

Unusually early flowering of alder in Vienna: first report of *Alnus* × *spaethii* in Austria, combined LM and SEM study of alder species and impact on pollen allergy sufferers

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Abstract Different species of alder cause hay fever symptoms in pollen allergy sufferers within Europe: *Alnus glutinosa* (black alder), *A. incana* (grey alder), *A. viridis* (green alder) and more recently *A. × spaethii* (*japonica* × *subcordata*; Spaeth's alder). In Austria, the alder pollen season usually lasts from January to April (until June in the mountains due to later flowering of *A. viridis*). The pollen season started very early in 2014, namely by the end of December 2013 in Vienna with the first pollination of alder. Such an early flowering around Christmas is known for *A. × spaethii* and was first recognized as problem in Switzerland. Up to now, this alder species was not reported in Austria. A sample including twigs, leaves, catkins and pollen from Switzerland was compared with an unusually early flowering alder in Vienna. The pollen of these (*Alnus* × *spaethii*) and in addition from black and green alder was analysed by light microscopy and scanning electron microscopy to find possible morphological differences or similarities in their pollen morphology. The general morphology revealed that *A. × spaethii* is present in Austria

(planted in a park) and was responsible for the first burden on pollen allergy sufferers during the alder pollen season in Vienna, although pollen data revealed no significant alder pollen concentrations. The results emphasize the importance of monitoring phenology for pollen information.

Keywords *Alnus* · Phenology · Palynology · Symptom load index · Patient's hayfever diary

Abbreviations

EAN European aeroallergen network
PHD Patient's hayfever diary
SLI Symptom load index

1 Introduction

According to the first Austrian allergy report (Dorner et al. 2006), about one million people suffer from pollen allergy in Austria; especially, tree pollen attributed to the order of Fagales such as birch, alder and hazel are responsible for pollinosis in spring (Wahl et al. 1996). Alder pollen has a considerable impact on pollen allergy sufferers due to (1) the wide distribution of alder within Europe (e.g. *A. glutinosa*; Fig. 1; EUFORGEN 2009, <http://www.euforgen.org/distribution-maps>), (2) cross-reactivity of alder pollen allergens (Aln g1) with, for

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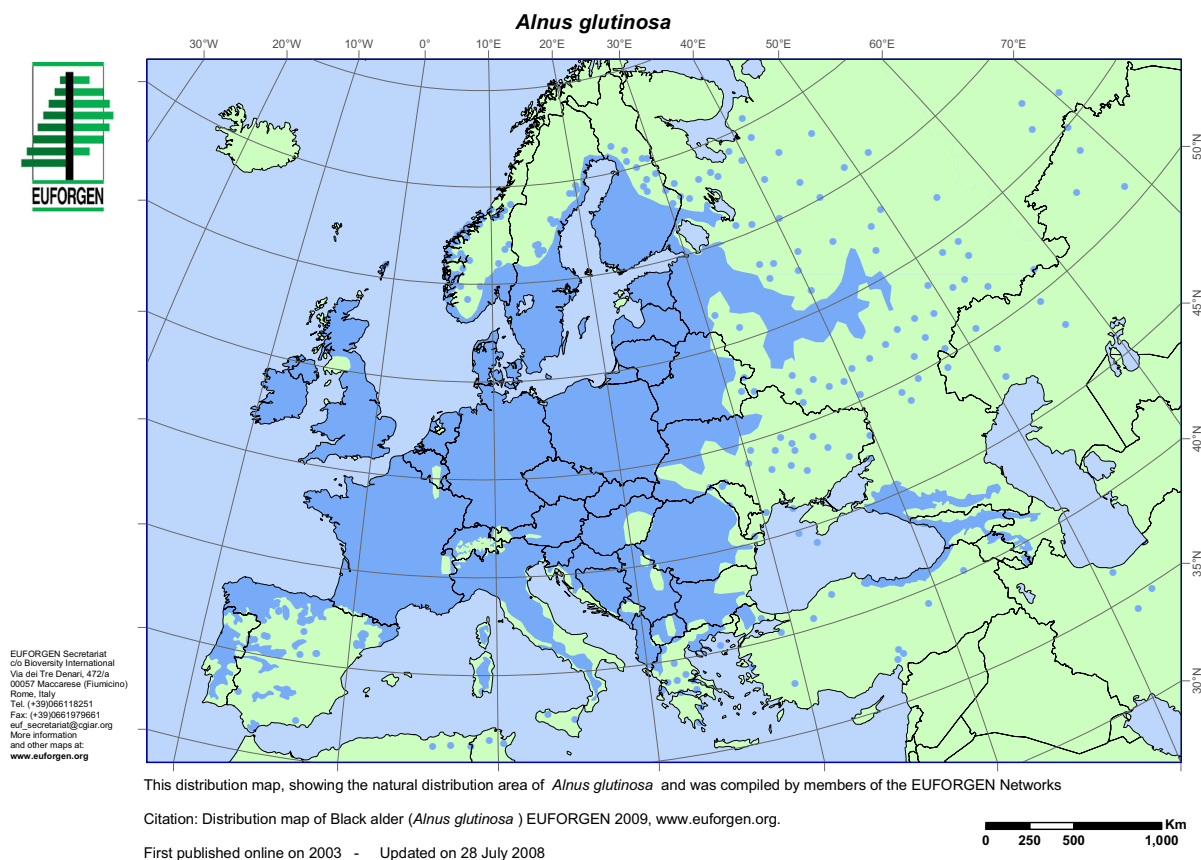


Fig. 1 Distribution of black alder (*A. glutinosa*) in Europe according to EUFORGEN EUFORGEN 2009. <http://www.euforgen.org/distribution-maps>, requested on 23 September 23, 2014

example, birch pollen allergens (Bet v1; Ebner et al. 1993) and (3) the early occurrence of the beginning of pollen season in January/February. Up to now, three species of alder have been known to be distributed in Austria: *Alnus glutinosa* (black alder), *A. incana* (grey alder) and *A. viridis* (green alder). According to the Bundesamt und Forschungszentrum für Wald (http://bfw.ac.at/700/2092_1.html, Schadauer 1994), black alder is more dominant in the northern and eastern parts of Austria, whereas grey alder dominates in the south, central and western parts. Moist habitats along rivers are preferred, but both black and grey alder reach subalpine regions in their distribution. However, green alder is the dominating alder species at higher elevations and grows in the Alps and Prealps. It grows at higher altitudes (subalpine to montane regions) in the more mountainous regions of Austria and flowers later than the other alder species, i.e. from May to June. Nonetheless, pollination

from green alder was recognized as a considerable threat to alder pollen allergy sufferers in valleys due to medium range transport in Switzerland (Gehrig and Peeters 2000). Just recently, an additional alder species was detected to cause discomfort in pollen allergy sufferers (Gassner et al. 2013, 2014; Gehrig et al. 2014). The hybrid species *A. × spaethii* (*A. japonica* × *A. subcordata*) shows high winter resistance and was therefore planted along a main boulevard in Buchs, a city in Switzerland close to Austria (Gassner et al. 2013, 2014; Gehrig et al. 2014). The authors (2013, 2014) observed unusual winter allergic symptoms around Christmas and detected increased prevalence of IgE antibodies against the main allergen Aln g1 in school children (1986–2010) caused by the increased plantation of this hybrid tree. Thus, it has to be kept in mind that there are different species of alder that release pollen early in the season, depending on the meteorological conditions.

Alder is grouped together with birch and hornbeam in the family Betulaceae, but is considered to be more closely related to birch than to hazel or hornbeam (Chen et al. 1999). The genus expanded during the late glacial and early Holocene periods in Europe (Douda et al. 2014). Different species-specific requirements in meteorological and ecological parameters have been discussed, but not confirmed up to now (Douda et al. 2014). Concerning the variations in alder pollen concentrations in the air, it is known that temperature is the most important factor (Frenguelli et al. 1991; Myszkowska et al. 2010; Puc and Kasprzyk 2013). Alder pollen was investigated in detail as early as 1953 (Erdtman 1953). More recent publications reworked the morphological details (Blackmore et al. 2003; Leopold et al. 2012). It is possible to discriminate *A. viridis* pollen from *A. glutinosa* and *A. incana* based on micromorphological features (e.g. Blackmore et al. 2003). However, the latter two species are united in the *A. glutinosa* morphotype (Blackmore et al. 2003). Leopold et al. (2012) defined four categories for morphological differences for *Alnus*, including the number of pores (dominance and range), strength/thickness of arci, the presence/absence of polar arci and the size of pollen grains. None of the recent publications included the hybrid species *A. × spaethii* (*A. japonica* × *A. subcordata*). The pollen morphology of *A. × spaethii* was observed herein in detail to assess possible determination features (LM and SEM) to evaluate whether hitherto undetermined trees/population could be identified in the time of their flowering in winter (before leaf sprouting). Therefore, pollen sampled from the first observed *A. × spaethii* trees in Vienna were compared with a sample from Switzerland, as well as samples from *A. glutinosa* and *A. viridis* by light microscopy (LM) and scanning electron microscopy (SEM) to complete the palynological knowledge and to test determination on species level based on pollen morphology as well as intraspecific variability of *A. × spaethii* pollen.

2 Materials and methods

For this study, an alder tree population in the north-eastern part of Vienna was observed and documented (Wasserpark location). The population included four individuals of *A. glutinosa*, two individuals of *A. incana* and two individuals of *A. × spaethii*. In

addition, the flowering phases of *Corylus avellana* were monitored in addition (20 individuals). The observations included a detailed pollen analysis, phenological observations during the flowering period as well as comparison with symptom and pollen data (alder and hazel pollen).

2.1 Phenological observations

The individuals of *A. glutinosa* Gaertner found in the Wasserpark location (Vienna) exhibited the species characteristic features as described in Banaev and Bažant (2007) during its flowering period: bark dark brown and cracked, staminate inflorescences (3–5 in a cluster), mature infructescences on stalks (3–5). Later in the season, the typical glabrous leaves appeared.

The individuals of *A. incana* Moench found in the Wasserpark location (Vienna) and observed for the phenological part of this study exhibited the species characteristic features as described in Banaev and Bažant (2007) during its flowering period: bark smooth, grey or brown, mature infructescences commonly sessile (ten and more). Later in the season, the typical leaves (sharp-pointed tip, egg shaped to elliptic) appeared.

Natural hybrids of black and grey alder appear only rarely (Banaev and Bažant, 2007).

The earliest flowering tree under study here was previously determined as *A. × spaethii* Callier, not only due to the state of the season. Later in the season, the typical elongate leaves appeared that reinforced the identification.

The trees in Vienna (Wasserpark) were observed once a week. They were assessed based on the traits discussed and described in Banaev and Bažant (2007).

We applied the method used in the study of (Dabrowska and Kaszewski 2012) based on Łukasiewicz (1984) for phenological observations of the different flowering stages that are detailed in the following:

- F2 Appearance of the first flowers
- F3 Onset of full bloom
- F4 Appearance of first faded flowers
- F5 End of full bloom
- F6 Last blooming flower
- F7 End of flowering

In addition, we included the phase F1 (catkins closed). The dates of these stages were determined for

all alder species included in the phenological study and present in Vienna (*A. glutinosa*, *A. incana*, *A. × spaethii* and *Corylus avellana*).

2.2 Pollen analysis and determination

Material from the *Alnus × spaethii* population and black alder (*A. glutinosa*) were collected close to each other in the Wasserpark (Floridsdorf, Vienna) in 2014. A sample from an *A. × spaethii* tree in Switzerland (Gassner et al. 2013, 2014) was provided by Markus Gassner for comparison of intraspecific variability. All samples (including twigs, catkins) were registered and are now stored in the Herbarium of the Department for Botany (University Vienna). A green alder (*A. viridis*) sample was requested from the Herbarium and included in the pollen analysis.

The following pollen samples were used for the LM and SEM study:

Alnus glutinosa WU 0074852 (Vienna, Floridsdorf, Wasserpark; Bastl et al. 2014)

Alnus × spaethii WU 0074854 (Vienna, Floridsdorf, Wasserpark; Bastl et al. 2014)

Alnus × spaethii WU 0074855 (Bahnhofstraße, Buchs, Kanton Sankt Gallen, Switzerland; Gassner et al. 2013)

Alnus viridis WU 0074681 (1064; Gölles and Gölles 1992)

A detailed palynological investigation using LM and SEM was performed to gain the maximum of information on the pollen morphology. For photograph, documentation in LM and SEM the single-grain technique (Zetter 1989; Ferguson et al. 2007; Hesse et al. 2009) was applied. Therefore, small amounts of pollen from each sample were transferred onto a microscopic slide in a drop of acetolyzed solution (Erdtman 1954) with a dissecting needle. Afterwards, the slides were heated for a few minutes to remove cell contents and single grains were transferred with a micromanipulator on a separate slide containing a single drop of clean glycerol for photographic documentation in the LM. In the next step, the pollen grains were transferred onto a SEM stub. The grains were washed with drops of absolute alcohol to dissolve the remains of glycerol and then sputter-coated with gold and studied as well as photographed with the SEM. All taxonomic descriptions presented here include the most diagnostic features observed

both by LM and SEM. The terminology follows mostly Punt et al. (2007) for LM and Hesse et al. (2009) for SEM. We used the following sample sizes for *Alnus glutinosa* (50 pollen grains), *Alnus × spaethii* (sampled in Austria; 50 pollen grains), *Alnus × spaethii* (sampled in Switzerland; 50 pollen grains) and *Alnus viridis* (50 pollen grains).

2.3 Airborne pollen concentrations

Pollen data were requested from the European Aeroallergen Network (EAN) for the pollen monitoring station in Vienna. These data were collected with a volumetric spore trap of the Hirst design (Hirst 1952) located at the rooftop of the Zentralanstalt für Meteorologie und Geodynamik (ZAMG) in Vienna. The distance between the location of the pollen trap and the area of phenological observations (Wasserpark) is about 3 km. The device sucks in ambient air at 10 l/min through a 2 mm × 14 mm orifice, transferring airborne particles to a plastic tape that is wound up on a rotating drum. After 1 week of sampling, the tape is divided into sections corresponding to 24-h periods. Each section of the tape is mounted between a light microscopy slide and a cover slip using a mixture of distilled water, glycerol, phenol, Gelvatol® and basic fuchsin (Jäger et al. 1995). The samples were examined by LM (40× magnification), and three longitudinal transects were counted to express airborne pollen as mean daily pollen concentration per cubic metre of air, following the minimum requirements of the EAN (Jäger et al. 1995; Galan et al. 2014).

2.4 Symptom data and symptom load index

For the calculation of the impact on pollen allergy sufferers, we used the data pool of the patient's hayfever diary (PHD; www.pollendiary.com). It is not known if users of the diary are doctor diagnosed, since the PHD corresponds to the latest data use policy guidelines and only the minimum of personal data is collected. However, background statistics included in the PHD was used and calculated the correlation of each user to the pollen concentrations of hazel and alder. The symptom load was expressed as the symptom load index (SLI) and calculated accordingly to Bastl et al. (2014). The basis was all symptom data on the following organs: eyes, nose and lungs. In addition, we included medication to consider the changed

allergic reaction pattern when users take in antihistamines, sprays, drops or other medication. The total symptom score based on this data set was normalized to attain a value between 0 and 10 (thus there is no measurement unit). Zero values occur when users do not experience any problems at all. The value of 10 is unrealistic and would only appear when problems for all organs in the most severe way possible occur. We only used data from users in Vienna during the time period that was observed in this study to ensure to include only a population potentially exposed to the flowering of *Alnus* × *spaethii*.

3 Results

3.1 Morphology

3.1.1 *Alnus glutinosa* Gaertner (Fig. 2a–c)

Description: Pollen, monad, oblate, quadr-, pent- or hexangular in polar view (LM); equatorial diameter 21–23 µm by LM, 23–25 µm by SEM, stephanoporate (4–6), pores elliptic (SEM), thickening of the nexine (arci) connecting pores (LM), exine <1 µm thick, nexine as thick as sexine (LM); tectate, sculpturing psilate by LM, microrugulate and microechinate by SEM, microechinae positioned on microrugulae, microrugulae 0.1–0.3 µm wide and cross each other (SEM).

3.1.2 *Alnus* × *spaethii* Callier from Austria (Fig. 2d–f)

Description: Pollen, monad, oblate, quadr-, pent- or hexangular in polar view (LM); equatorial diameter 22–24 µm by LM, 23–25 µm by SEM, stephanoporate (4–6), pores elliptic (SEM), thickening of the nexine (arci) connecting pores (LM), exine <1 µm thick, nexine as thick as sexine (LM); tectate, sculpturing psilate by LM, microrugulate and microechinate by SEM, microechinae positioned on microrugulae, microrugulae 0.1–0.3 µm wide and cross each other (SEM).

3.1.3 *Alnus* × *spaethii* Callier from Switzerland (Fig. 2g–i)

Description: Pollen, monad, oblate, quadr-, pent- or hexangular in polar view (LM); equatorial diameter

24–27 µm by LM, 23–25 µm by SEM, stephanoporate (4–6), pores elliptic (SEM), thickening of the nexine (arci) connecting pores (LM), exine <1 µm thick, nexine as thick as sexine (LM); tectate, sculpturing psilate by LM, microrugulate and microechinate by SEM, microechinae positioned on microrugulae, microrugulae 0.2–0.4 µm wide, elevated and cross each other (SEM).

3.1.4 *Alnus viridis* de Candolle (Fig. 2j–l)

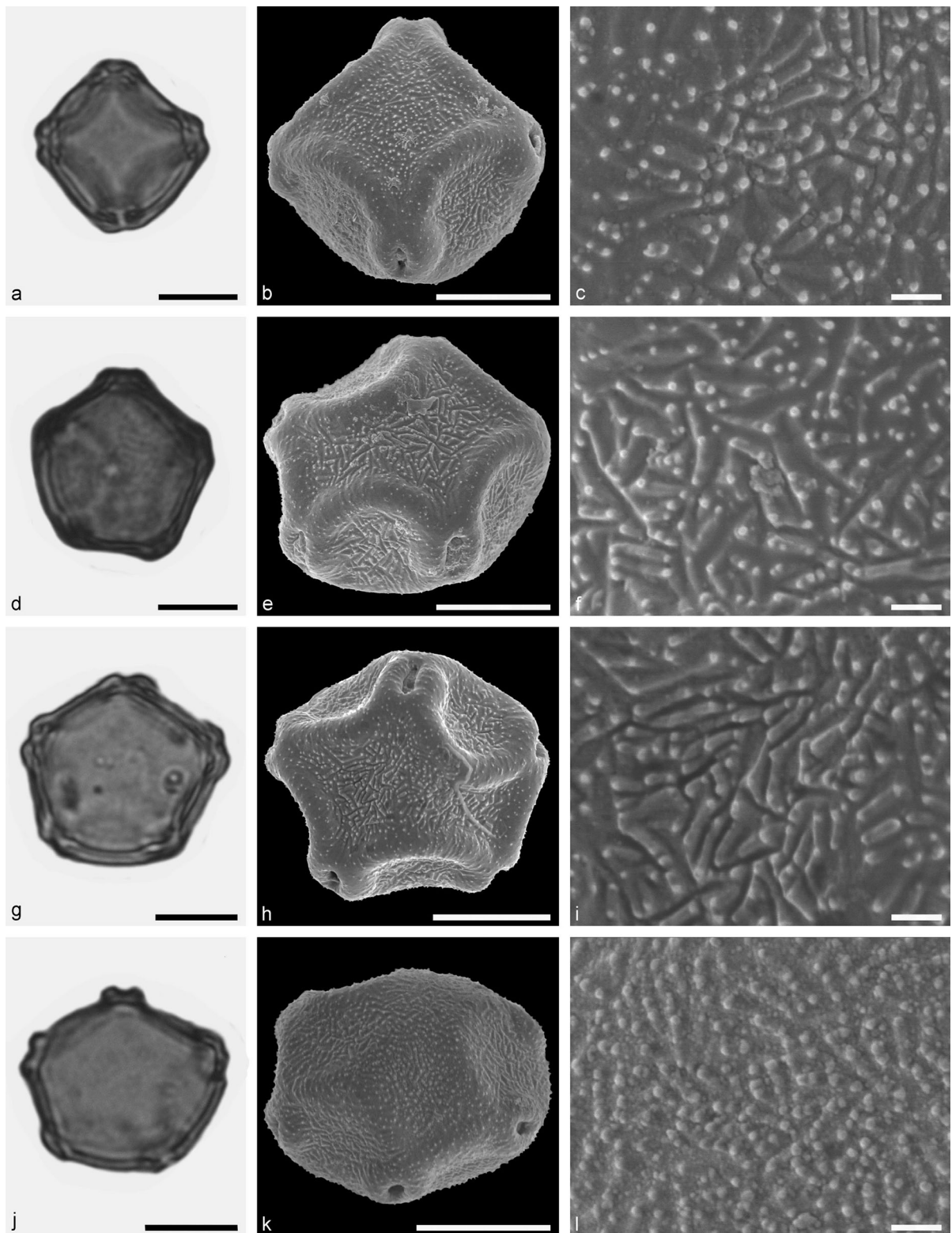
Description: Pollen, monad, oblate, quadr-, pent- or hexangular in polar view (LM); equatorial diameter 22–24 µm by LM, 21–23 µm by SEM, stephanoporate (4–6), pores elliptic (SEM), thickening of the nexine (arci) connecting pores (LM), exine <1 µm thick, nexine as thick as sexine (LM); tectate, sculpturing psilate by LM, microrugulate and microechinate by SEM, microechinae not only positioned on microrugulae but also between them, microrugulae 0.08–0.15 µm wide, flatter and finer than in the other species, shortened and cross each other (SEM).

3.2 Remarks

The first three species were not distinguishable, either by light or by electron microscopy. We followed Blackmore et al. (2003) and attributed, in addition, the pollen of *A.* × *spaethii* to the *A. glutinosa* type. Only *A. viridis* showed noticeable differences in morphology, i.e. circular pores and flatter and finer microrugulae as suprasculptural elements.

3.3 Phenology

A. × *spaethii* did not exhibit its typical leaves when it was flowering as other alder species. In addition to other features, we observed a slightly different morphology of the catkins. Whereas the scales of the catkins in *A.* × *spaethii* are more rhombic in shape, they appear more rounded in *A. glutinosa* and *A. incana*. The flowering period of *A.* × *spaethii* was earlier than the flowering period of *A. incana* and *A. glutinosa*. The first pollen of *A.* × *spaethii* was released around Christmas (52nd week of 2013), when the flowers reached stage F2. The majority of catkins were in full flower by the beginning of 2014 (1st week of 2014), which corresponds to stage F3. In the following week, stages F4 and F5 were reached



◀ **Fig. 2** LM (a, d, g, j) and SEM (b, c, e, f, h, i, k, l) micrographs of observed alder pollen, a–c *Alnus glutinosa* WU 0074852 (Vienna, Floridsdorf, Wasserpark; Bastl et al. 2014), d–f *Alnus × spaethii* WU 0074854 (Vienna, Floridsdorf, Wasserpark; Bastl et al. 2014), g–i *Alnus × spaethii* WU 0074855 (Bahnhofstraße, Buchs, Kanton Sankt Gallen, Switzerland; Gassner et al. 2013), j–l *Alnus viridis* WU 0074681 (1064; Gölles and Gölles 1992), Scale bar 10 µm in panels a, b, d, e, g, h, j, k, and 1 µm in c, f, i and l

when the first flowers began to fade and the full bloom ended. Pollination ceased the week after (F6 and F7) with the last blooming flower and the end of flowering, right when the first flowers of *A. incana* and *C. avellana* appeared (F2; Table 1). By the 5th week of 2014, the first catkins of individuals of *A. glutinosa* opened (F2). The flowering of *A. glutinosa* finished in the 11th week of 2014 (F6 and F7).

3.4 Pollen and symptom load

In Vienna, only rare occurrences of alder pollen were monitored for the flowering period of *A. × spaethii* (Table 1). The same phenomenon occurred for the flowering of the grey alder. Relevant pollen loads appeared only during the flowering period of *A. glutinosa* (Fig. 3). This is also true to a minor extent for the symptom load. Seventy-three users were recorded in Vienna entering data on a regular (daily) basis within the flowering period of *A. × spaethii*. The total symptom score of 57 users shows a positive correlation with alder and/or hazel pollen concentrations. The remaining 16 users entered symptom data, but not in a sufficient extent to be correlated with pollen concentrations. We found 21 users entering symptoms within the observed period during the flowering of *Alnus × spaethii* in winter 2013/2014 and 36 users which started to enter data, but did not experience any symptoms during the *Alnus × spaethii* flowering period (0 values) but in the flowering period of *Alnus glutinosa*. It is clear that pollen allergy sufferers react during the flowering period of the black alder (Fig. 3). However, there is also an increase and a peak of the symptom load index during the full flowering towards the end of flowering of *A. × spaethii*, when the SLI rises above the level of 2 (8–13 January; Fig. 3). Hazel pollen was on a relatively low level during the season, and its pollination started after the first symptom load peak.

4 Discussion

Distinction of the alder species under study here, i.e. those that have an impact on pollen allergy sufferers in Austria, confirm that only the pollen of *A. viridis* is discernible at SEM only. The differences in pollen morphology are comprised of circular pores as well as flatter and finer microrugulae as suprasculptural elements. In addition, we can report for the first time that *A. × spaethii* can be assigned to the *A. glutinosa* morphotype. Pollen morphology variability is little as observed in other alder species (Fig. 2). Alas, *A. × spaethii* cannot be separated on the basis of pollen morphology alone (LM and SEM) to come to a safe determination during its flowering period. Later in the season at leaf sprouting, it is easier to confirm its determination, when the typical elongate leaves appear. However, the species could be separated by the morphology of its catkins, which is different from those of black and grey alder. *A. × spaethii* started flowering weeks earlier than the other species, especially compared with *A. glutinosa*. However, *A. × spaethii* is known to flower around Christmas in Switzerland (Gehrig et al. 2014), and *A. incana* is known to flower up to 3 weeks prior to *A. glutinosa* if grown in the same habitat (Pancer-Kotejowa and Zarzycki 1980). Moreover, the temperature influences the alder pollen concentration in the air (Frenguelli et al. 1991; Myszkowska et al. 2010; Puc and Kasprzyk 2013). Conditions that favour earlier flowering are exposure to sunlight and isolation from wind (Puc and Kasprzyk 2013). Despite the early flowering of *A. × spaethii*, pollen counts did not show relevant pollen concentrations during the respective period (Table 1). It is likely that meteorological parameters such as wind direction kept the pollen from being monitored. A similar situation is known for Zürich (Switzerland) where the pollination of *Alnus × spaethii* trees is not always mirrored in the pollen counts (Gehrig et al. 2014). An additional explanatory approach could be the less frequent distribution of *A. × spaethii* and *A. incana* in eastern Austria. The distribution of *A. × spaethii* was formerly unknown in Vienna and Austria in general. In this study, we observed two individuals of *A. × spaethii* in the Wasserpark location (Vienna), 3 km from the catchment area of the pollen trap. Its overall distribution remains up to now unknown in Austria. The distribution in Vienna is probably limited to private

Table 1 Data set for the time period of the flowering of *A. × spaethii* in Vienna presenting alder and hazel phenology, measured alder pollination (pollen per m³) in Vienna and the symptom load (SLI) of pollen allergy sufferers in Vienna

Week	Date	Phenology	<i>Alnus</i> pollen per m ³	SLI
2013				
51st week	2013-12-16	<i>A. × spaethii</i> Stage F1	0	0.5
	2013-12-17		0	0.0
	2013-12-18		0	0.0
	2013-12-19		0	0.0
	2013-12-20		0	0.0
	2013-12-21		0	0.6
	2013-12-22		0	0.0
52nd week	2013-12-23	<i>A. × spaethii</i> Stage F2	0	0.0
	2013-12-24		0	0.4
	2013-12-25		0	0.4
	2013-12-26		0	0.2
	2013-12-27		0	0.4
	2013-12-28		0	0.7
	2013-12-29		0	0.9
2014				
1st week	2013-12-30	<i>A. × spaethii</i> Stage F3	0	1.2
	2013-12-31		0	1.2
	2014-01-01		0	1.0
	2014-01-02		0	0.9
	2014-01-03		1	0.9
	2014-01-04		0	1.0
	2014-01-05		0	1.4
2nd week	2014-01-06	<i>A. × spaethii</i> Stage F4 and F5 <i>A. incana</i> , <i>C. avellana</i> Stage F1	1	1.5
	2014-01-07		0	1.2
	2014-01-08		0	2.0
	2014-01-09		0	2.2
	2014-01-10		0	2.2
	2014-01-11		0	2.5
	2014-01-12		1	1.9
3rd week	2014-01-13	<i>A. × spaethii</i> Stage F6 and F7 <i>A. incana</i> , <i>C. avellana</i> Stage F2 <i>A. glutinosa</i> Stage F1	0	1.9
	2014-01-14		2	1.2
	2014-01-15		0	0.9
	2014-01-16		2	1.2
	2014-01-17		0	0.7
	2014-01-18		1	1.0
	2014-01-19		2	1.8

Phenological stages used are as follows: *F1* catkins closed, *F2* appearance of the first flowers, *F3* onset of full bloom, *F4* appearance of first faded flowers, *F5* end of full bloom, *F6* last blooming flower and *F7* end of flowering

Note the increase in symptom load during the full flower of black alder. Alder pollen grains are observed on a rare basis (3rd and 6th of January, 2014) during the flowering period of *A. × spaethii*

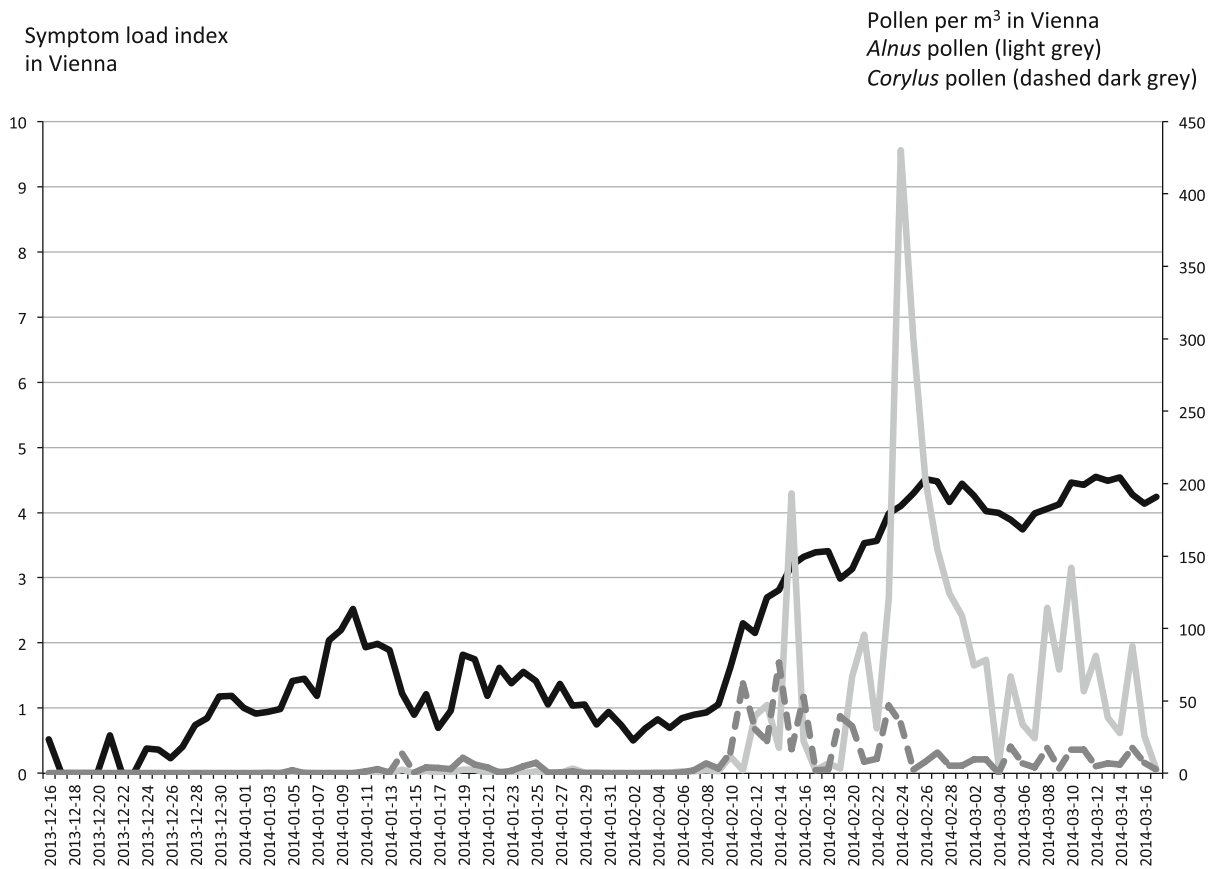


Fig. 3 Comparison of *Alnus* and *Corylus* pollen data (pollen per m³; Vienna pollen trap; EAN) with symptom data (SLI; PHD) from Vienna in black. The increase in the symptom load is

obvious during the flowering period of *A. × spaethii* (52nd week in 2013 to 1st week of 2014). This peak is not reflected in the pollen data, but in the phenological observations

gardens and public parks, and thus, no large number of trees is expected. Alas, the orchid register in Vienna excludes such regions, e.g. natural monuments and public parks. Hence, the stock size of *Alnus × spaethii* in Vienna cannot be estimated. The observed phenological development might be a local phenomenon, and the observed trees might deviate in the flowering from other stocks. Nonetheless, the impact on pollen allergy sufferers can be observed (Fig. 3), even if the increase is only slight. A first peak in the SLI was present in 2014 before the flowering of *A. glutinosa*. At this time, *A. × spaethii* is coming between full flower (F3) and fading of flowering (F4 and F5). It can only be speculated if (1) other *A. × spaethii* trees flowered later as the observed population or (2) users of the PHD had a postponed allergic reaction. The SLI peak is relatively low, but this can be explained by the 0 values of many users during that time, since averages decrease in the course

of normalization. *Corylus* pollen can be excluded as allergy trigger for the first SLI peak, because its pollination started right afterwards (Fig. 3). Ultimately, we cannot prove that every single user entered data because of allergic problems, and thus, other sources such as house dust mite allergy, colds or other influencing factors cannot be strictly excluded, but we see the validity of the calculation; since (1) users commonly only make the effort to enter data when they are experiencing allergic problems, (2) the majority of users could be correlated with alder pollen loads later in the season during the flowering of black alder and (3) the SLI calculation as an average shows high robustness. Therefore, we emphasize the importance of phenological monitoring for pollen information systems and thus proper and extensive warning for affected people that would be overlooked by common aerobiological monitoring (Hirst-type pollen traps) alone.

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