability (as seen in the seasonality and year in Figure 2 and in the station and yearly differences in Tables II, IV).

Discussion

Considerable variation is observed for the first part of the season for wind pollinated pollen concentrations in Figure 2. This corresponds to the variable intense flowering of trees during this time period such as *Betula*, *Quercus* and *Fagus* (see also Figure 5). All of them are widely distributed in Austria, wind-pollinated and capable of producing high pollen concentrations. Particularly *Betula* is known for a highly variable yearly pollen production and a high total amount of pollen in general: a less intense year may result in a seasonal pollen index of 3487 (2017 Vienna) and an intense year reaches values of 9233 (2016 Vienna; taken from Bastl et al. 2018). The flowering of these trees is responsible for the peak of wind pollinated pollen during April, whereas the peak of insect pollinated pollen occurs later. Major contributors of insect pollinated pollen are *Aesculus*, *Sambucus*, and depending on the location also Rosaceae, Brassicaceae and *Tilia* (Figures 3–5).

The increase of wind pollinated pollen in the end of August is not unexpected. This phenomenon is linked to the full flowering of *Ambrosia*. Long distance pollen transport plays a significant role in Austria and adds to the pollen concentrations deriving from the local flowering. The pollen season would be coming to an end during this time, if the late bloomer *Ambrosia* would not have been introduced to Austria. *Ambrosia* is a significant source of wind-pollinated pollen during late summer/autumn and may burden concerned pollen allergy sufferers until October.

Comparing these results to the actual vegetation in the vicinity of the selected pollen traps (Figure 1; for a description of the predominating vegetation types for each sample locality see Material and methods section) the following patterns become apparent: Wind pollinated pollen is overrepresented, while insect pollinated pollen is underrepresented. The surrounding vegetation is reflected in the pollen spectrum, but biased. This is best illustrated by pollen trap specific signals (e.g. *Tilia* in Vienna, discussed in the following). Moreover, it appears from our results that different climatic influences do not alter the ratios of insect to wind pollinated pollen, although our sample is too limited to allow a general conclusion on this aspect.

It is noteworthy that Vienna shows higher percentages and higher daily mean pollen concentrations (Tables II, III) for insect pollinated pollen than all other stations. We can attribute this finding to the local situation of the pollen trap in Vienna. The station is placed in the suburbs and *Tilia* is planted next to the building that hosts the pollen trap on its

roof. Therefore, *Tilia* pollen has a strong local impact, which was already observed for outdoor soil samples from the same area (Bastl et al. 2019) as well as for indoor dust samples from the same building (Bastl et al. 2017). The influence of local conditions can also account for differences in pollen concentrations in soil and air samples. For example, Bastl et al. (2019) observed higher frequency of *Acer* pollen in soil than in air samples as a result of accumulation of withered flowers.

It should be noted that the attribution of a pollen type to the local or regional flora is not always clear: This was illustrated by Van Zeist et al. (1975) by the description of a peak of insect pollinated Asteroideae and Cichorioideae. The increased occurrence of Asteraceae pollen might just be taken as part of a typical steppe signal. In fact, Van Zeist et al. (1975) suggested that it indicated the temporal exposure of a lake shore. In contrast, the steppe signal would not have that high concentrations of Asteroideae and Cichorioideae pollen and different overall pollen composition of the pollen diagram. In this latter case, the same insect pollinated pollen would have been of a different origin further away from the lake. Taxa being insect pollinated are not expected to dominate a pollen sample if they are part of the hinterland (i. e. the steppe vegetation). However, they may dominate if they are growing very locally (as seen also herein for *Tilia* in Vienna).

Certain characteristics such as the production of pollen in high quantities (Hesse 1981; Culley et al. 2002) predetermine wind pollinated pollen as a more likely cause of pollen allergies in comparison to insect pollinated pollen. However, it is important to record all identifiable pollen, because allergies to insect pollinated plants such as *Solidago* (de Jong et al. 2007) and the Asteraceae (Feinberg 1934) were reported and the allergic response to ornamental plants is increasing (Goldberg et al. 1998).

Modern aerobiological studies are important, because they aid in the interpretation of historical pollen archives ranging from the past decades to millions of years. To name a few concrete and recent insights: Within a forest–savannah transition in west Africa a Poaceae abundance of more than 40% has been considered as indicative of a savannah-type vegetation (Julier et al. 2018). Another modern pollen-rain study revealed that considerable pollen producers such as teak with a dominant frequency (80–95% of the total forest constituents) may still result in a low recorded frequency in pollen diagrams due to entogamy (Quamar & Bera 2014) and thus introduce a substantial bias to pollen diagrams. In contrast, a complex mangrove environment in eastern India is well reflected in the pollen record and useful for the interpretation of the palaeoenvironment and palaeoecology of tropical

marshes and similar environments (Pandey & Minckley 2019). Thus, the results presented herein deriving from air samples are also relevant to other disciplines than aerobiology such as, e.g. palaeoecology, archeology or forensic sciences.

The present study is the first study to our knowledge that evaluates the relationship between insect and wind pollinated pollen in air samples of the Hirst-trap design and documents the dominance of wind pollinated pollen and variability of taxa in recent pollen rain (Figures 3–5). Outdoor soil and air samples assessed with the pollen trap in Vienna record similar signals during the pollen season and the impact of local vegetation (Bastl et al. 2019).

Conclusions

The evaluation of insect pollinated pollen in comparison to wind pollinated pollen focusing on the progress during the season and the quantitative aspects indicate (1) different seasonal occurrence of insect and wind pollinated pollen concerning progress and peaks, (2) low quantities of insect pollinated pollen in comparison to wind pollinated pollen with daily means between 0 and 22 pollen/m³ air or 0.6–12.1% of wind pollinated pollen and (3) a significant dependence on the station, year and station × year.

Insect pollinated pollen accounts for a small fraction in air samples attained by Hirst-type traps usually used in aerobiology but may give some useful indication concerning the relationship of insect and wind pollinated pollen in general and should be evaluated in the aerobiological routine, since pollen allergies may be caused also by insect pollinated pollen and results are an important source of recent comparisons to other fields of study.

Acknowledgements

The authors are grateful to all the data suppliers including Roland Schmidt, Helmut Zwander, Ulrike Langmann, Margit Langanger, Siegfried Jäger, Notburga Oeggel-Wahlmüller and the local pollen information services in Austria: The pollen information service in Carinthia, Upper Austria, Salzburg and Tyrol, who provided the fundament for the evaluation herein with their aerobiological routine work in addition to the evaluations performed in Vienna by our team. The authors also thank Alexander Kowarik (Statistics Austria, Vienna, Austria) for the support in statistical analyses. We thank two anonymous reviewers for improving our manuscript and valuable input, especially one of them, whose efforts smoothened the manuscript text. In addition, the authors are grateful for the improvements, discussions and the feedback

from the handling editor, Thomas Denk, for this publication.

Furthermore, it is our wish to dedicate this work to our teacher in (palaeo)palynology, Ao.Univ.-Prof. Mag. Dr Reinhard Zetter, who has guided two of us (KB and MaBa) on our way into the world of pollen.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Katharina Bastl  <http://orcid.org/0000-0002-8200-9708>

Maximilian Bastl  <http://orcid.org/0000-0002-2182-0409>

Uwe Berger  <http://orcid.org/0000-0002-9265-2131>

References

- Bastl K, Bastl M, Berger U, Weber M. 2019. Air and surface soil samples – two different pair of shoes? Comparing the pollen spectrum on different days of the pollen season. *Grana* 58: 371–382. doi:10.1080/00173134.2019.1615985.
- Bastl K, Berger U, Kmenta M, Weber M. 2017. Is there an advantage to staying indoors for pollen allergy sufferers? Composition and quantitative aspects of the indoor pollen spectrum. *Building and Environment* 123: 78–87. doi:10.1016/j.buildenv.2017.06.040.
- Bastl K, Kmenta M, Berger M, Berger U. 2018. The connection of pollen concentrations and crowd-sourced symptom data: New insights from daily and seasonal symptom load index data from 2013 to 2017 in Vienna. *World Allergy Organization Journal* 11: 24. doi:10.1186/s40413-018-0203-6.
- Blattner FR, Kadereit JW. 1999. Morphological evolution and ecological diversification of the forest-dwelling poppies (Papaveraceae: Chelidonioideae) as deduced from a molecular phylogeny of the ITS region. *Plant Systematics and Evolution* 219: 181–197. doi:10.1007/BF00985578.
- Crouzy B, Stella M, Konzelmann T, Calpini B, Clot B. 2016. All-optical automatic pollen identification: Towards an operational system. *Atmospheric Environment* 140: 202–212. doi:10.1016/j.atmosenv.2016.05.062.
- Culley TM, Weller SG, Sakai AK. 2002. The evolution of wind pollination in angiosperms. *Trends in Ecology and Evolution* 17: 361–369. doi:10.1016/S0169-5347(02)02540-5.
- de Jong NW, Vermeulen AM, Gerth van Wijk R, de Groot H. 2007. Occupational allergy caused by flowers. *Allergy* 53(2): 204–209. doi:10.1111/j.1398-9995.1998.tb03872.x.
- Essl F. 2003. Zur Vegetation Oberösterreichs – Ein Überblick. *Denisia* 0007: 31–41.
- Faegri K, van der Pijl L. 1979. The principles of pollination ecology. 3rd ed. Oxford: Pergamon Press.
- Feinberg SM. 1934. Pyrethrum sensitization. Its importance and relation to pollen allergy. *Journal of the American Medical Association* 102(19): 1557–1558. doi:10.1001/jama.1934.02750190015006.
- Frenz DA. 1999. Comparing pollen and spore counts collected with the Rotorod Sampler and Burkhard spore trap. *Annals of Allergy, Asthma and Immunology* 83: 341–347. doi:10.1016/S1081-1206(10)62828-1.
- Friedman J, Barrett SCH. 2009. Wind of change: New insights on the ecology and evolution of pollination and mating in wind-pollinated plants. *Annals of Botany* 103: 1515–1527. doi:10.1093/aob/mcp035.
- Galán C, Smith M, Thibaudon M, Frenguelli G, Oteros J, Gehrig R, Berger U, Brandao R, EAS QC Working Group. 2014. Pollen monitoring: Minimum requirements and reproducibility of analysis. *Aerobiologia* 30: 385–395. doi:10.1007/s10453-014-9335-5.
- Goldberg A, Confino-Cohen R, Waisel Y. 1998. Allergic responses to pollen of ornamental plants: High incidence in the general atopic population and especially among flower growers. *Journal of Allergy and Clinical Immunology* 102(2): 210–214. doi:10.1016/S0091-6749(98)70088-0.
- Greimler J. 1997. Pflanzengesellschaften und Vegetationsstruktur in den südlichen Gesäusebergen (nordöstliche Kalkalpen, Steiermark). *Mitteilungen Der Abteilung Für Botanik Am Landesmuseum "Joanneum" Graz* 25–26: 1–238.
- Hesse M. 1981. Pollenkitt und viscin threads: Their role in cementing pollen grains. *Grana* 20: 145–152. doi:10.1080/00173138109427656.
- Hicks S, Hyvärinen H. 1999. Pollen influx values measured in different sedimentary environments and their palaeoecological implications. *Grana* 38(4): 228–242. doi:10.1080/001731300750044618.
- Hirst JM. 1952. An automatic volumetric spore trap. *Annals of Applied Biology* 39(2): 257–265. doi:10.1111/aab.1952.39.issue-2.
- Julier ACM, Jardine PE, Adu-Bredu S, Coe AL, Duah-Gyamfi A, Fraser WT. 2018. The modern pollen-vegetation relationships of a tropical forest-savannah mosaic landscape, Ghana, West Africa. *Palynology* 42(3): 324–338. doi:10.1080/01916122.2017.1356392.
- Kilian W, Müller F, Starlinger F. 1994. Die forstlichen Wuchsgebiete Österreichs. Eine Naturraumgliederung nach waldökologischen Gesichtspunkten. Wien: Forstliche Bundesversuchsanstalt.
- Li J, Bogle AL, Donoghue MJ. 1999. Phylogenetic relationships in the Hamamelidoideae inferred from sequences of TRN non-coding regions of chloroplast DNA. *Harvard Papers in Botany* 4: 343–356.
- Linder HP. 2000. Pollen morphology and wind pollination in angiosperms. In: Harley MM, Morton CM, Blackmore S, eds. *Pollen and spores: morphology and biology*, 73–88. Kew: Royal Botanic Gardens.
- Oteros J, Pusch G, Weichenmeier I, Heimann U, Möller R, Röseler S, Traidl-Hoffmann C, Schmidt-Weber C, Buters JTM. 2015. Automatic and online pollen monitoring. *International Archives of Allergy and Immunology* 167(3): 158–166. doi:10.1159/000436968.
- Pandey S, Minckley TA. 2019. Modern pollen-vegetation studies from the Sajnekhali Island Wildlife Sanctuary, Sundarbans, Eastern India. *Palynology* 43(2): 213–222. doi:10.1080/01916122.2018.1451786.
- Prentice IC. 1988. 2. Records of vegetation in time and space: The principles of pollen analysis. In: Huntley B, Webb T III, eds. *Vegetation history*, 17–42. Dordrecht: Kluwer Academic Publishers.
- Quamar MF, Bera SK. 2014. Surface pollen and its relationship with modern vegetation in tropical deciduous forests of south-western Madhya Pradesh, India: A review. *Palynology* 38(1): 147–161. doi:10.1080/01916122.2013.875491.
- Quamar MF, Bera SK. 2019. Aerobiological implications of the extracted palynomorphs from the modern tree barks of the

- Korba District, Chhattisgarh, central India. *Palynology* 43(1): 34–42. doi:[10.1080/01916122.2018.1430069](https://doi.org/10.1080/01916122.2018.1430069).
- R Core Team. 2019. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. <https://www.R-project.org/>.
- Reeves PA, Olmstead RG. 1998. Evolution of novel morphological and reproductive traits in a clade containing *Antirrhinum majus* (Scrophulariaceae). *American Journal of Botany* 85: 1047–1056. doi:[10.2307/2446338](https://doi.org/10.2307/2446338).
- Ricek EW. 1982. Die Flora der Umgebung von Gmünd im niederösterreichischen Waldviertel. *Abhandlungen Der Zoologisch-Botanischen Gesellschaft in Österreich* 21: 1–204.
- Rubel F, Brugger K, Haslinger K, Auer I. 2017. The climate of the European Alps: Shift of very high resolution Köppen–Geiger climate zones 1800–2100. *Meteorologische Zeitschrift* 26: 115–125. doi:[10.1127/metz/2016/0816](https://doi.org/10.1127/metz/2016/0816).
- Van Zeist W, Woldring H, Stapert D. 1975. Late Quaternary vegetation and climate of southwestern Turkey. *Palaeohistoria* 14: 35–143.
- Vansell GH, Griggs WH. 1952. Honey bees as agents of pollination. In: *Yearbook of Agriculture*, 88–107. Washington, DC: US Government Printing Office.
- WAO (World Allergy Organisation). 2013. White book on allergy: update 2013. Pawankar R, Canonica GW, Holgate ST, Lockey RF, Blaiss MS. www.worldallergy.com.
- Wickham H. 2016. *ggplot2: elegant graphics for data analysis*. New York: Springer.
- Willner W. 2013. Pannonische Steppenrasen in Österreich. In: Druckhaus Gera GmbH, Gera, ed. *Steppenlebensräume Europas – Gefährdung, Erhaltungsmaßnahmen und Schutz*. Thüringer Ministerium für Landwirtschaft, Forsten, Umwelt und Naturschutz, 151–162. Erfurt: Druckhaus Gera GmbH.