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Late exposure to grass pollen in September: the case of *Phragmites* in Burgenland

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Abstract
The grass pollen season was monitored at the Biological Station Lake Neusiedl (Burgenland, Austria) during 2017, 2018 and 2019 by means of a Hirst-type volumetric pollen and spore trap. While the start, end, duration and peaks of the pollen seasons varied between the years, both showed a late occurrence of grass pollen in the air with more than 20 grass pollen/ m^3 air in September. This late flowering event is unusual for Austria and was only noticed at the pollen monitoring site in Illmitz. The site is characterised by reed vegetation surrounding the station. The reed plants were determined as *Phragmites australis* and phenologically monitored in September 2018. The local plants flowered during this period. Thus, the source of the significant grass pollen exposure in September could be attributed to a local phenomenon. Other places surrounded by reed grasses should be monitored to assess the consistency of the observed pattern and variation of the flowering of *Phragmites*. The exposure to grass pollen has to be communicated to persons affected by grass pollen allergy including the incorporation of such events into pollen forecasting routines.

Keywords: *grass pollen, late flowering of grasses,* Phragmites, *Illmitz, Austria*

The World Allergy Organisation (WAO) recognises allergies as a global public health issue (Pawankar et al. 2013) since Immunoglobulin E (IgE)-associated allergies in Europe are a growing problem and already show a considerable socioeconomic impact (Asher et al. 2006; D'Amato et al. 2007). Approximately one million people are suffering from pollen allergy in Austria (Dorner et al. 2006). Moreover, grass pollen allergy is the most common pollen allergy in Austria with sensitisation rates of more than 50% (Hemmer et al. 2010). The grass pollen season involves various grass species flowering during different periods of the grass pollen season (Kmenta et al. 2016, 2017). The average duration of the main grass pollen season in the eastern part of Austria is from the beginning of May until end of July (Kmenta et al. 2016, 2017). However, late flowering grasses of local character, such as common reed, might be an underestimated additional allergen source for grass pollen allergy sufferers in the region of Burgenland after the main grass pollen season. At the end of 2016, a volumetric pollen-monitoring station was placed on the rooftop of the Biological Station Lake Neusiedl (Illmitz, Burgenland) by the Austrian pollen information service in order to establish continuous pollen monitoring in the Pannonian plains. Various types of landscapes such as the Lake Neusiedl, meadows and pastures, alkaline ponds, forest, vineyards and fields and reed belt vegetation characterise the vicinity of the station and the national park Lake Neusiedl-Seewinkel. The reed belt surrounding Lake Neusiedl has only developed since the mid-nineteenth century and today comprises approximately 178 km^2 rendering this lake as one of the biggest uninterrupted reed bed vegetation areas of Europe. Reed beds are characterised by the monoculture of common reed, *Phragmites australis* (Cav.) Trin. ex Steud. (Poaceae). Poaceae are one of the

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largest plant families with a global distribution and more than 10 000 species (The Angiosperm Phylogeny Group 2009). Grass dominated habitats cover about 40% of the Earth's vegetation (Gibson 2009) and the grass family includes the most important crops in the world (Grass Phylogeny Working Group II 2012). Determination of different grass species contributing to seasonal pollen peaks is only possible by means of phenological studies due to the uniform morphology of grass pollen grains (Perveen 2006; Kmenta et al. 2016). Therefore, determination of grass species is the basis for phenological routines. The morphology of *Phragmites australis* can be described as follows: *Phragmites australis* has erect stems that can grow up to 6 m tall. Its leaves are up to 50 cm long and 2–3 cm wide. The flowers are produced in late summer in a long, dark-purple panicle and the pollination of common reed usually takes place in August or September in Central Europe (Lauber et al. 2018). Thus, it can be easily identified in phenological routines. Furthermore, common reed is an important ecological factor in the region of Lake Neusiedl. It is used as roof covering material, building material for low-energy consumption houses and has potential as a biomass plant (Dillinger et al. 2012).

However, large amounts of *Phragmites australis* can be a problem for pollen allergy sufferers since sensitisations for common reed are common (Van Ree et al. 1998) and can reach 90% in *in vitro* immunological approaches in grass pollen allergy sufferers in general (López-Matas et al. 2016). Globally, grasses are among the most important aeroallergens with sensitisation rates to 30% depending on the climate and region (Andersson & Lidholm 2003; Kleine-Tebbe & Davies 2014). In addition, the Poaceae are known for their extensive cross-reactivity (Johansen et al. 2009), different allergenicity of grass species (Bullimore et al. 2012) and a range of major and minor allergens already identified (Andersson & Lidholm 2003).

This study describes late flowering events from *Phragmites australis* in Illmitz during the years 2017, 2018 and 2019 and highlights the importance of local grass pollen loads in areas with a high density of common reed for grass pollen allergy sufferers after the main grass pollen season.

Material and methods

Daily mean pollen concentrations were acquired by a volumetric sampler of the Hirst design (Hirst 1952), produced by Burkard Scientific, which is located at the roof of the Biological Station Lake Neusiedl, Illmitz, Burgenland (47° 46′ 07.5″ N, 16° 45′ 58.3″ E). Air and airborne particles are sucked into the device through an orifice with a constant flow rate of 10 l/min. Inside the device the air streams past a cellophane tape coated by a mixture of vaseline and paraffin, which is mounted on a rotating drum. Such a drum can be used to record up to seven sampling days before it has to be exchanged. Days with missing data may occur if the drum is not exchanged correctly on a regular basis.

After exposure the tape was prepared and examined in a laboratory according to the minimum requirements published by Galán et al. (2014). Therefore, the tape was cut into pieces, each representing one day. Then these pieces were transferred to microscopic slides and embedded in a mixture of polyvinyl alcohol (Mowiol®, Sigma-Aldrich) and basic fuchsine as staining agent. For examination and slide analysis an Olympus BX51 light microscope (correction factor 0.5 for pollen and 1.5 for spores, total area of analysis 13.9%) was used in combination with the PollRec web-tool for uploading bihourly pollen counts and the resulting daily mean pollen concentration to the EAN database. The applied terminology for terms in aerobiology follows Galán et al. (2017). The grass pollen seasons were calculated with the standard EAN season definition, starting with 1% and ending with 95% of the yearly grass pollen amount.

Results

Data on the grass pollen seasons is summarised in Table I, whereas the progress of the grass pollen seasons is shown in Figure 1. The data shows similar patterns as well as differences as described in the following.

The grass pollen season started in May in 2017 and ended in September of the respective year (Table I). This long flowering period of grasses was characterised by multiple peaks, two in May, two in June and one in September. The grass pollen concentrations decreased considerably after the main flower in May and June during the summer months of July and August in 2017 (Figure 1A). Pollen concentrations notably increased during September and caused a conspicuous late peak in grass pollen concentrations. In total four days of data are missing in the grass pollen season of 2017 due to drum change delays (Table I).

In 2018, the grass pollen season started in April and ended in August when calculated with the 1–95% method (Table I). The grass pollen season of 2018 was characterised by multiple peaks with most and the highest of them occurring in May and in June. However, relevant pollen concentrations were observed in July and September as well (Figure 1B).

Table I. Data on the grass pollen seasons of 2017, 2018 and 2019 in Illmitz (Burgenland, Austria).

Year of the grass pollen season	Season data
2017	Start day: 1 May 2017 End day: 14 September 2017 Number of days in the season ≥ 20 pollen/m^3 air: 48 Peak day: 9 June 2017 Peak concentration: 97.5 pollen/m^3 APIn: 2831 Gap days within the grass pollen season: 4 (31 May–1 June 2017; 7–8 June 2017) Gap days during September: —
2018	Start day: 28 April 2018 End day: 26 August 2018 Number of days in the season ≥ 20 pollen/m^3 air: 47 Peak day: 8 May 2018 Peak concentration: 109 pollen/m^3 APIn: 2889 Gap days within the grass pollen season: 8 (30 May–6 June 2018) Gap days during September: 5 (9–13 September 2018)
2019	Start day: 2 May 2019 End day: 15 September 2019 Number of days in the season ≥ 20 pollen/m^3 air: 57 Peak day: 12 September 2019 Peak concentration: 220 pollen/m^3 APIn: 3971 Gap days within the grass pollen season: 8 (22–23 May 2019; 29–31 May 2019; 12–14 June 2019) Gap days during September: —

Significant grass pollen concentrations occurred again during September 2018 (Figure 1B) and suggested a similar trend. However, two weeks of data are missing in the grass pollen season of 2018 due to drum change errors, five days of them in September. Therefore, the precise intensity and flowering activity of *Phragmites* during September 2018 remained unclear.

The grass pollen season of 2019 started in May and ended in September of the same year. Moreover, it was the most intense season regarding the total pollen amount and the number of days above 20 pollen grains/m^3 air (Table I). Also, this season was characterised by several peaks in May and June and one small peak in the beginning of July (Figure 1C). However, the two highest peaks of the grass pollen season of 2019 were recorded in September. These peaks also include the highest grass pollen concentrations of all years under observation with a maximum of 220 grass pollen/m^3 air on 12 September 2019 (Figure 1C, Table I). In total eight days of data are missing in the grass pollen season of 2019 due to drum change delays (Table I).

The following has to be kept in mind when all grass pollen seasons are compared: the grass pollen season of 2018 was the shortest grass pollen season, started and ended earlier, and was the season with the earlier peak day (May instead of June). The grass pollen seasons of 2017 and 2019 showed a similar seasonal pattern, however the season of 2019 had a higher annual pollen integral (APIn), was the one with the higher peak day, and the one with more gap days (Table I). Relevant grass pollen concentrations were measured with a Hirst-type volumetric pollen and spore trap in September in all years (Figure 1). The grass pollen concentrations in September increased later in 2018 than in 2017 but showed a very similar pattern after the gap in 2018 and towards the end of this late exposure. The season of 2019 showed the highest grass pollen concentrations in September with an earlier start compared to the years 2017 and 2018 and recorded the highest grass pollen peak of 220 grass pollen/m^3 air during the whole observation period (2017–2019).

Short-term phenological monitoring revealed that the Biologische Station Illmitz is surrounded by common reed (*Phragmites australis*; Figure 2).

Phenological observations were performed in 2018 on 30 August and on 13 September. Flowering of a small fraction of *Phragmites* was observed on 30 August, whereas a decreasing flowering trend was observed on 13 September. *Phragmites* grows in high

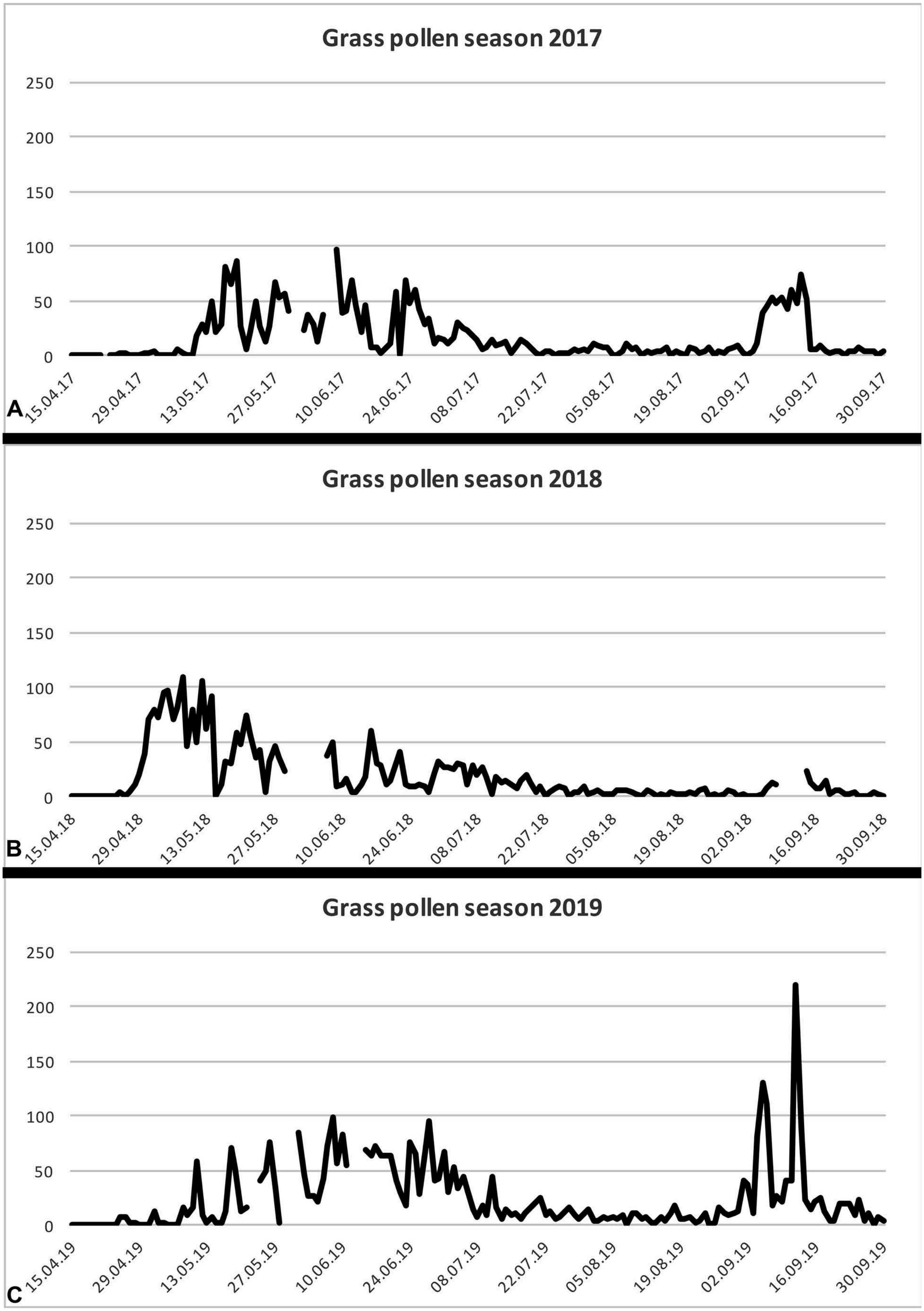

Figure 1. Daily mean grass pollen concentrations during the grass pollen season of 2017 (**A**), 2018 (**B**) and 2019 (**C**) measured in Illmitz (Burgenland, Austria). Note the differences in the grass pollen season in general (start, end, peaks) and especially the occurrence of relevant grass pollen concentrations in September in all years.

Figure 2. *Phragmites australis* at Illmitz (Burgenland, Austria). **A.** Biologische Station Illmitz. **B.** Surrounding area with reed. **C.** Flowering inflorescence of *Phragmites australis*. **D.** White whorl of hair as typical morphological feature of *Phragmites australis.*

numbers around the Biologische Station Illmitz (Figure 2A, B). Illmitz and the area around Illmitz was observed for possible other flowering grass species. However, no other flowering grass species could be detected in larger amounts around Illmitz except for cornfields (*Zea mays*) that had already finished flowering and were at the fruit stage at that time. The phenological monitoring was performed in order to determine the source of the late grass pollen exposure in Illmitz. Therefore, no detailed phenophases were described and phenological observations were not done for the years 2017 and 2019.

Discussion

Grass pollen concentrations measured in September (2017, 2018 and 2019) exceeded a value of 20 pollen/m^3 air. This level is considered as relevant exposure in the forecasting and aerobiological routines of the Austrian pollen information service of the Medical University of Vienna and corresponds to a moderate burden for pollen allergy sufferers. Comparable grass pollen concentrations were not measured at any other pollen monitoring station within Austria during this period. Only low grass pollen concentrations were recorded in some of the stations in the east of Austria. Therefore, after the first observation of this event in 2017, additional phenological observations were done in 2018 to determine the possible source of the exposure to grass pollen. Common reed, *Phragmites australis*, flowered during the end of August and September in 2018 and is vastly growing near the Biologische Station Illmitz (Figure 2A, B).

We assumed that the late grass pollen exposure in September lasted shorter in 2018 than in 2017 with a probable lower total exposure than during 2017 when over 70 grass pollen/m^3 were measured. However, the missing data in 2018 for the crucial period rendered a detailed comparison impossible. Hence, to confirm the late flowering of *Phragmites australis* in Illmitz the season of 2019 was included into the evaluation and revealed the most intense flowering of *Phragmites* in the three years including two peaks exceeding 100 and even 200 grass pollen/m^3 air. The monthly weather report for Burgenland in September 2018 summarises the respective month with 29% more precipitation and +1.7 °C mean monthly temperature, whereas the report presents 49% more precipitation and −0.8 °C for September 2017 as compared to the long-term average (1981–2010) (H. Seidl, pers. comm., 28 September 2018). The monthly weather report of 2019 summarises the respective month with −2% precipitation and +0.9 °C as compared to the long-term average (1981–2010) (H. Seidl, pers. comm., 30 September 2019). Although higher temperatures seem to be beneficial for *Phragmites*, low precipitation during August and more precipitation during September are as well important for seed production (McKee & Richards 1996), which, in turn, depends on pollen production. Precipitation was lower than average in August in all years according to the Austrian Weather Service, ZAMG (Zentralanstalt für Meteorologie und Geodynamik), (2017: −19%; 2018: −24%; 2019: −21%; H. Seidl, pers. comm., 30 September 2019) and it was higher during September except for the year 2019. However, a clear meteorological pattern is not apparent comparing the three years and does not explain the high grass pollen concentrations caused by *Phragmites* in the year 2019.

The grass pollen concentrations measured in Illmitz in September 2017–2019 are of importance for grass pollen allergy sufferers. Cross-reactivity is high in grass pollen from different species (Bullimore et al. 2012), especially with the Pooideae and to a lesser extent with Chloridoideae grasses (Tiwari et al. 2009). Although immune responses are lower to *Phragmites australis*, *Zea mays* and *Cynodon dactylon* as compared to *Poa pratensis*, *Festuca rubra*, *Phleum pratense* and *Dactylis glomerata*, they are present (Van Ree et al. 1998). In addition, López-Matas et al. (2016) found that 90% of people suffering from grass pollen were also sensitised to *Phragmites* in an *in vitro* immunological test. Thus, the grass pollen concentrations measured in Illmitz most likely affect local grass pollen allergy sufferers. Moreover, an European Academy of Allergy and Clinical Immunology (EAACI) position paper of 2017 described pollen exposure times for clinical trials and season definitions for the most important aeroallergens in Europe (Pfaar et al. 2017). If the season definition for grasses in clinical trials is applied on the data of the present study the pollination period of common reed is included in the peak pollination period in all years under study even considering the data gap in 2018. These outcomes support a possible impact and a potential clinical relevance of *Phragmites australis* for grass pollen allergy sufferers. This local phenomenon observed in Burgenland should be monitored in other locations surrounded by *Phragmites* belts since pollen information is crucial for pollen allergy sufferers (Kiotseridis et al. 2013). Continuous pollen monitoring in Illmitz will provide more information in the future on the variability, consistency and intensity of this late exposure to grass pollen.

Conclusions

The grass pollen season showed a relevant exposure to grass pollen in September in the years 2017, 2018 and 2019 in Illmitz (Burgenland, Austria). Usually grass pollen is not expected in significant amounts during autumn in Austria. The exposure is attributed to the flowering of common reed, *Phragmites australis*, which grows in large quantities around the Biologische Station Illmitz where the pollen trap is located. In general, the grass pollen season of 2018 was earlier, shorter, and showed more peaks, whereas the seasons of 2017 and 2019 in Illmitz were more similar although the season of 2019 was much more intense.

The presence of reed should be considered in aerobiological routines including pollen forecasting to inform grass pollen allergy sufferers about a possible cause for their allergy and to help them avoid a burden (e.g. avoiding proximity to reed) in autumn when grass pollen is not expected in the amounts presented in this investigation. Certain weather conditions such as precipitation may affect the intensity of pollen release from *Phragmites*.

Furthermore, local peculiarities such as late flowering of a grass species should be monitored to assess its variability and influence on the local population. Such phenomena might be of crucial importance to the public and pollen information services due to being a cause for possible allergy symptoms in autumn.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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