



Amaranthaceae pollen grains as indicator of climate change in Lublin (Poland)

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ABSTRACT

Previous studies have demonstrated that plants are a very good indicator of global environmental variations. The responses of many plant species to climate change are confirmed by aerobiological research. This paper presents an analysis of many parameters of pollen seasons in the Amaranthaceae family based on measurements of pollen concentrations in atmospheric air. Pollen samples were collected with the volumetric method at a sampling site in Lublin (Poland) in 2001–2019. The obtained data were verified using statistical analyses. Moreover, the presence of pollenkit on the pollen grain surface was examined in fresh anthers using scanning electron and light microscopes, since there are some difficulties in identification of Amaranthaceae pollen grains deposited on microscopic slides in aerobiological analysis.

The pollen season in Amaranthaceae began on average on June 23 and ended on October 5, i.e. it lasted 105 days. The peak value and annual pollen sum were characterized by the highest variability in the study years in comparison with other season characteristics. The annual pollen sum was in the range from 183 to 725. Maximum concentrations were most often recorded in the second half of August, which is associated with the greatest risk of development of pollen allergy symptoms in sensitive subjects during this period. The results obtained in the 19-year study revealed that the pollen seasons began 14 days earlier. Similarly, the end of the season was accelerated by 24 days. The response of these plants to climate change also include the reduced pollen production by representatives of this family, which was manifested by a decrease in the annual sum of daily airborne pollen concentrations, on average by 35%, and a reduction in the maximum pollen concentration, on average by more than 60%. We found that temperature in May and June had an effect on pollen release, and relative air humidity in May influenced pollen concentrations. We noted significant similarities in the pollen release rate during the last 8 years of the study. The scanning electron microscopy examinations showed that the pollen grain surface in the representative of this family was covered completely or partially with pollenkit. Hence, the apertures characteristic for pollen in this family were poorly visible. The presence of pollenkit on the surface of these polyaperturate pollen grains may play an important role in preventing water loss during pollen migration in the air. Our research has demonstrated the response of plants flowering in summer to climate change. The results not only have practical importance for public health in the aspect of allergy risk but can also help to assess environmental changes.

1. Introduction

Species belonging to the family Amaranthaceae, which includes the subfamily Chenopioideae, occur in almost all parts of Europe (Akeroyd 1993; Pyšek et al., 2009). Many of them are halophytes, weeds, and ruderal plants that are found in marine habitats, crop fields, steppe, and

semi-desert regions (Edmondson 1993). In Poland, the family Amaranthaceae is primarily represented by weeds of the genera *Atriplex* L., *Chenopodium* L., and *Amaranthus* L. and by crop plants *Beta vulgaris* L. and *Spinacia oleracea* L. (Rutkowski 2008).

Pollen grains in species of this family growing in Europe are spherical, polyaperturate, and different sized (21–43 µm). They represent the

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panoporate type, which is characterized by a large number of small pores (25–110) located at small distances (4–7 µm). The pore diameter is in the range of 1.1–3.0 µm. The smallest pollen grains are produced by plants of the genus *Beta*, while the largest sizes are noted in the genus *Spinacia*. The lowest number of pores was recorded in *Beta* pollen grains (35–40), whereas *Spinacia* pollen grains are characterized by the greatest number of these structures (90–110) (Beug 2004). *Spinacia oleracea* produces a certain number of dwarf pollen grains with much smaller dimensions than those of other grains (Wodehouse 1965).

Chenopoideae are a subfamily originating from an entomophilous ancestor, and their development has progressed towards anemophily (Wodehouse 1965). Various degrees of balance between anemophily and self-pollination can be found in this subfamily (Proctor et al., 1996). As in some anemophilous plants, the pollen grain exine in *Amaranthaceae*, including *Chenopoideae*, is covered with pollenkit (Francki and Miles 1985; Borsch 1998; Lumaga et al., 2016), i.e. a sticky pollen coat material produced by the anther tapetum. Tapetal cell protoplasts degenerate and pollen grains are surrounded by the gluing material. In anemophilous plants, pollenkit serves many functions, e.g., it protects pollen from water loss and ultraviolet radiation and facilitates pollen rehydration. Additionally, it facilitates adhesion to the stigma, is responsible for pollen-stigma recognition, and facilitates self-pollination (Paccini and Hesse 2005; Rejón et al., 2016; Chichiricò et al., 2019; Shin et al., 2019).

In aerobiological studies, pollen grains of *Amaranthaceae*, previously termed *Chenopodiaceae/Amaranthaceae*, have been recorded at different concentrations in the air in many countries in Europe (Alcázar et al., 2009), excluding most areas in Scandinavia and the British Isles (Skjøth et al., 2013). At some monitoring sites, their content in the pollen spectrum did not exceed 2.0%. For example, it constituted 1.7% in Toledo (Spain) (Pérez-Badía et al., 2010), 1.2% in Salamanca (Spain) (Rodríguez de la Cruz et al., 2012), 0.7% in Cordoba (Spain) (García-Mozo et al., 2007), 1.2% in Bratislava (Slovakia) (Ščevková et al., 2010), 0.9% in Cracow (Poland) (Szczepanek 1994), 0.6% in Lublin (Poland) (Piotrowska-Weryszko and Weryszko-Chmielewska 2014), 0.3% in Vincovici (Croatia) (Stefanic et al., 2007), 0.2% in Skien (Norway) (Piotrowska 2004), and 0.1% in Münster (Germany) (Melgar et al., 2012). In turn, much higher contents of pollen of these plants were recorded in Murcia (south-eastern Spain), where it accounted for almost 12% of the annual total, and 49.5% of pollen allergic people were sensitized to these pollen grains (Giner et al., 2002). In Kuwait, *Chenopodiaceae/Amaranthaceae* pollen grains dominated (on average 31%) and were the most common triggers of allergy in this country, alongside *Malvaceae* and *Asteraceae* pollen (Al-Dousari et al., 2018). Since the family *Amaranthaceae* is represented by many species, it is characterized by a long pollen season with many maximum values (Kasprzyk 2007; Alcázar et al., 2009; Puc 2009).

A substantial number of cases of allergy to *Chenopodiaceae/Amaranthaceae* pollen has been found in Greece (Gioulekas et al., 2004), Hungary (Kadocsa and Juhasz 2002), and Spain (Arias and Amat 2003). The allergenicity of pollen of plants belonging to *Chenopodiaceae/Amaranthaceae* is regarded as low (Rapiejko et al., 2001; Silny and Czarnecka-Operacz 2001), moderate (Smith 1990), or high (Ruiz-Valenzuela and Aguilera, 2018). Cross reactions between allergens of different species may occur within this family (Rapiejko 2008).

In the last decades, human allergic diseases have been observed to increase. These diseases are largely a result of climate change associated with increased pollen production by wind-pollinated plants and with the shifts in airborne pollen seasons (Damialis et al., 2019). After 1990, phenological studies have paid attention to the strong biological signal of climate change (Fitter and Fitter 2002; Czernecki and Jabłońska 2016). Plants have been shown to be a good indicator of global environmental variations (Primack et al., 2004; Chmielewski et al., 2005; Menzel et al., 2006; Carroll et al., 2009; Luedeling et al., 2013). An earlier onset of pollen seasons has been found in many anemophilous plant species, especially in early spring flowering plants (Rasmussen

2002; Emberlin et al., 2002; van Vliet et al., 2002; Frei and Gassner 2008; Malkiewicz 2014; Puc 2014; Weryszko-Chmielewska et al., 2016; Gross et al., 2019). The annual totals of daily counted airborne pollen have also been demonstrated to increase (Levetin 2001; Spieksma et al., 2003; Frei and Gassner 2008; Ziello et al., 2012; Zhang et al., 2015; Mercuri et al., 2016; Weryszko-Chmielewska et al., 2016; 2019; D'Amato and Akdis 2020).

Aerobiological studies have shown that an increased annual pollen load in various plant taxa is associated with a rise in temperature (Zhang et al., 2014; Myszkowska et al., 2015; Puc et al., 2015; Ziska et al., 2019). Daily concentrations of airborne pollen significantly increase on days with higher temperatures (Stepalska et al., 2016; Grewling et al., 2019). Moreover, Puc et al. (2015) demonstrated a positive correlation of *Betula* pollen count with wind speed and total radiation and a negative correlation between the pollen count in this taxon and relative humidity. Majeed et al. (2018) investigated the correlation between the pollen season parameters of 22 taxa and winter values of meteorological variables in Catalonia. The authors found that temperature exerted a greater influence than the other variables on the pollen concentration and the onset of the pollen season. In turn, precipitation usually had a negative effect, whereas insolation exerted a slight effect on the course of the pollen seasons in the analyzed plant taxa.

The aim of the present 19-year research was to analyze *Amaranthaceae* pollen seasons in central-eastern Poland. This included the determination of the start and end dates of pollen seasons, season duration, maximum pollen concentration values, and annual pollen sums. We also compared the dynamics of individual pollen seasons and attempted to distinguish similar seasons. We analyzed the obtained results in the aspect of changes taking place in climate warming conditions. During the microscopic analysis, it was difficult to identify some pollen grains due to the poor visibility of their apertures and exine surface. Therefore, we decided to examine fresh (*in vivo*) pollen grains of *Spinacia oleracea* located in bursting anthers.

2. Materials and methods

2.1. Study object

According to classical taxonomy systems, the family *Chenopodiaceae* and related *Amaranthaceae* used to be treated as separate taxonomic units (Cronquist 1981), whereas the modern phylogeny system APG IV (The Angiosperm Phylogeny Group, 2016) has combined the representatives of both these families into one taxon – the family *Amaranthaceae*. Polish flora is represented by 16 genera from this family (Rutkowski 2008). In the Lublin region, 66 *Amaranthaceae* species have been found, among which *Chenopodium glaucum*, *Ch. hybridum*, *Ch. album*, *Atriplex nitens*, *A. patula*, *Amaranthus hybridus*, and *A. retroflexus* occur most frequently (Fijałkowski 1994).

2.2. Sampling and morphometric analysis of pollen grains (LM)

Pollen grains of *Spinacia oleracea* cv. 'Olbrzym Zimowy' were collected from pot-grown plants. The plants grew in a humus-rich sandy loam substrate with a pH of 7. The following fertilization was applied: 80 kg N, 50 kg P₂O₅, and 90 kg K₂O.

Fresh pollen was examined under a light microscope. The pollen grain size was determined in glycerol-gelatin slides based on measurements of 300 palynomorphs. Acetocarmine was applied to determine pollen viability, while Sudan III was used to examine the extra-exinous layers. Photographs were taken with a Coolpix 4500 (Nikon, Japan) camera coupled to an Eclipse 400 Nikons light microscope (Nikon).

2.3. Scanning electron microscopy examination

The preparation technique usually used in examination of pollen grains involving material fixation and dehydration leads to the

dissolution of extra-exinous layers such as the pollenitt layer (Thani-kaimoni 1986). To preserve grains that probably had the pollenitt layer, fresh palynomorphs located in the pollen sacs were examined, without employing conventional preparation methods used in SEM studies, i.e. without fixation, dehydration, and gold coating. *In vivo* SEM observations of 50 pollen grains were conducted using mature anthers collected from flowers and parts of fresh fragments of male spinach inflorescences, which were transferred to the SEM tabletop covered with Colloidal Silver Paste from Energy Beam Sciences, USA (product no. P-CP-25). The paste made it possible to stick the plant samples to the tabletop and facilitated electron flow and observation of the images. The examination was carried out in a BS-300 TESLA scanning electron microscope and the photographs were taken using this microscope. This simplified method facilitated observations of the pollen grain surface without damage to any of the pollenitt layers. A drawback of this method is the slightly weaker sharpness of the images of pollen grains.

2.4. Aerobiological studies

Measurements of pollen concentrations in the air of Lublin, a city located in central-eastern Poland (Fig. 1), were carried out in 2001–2019. The volumetric method was applied using a Hirst-type pollen sampler (Lanzoni VPPS, 2000) operating on a 24-h basis (Hirst 1952). The pollen trap was placed on the roof of a building 18 m above the ground level in the Śródmieście district (51°14'37" N; 22°32'25" E). The method involved continuous suction of 10 l air/min (together with bioaerosol contained therein) according to the wind direction. A full rotation of the drum with the tape regulated by a precise clock mechanism lasted 7 days. The pollen grains were collected on an adhesive tape moving at 2 mm/h. Every Thursday at 12:00 a.m., the tape was replaced and cut into segments corresponding to 24-h periods. The qualitative and quantitative analysis of pollen grains in the aeroplankton was performed according to the recommendations of the International Association for Aerobiology (Mandrioli et al., 1998). Besides, our monitoring station is subject to quality inspections within the European network (Sikoparija et al., 2017).

After the slides were stained with basic fuchsin, a microscopic analysis of the surface of four longitudinal transects was performed at a magnification of 400 × under a light microscope. To collect data required for the characterization of the Amaranthaceae pollen seasons, 153 microscopic preparations were examined each year. From 101 to 141 results were analyzed in each study year. The preparation and counting methods followed the recommendations from the European

Aerobiology Society (Galan et al., 2014). Pollen concentration was expressed as the daily average of pollen grains per cubic meter of air (P/m^3). The length of pollen seasons was calculated by the 98% method, where the days on which 1% and 99% of the annual pollen count were recorded were considered the onset and end of the season, respectively (Emberlin et al., 1993). The start and end dates of plant pollen seasons, season duration, peak value, maximum concentration period, and annual total were analyzed.

The course of the Amaranthaceae pollen seasons in individual years is presented in graphs showing the dynamics in the achievement of the annual pollen total. The graphs show the duration of the successive stages of the season corresponding to the achievement of the successive values of the percentage contribution to the annual pollen sum (Latałowa et al., 2002; Weryszko-Chmielewska et al., 2019, Fig. 2).

Similarities between the pollen seasons were determined based on cluster analysis. The Shapiro-Wilk test was used to determine the normality of distribution of the seasonal characteristics. Correlation analysis was used to check whether the temporal variability of the specific seasonal characteristics was statistically significant. Linear regression equations were developed for the normal distribution characteristics.

Weather data obtained from the Meteorological Observatory of the Hydrology and Climatology Department, Maria Curie-Skłodowska University in Lublin, which is located at a distance of about 1.5 km from the pollen sampling site, were used to determine the effect of weather on the pollen seasons. The following meteorological factors were used in the analysis: mean, minimum, and maximum air temperature, relative air humidity, precipitation, cloud cover, and wind speed. Relationships between the meteorological factors and the pollen season parameters were investigated using Spearman's correlation analysis.

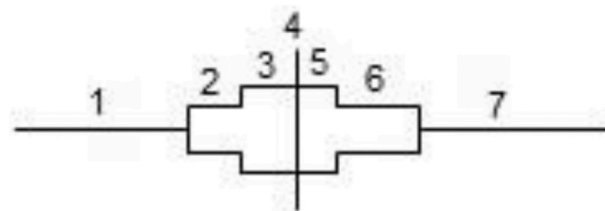


Fig. 2. Graphical representation of the dynamics in the achievement of the annual pollen sum. Stages of the pollen season: (1) 1–5%, (2) 5–25%, (3) 25–50%, (4) 50%, (5) 50–75%, (6) 75–95%, (7) 95–99%.

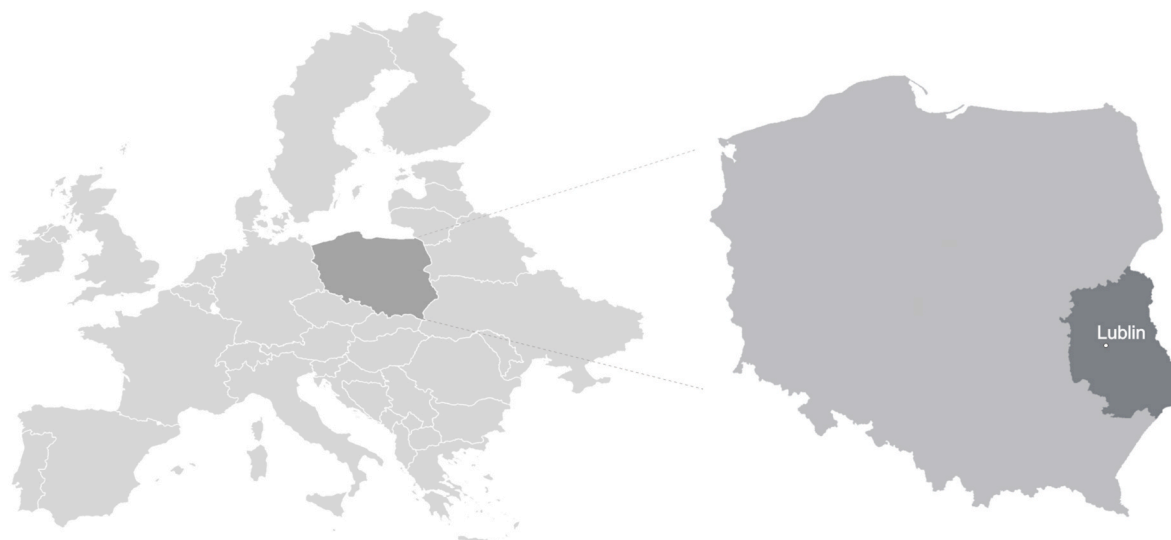


Fig. 1. Location of the study area and the Amaranthaceae pollen trap in Lublin (51°14'37" N; 22°32'25" E), Poland.

3. Results

3.1. Morphology of *Spinacia oleracea* pollen grains

Male inflorescences of spinach located at the tip of shoots are made up of numerous densely packed flowers (Fig. 3 A). Each staminate flower is composed of four greenish perianth segments (tepals) and four stamens that mature successively. Flowers located at the apex of the inflorescence open the earliest. Mature anthers dehiscence transversely, and pollen grains are located in the pollen sacs mainly in the vicinity of the stomium, as shown in Fig. 3 B & C). Fig. 4 A, B show morphological SEM images of fresh pollen grains. As can be seen, the pollen grains were surrounded by a layer of pollenkitt, which sometimes joined several pollen grains together. The pollenkitt coating sometimes surrounded some grains completely, while in some cases the grains were partly covered by pollenkitt, as illustrated in Fig. 4 C & D.

The pollenkitt, resembling a viscous fluid, covered a small surface area of grains and sometimes seemed to be flowing down the grains. The pollenkitt layer was homogenous (Fig. 4 A & B) and had an uneven thickness (Fig. 4 C & D). In other grains, the pollenkitt was visible only in the region of the pores and formed significant projections (Fig. 4 D & E). We observed conspicuous bridges among the pollen grains, (Fig. 4A–C). Small amounts of pollenkitt also remained in the loculus, where it was visible as flocculent material on the inner surface of the anther wall

(Fig. 4 A & B). Some pollen grains located closer to the center of the anther chamber were free of pollenkitt (Fig. 4 F). This may indicate that only small amounts of pollenkitt are produced from the tapetum residues, which are probably insufficient to cover all pollen grains. In this case, the pollenkitt is deposited only on grains located in the immediate vicinity of the tapetum.

The diameter of the spinach pollen grains ranged from 13.2 μm to 38.1 μm and its average value was $31.28 \pm 1.03 \mu\text{m}$. Dwarf grains accounted for about 6% of the total number of grains, with a diameter in the range of 13.2–17.3 μm . We established that these grains were sterile because their content did not stain with acetocarmine. Some dwarf grains were partially collapsed (Fig. 4 B), while some other ones were completely flattened. These facts confirm the sterility of the dwarf grains accompanied by degeneration of the protoplast resulting in grain deformation. The pollenkitt covering the pollen grain exine treated with Sudan III stained orange, which proves the presence of lipids.

The light microscopy observations of the Amaranthaceae pollen grains originating from the aerobiological collections revealed the presence of pollenkitt on the surface of many grains. Some pollen grains were covered by a thicker layer of pollenkitt, which masked the pores almost completely (Fig. 5 A). In turn, pores were poorly noticeable on the surface of other pollen grains (Fig. 5 B). We also found pollen grains with a slight layer of pollenkitt and better visible pores (Fig. 5 C & D). The pollenkitt present in the region of pores forms a specific barrier,

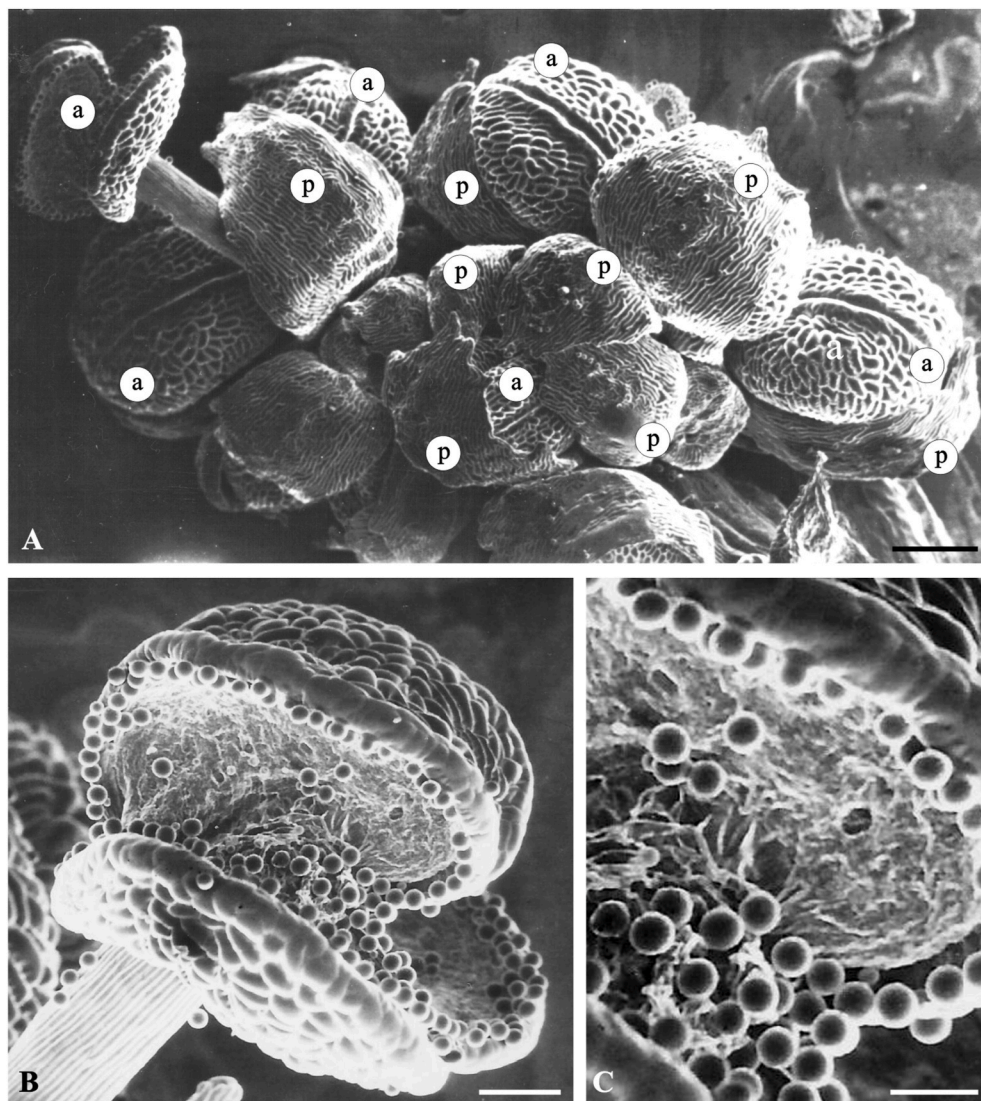


Fig. 3. Staminate inflorescence of *Spinacia oleracea* at the beginning of flowering and parts of the stamen (SEM) A – 4-fold staminate flowers at different development stages; a mature stamen in the oldest flowers can be seen at the top on the left; perianth segments p; anther a,B – Anther during the release of pollen grains, C – Enlarged detail of Fig. B: pollen grains inside the pollen chamber; the pollenkitt layer on their surface obscures the apertures, Scale bars: 500 μm (A), 200 μm (B), 100 μm (C).

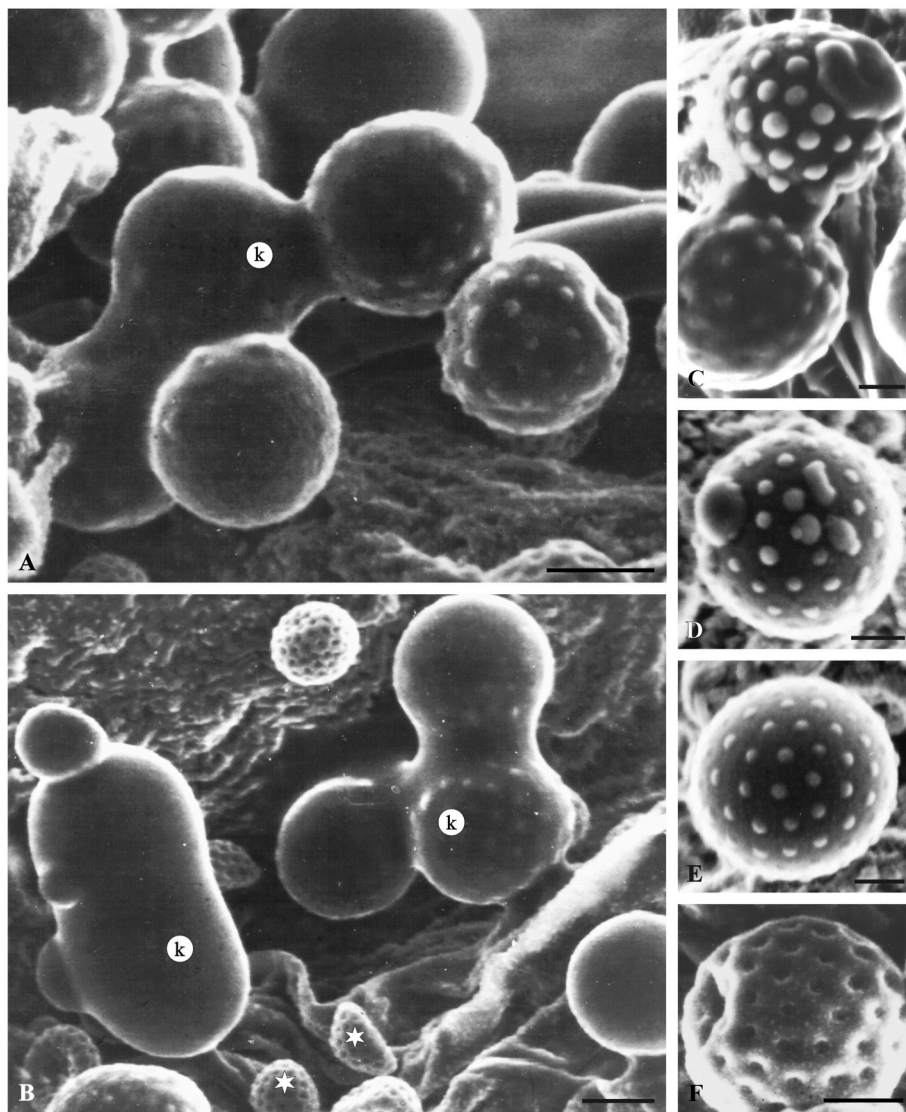


Fig. 4. *S. oleracea* pollen grains in the chamber of an open anther (SEM), A, B – Pollenkitt occurring abundantly on the surface of many pollen grains causes the formation of pollen conglomerates (k) and masks the surface of the exine and the aperture; dwarf pollen grains (asterisk) are frequently deformed and without pollenkitt; flocculent material from tapetum cell residues is visible on the surface of the inner wall of the anther. Scale bars: 20 μm , C, D, E – Pollen grains with pollenkitt deposited on some parts of their surface and near the pores. Scale bars: 10 μm , F – Pollen grain from the central part of the pollen chamber without pollenkitt on their surface with well visible pores. Scale bars: 10 μm .

which may be important during the migration of pollen grains in the air. This probably protects them against water loss, which may be intensified at higher temperatures and stronger winds.

3.2. Aerobiological studies

3.2.1. Onset, end, and duration of the pollen season

During the 19-year study, Chenopodiaceae/Amaranthaceae pollen season started between June 5 and July 17 in the different years, with the average date on June 19 (Table 1). The coefficient of variation for the onset date was 5.3%, which indicates low variation in the pollen season onsets. The correlation analysis revealed a statistically significant variation for the season start and end (0.4746; $p = 0.04$, 0.5716; $p = 0.01$, respectively). The analysis results showed a trend towards an earlier onset and end of the pollen seasons. It was calculated that the acceleration in the date of the onset of the Chenopodiaceae/Amaranthaceae pollen season was almost 14 days, but the fit of the regression model was 22% (Fig. 6). The end of the pollen season was recorded between September 19 and October 31, with the average date on October 5. The coefficient of variation for this parameter was 4.7%. The trend line showed a tendency towards an earlier end of the pollen season. A statistically significant variation in the pollen season end dates is almost 24 days, while the fit of the regression model is at a level of 33%

(Fig. 7). The Chenopodiaceae/Amaranthaceae pollen season was relatively long and lasted from 79 to 129 days, on average 105 days (Table 1). The variation coefficient value for the season duration reached 13.2%, which means that this parameter is less stable than the season start (Table 1). The analysis of the season duration did not show significant changes over the study period.

3.2.2. Annual pollen sum and maximum pollen concentration

During the study period, significant differences were found in the annual sums of the daily concentrations of Chenopodiaceae/Amaranthaceae pollen grains (Fig. 8). The average value of the annual total was 352 grains, with the extreme values ranging from 183 to 725 recorded in the 2013 and 2002 seasons, respectively (Table 1, Fig. 9). The relatively high coefficient of variation for the annual pollen sums (35.5%) confirms the existence of significant variation of this parameter over the study period (Table 1). The linear trend determined for the annual total shows a slight downward tendency in the Chenopodiaceae/Amaranthaceae pollen sums in 2001–2019 (Fig. 8). The annual total decreased by about 35% in relation to the value noted at the beginning of the study period. Nonetheless, this relationship was not statistically significant.

The maximum Chenopodiaceae/Amaranthaceae pollen concentrations were recorded between July 30 and August 28. The calculated

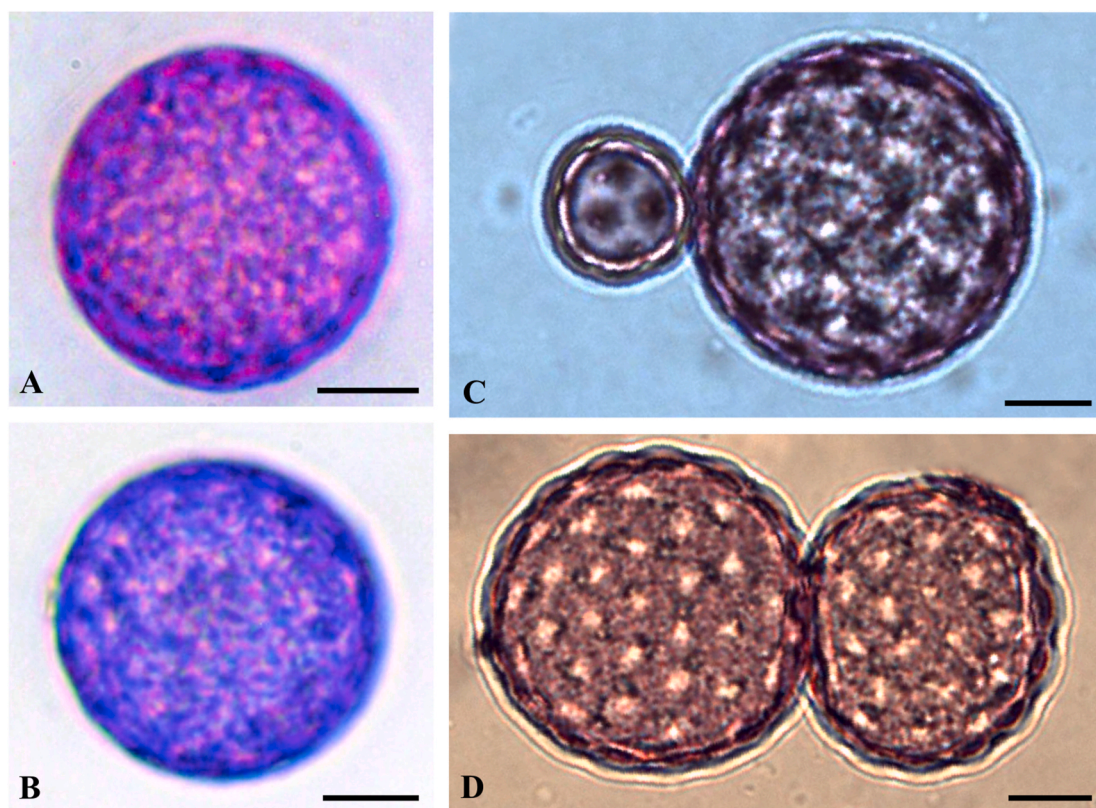


Fig. 5. Pollen grains of various Amaranthaceae representatives from aerobiological slides stained with basic fuchsin (LM), A-C – Pollen grains covered with pollenkitt to a different degree, partly obscuring the visibility of the pores, D – Pollen grain with clear pores, Scale bars: 10 μ m.

Table 1
Characteristics of Amaranthaceae pollen seasons in Lublin, 2001–2019.

Statistics	Pollen season		Duration (days)	Peak		Annual total (pollen sum)
	Start	End		P/m ³	Date	
Mean	23.06	5.10	105	22	15.08	352
Min	5.06	15.09	79	12	30.07	183
	2018	2013	2013	2009, 2012	2018	2013
Max	17.07	31.10	129	60	27.08	725
	2001	2010	2007	2001	2019	2002
SD	9.2	13.1	13.8	12.1	8.0	125.0
V (%)	5.3	4.7	13.2	54.7	3.5	35.5

SD – standard deviation, V – coefficient of variation.

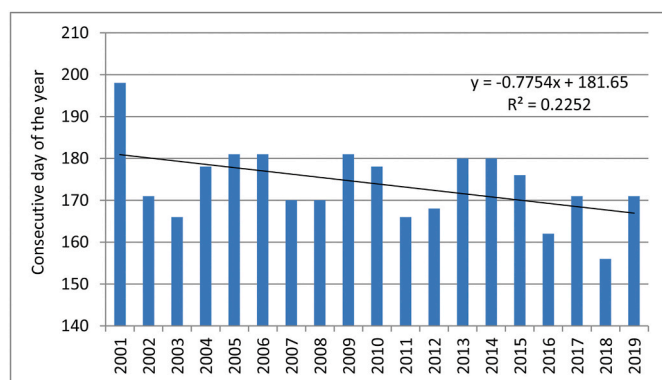


Fig. 6. Comparison of the dates of the onset of the Amaranthaceae pollen season in Lublin in 2001–2019 and the trend line.

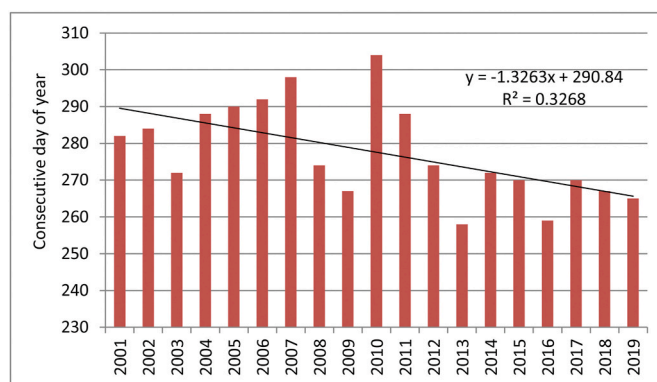


Fig. 7. Comparison of the dates of the end of the Amaranthaceae pollen season in Lublin in 2001–2019 and the trend line.

average peak was noted on August 18 and the coefficient of variation for this date was low (3.5%), which indicates low year-to-year variation of this pollen season characteristic of the studied taxon. The maximum concentration values ranged from 12 to 60 P/m³, with an average value of 22 P/m³. We found a statistically significant variation for the maximum pollen concentration (0.6451; $p = 0.003$). The linear trend determined for the maximum pollen concentration reveals a significant downward tendency ($R^2 = 0.42$). The trend line shows that the maximum pollen concentrations decreased by more than 60% during the last years (Fig. 10).

Spearman's rank correlation analysis showed the highest correlations for the Chenopodiaceae/Amaranthaceae pollen season parameters between the end date and the season duration. The season is statistically significantly longer if its end date occurs later. A positive correlation was also found between the peak value and the annual total (Table 2).

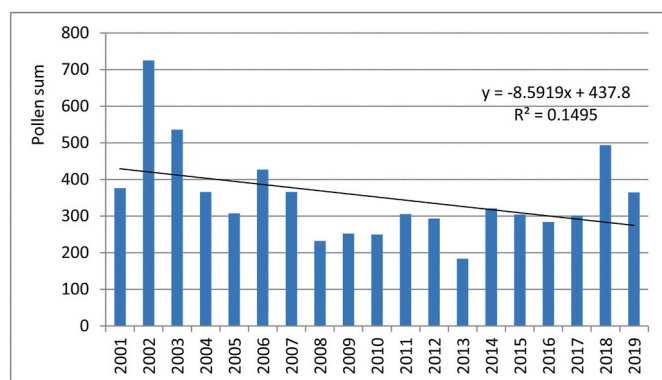


Fig. 8. Variation in the annual sums of Amaranthaceae pollen grains in Lublin in 2001–2019 and the trend line.

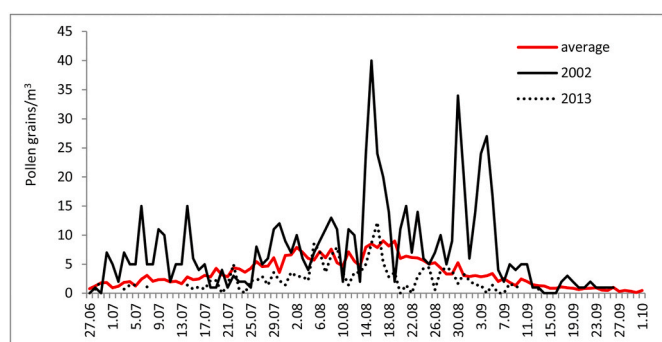


Fig. 9. Amaranthaceae pollen season in the years with the highest (2002) and lowest (2013) pollen sum relative to the average for 2001–2019.

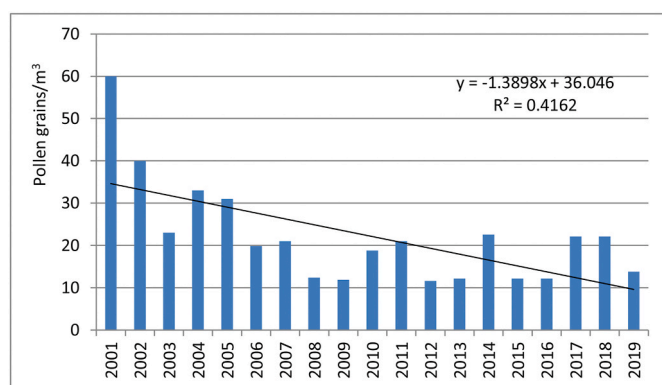


Fig. 10. Maximum concentrations of Amaranthaceae pollen grains in Lublin in 2001–2019 and the trend line.

Table 2

The list of significant Spearman's correlations between the parameters of the Amaranthaceae pollen season in Lublin (2001–2019).

Parameters of pollen season	Spearman coefficient
Duration & End	0.7315
Peak value & Annual total	0.7282

Level of significance 0.01.

3.2.3. Dynamics of pollen seasons

There were differences in the Chenopodiaceae/Amaranthaceae pollen concentrations not only between the years but also between the pollen season months (Fig. 11). Pollen produced by representatives of

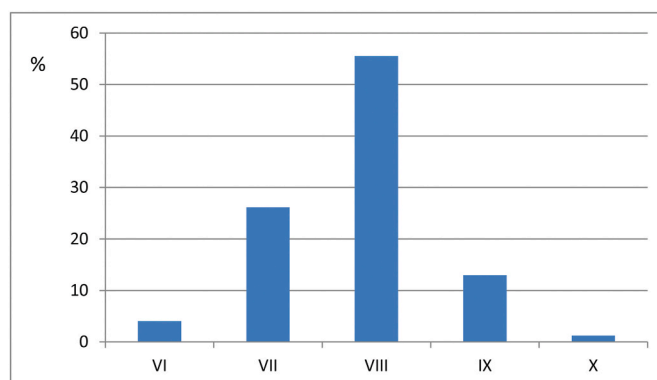


Fig. 11. Percentage of Amaranthaceae pollen grains in particular months of the year.

this family is present in the air of Lublin from June to October. The highest mean percentage of total pollen grains was noted in August (55.5%). Substantially lower average pollen content was recorded in July (26.2%) and September (13%), while in June and October it was only 4.1% and 1.2%, respectively. In the multimodal curves illustrating the dynamics of the content of pollen of plants from this family, several peaks can be seen, primarily in the middle of August and at the end of August and beginning of September in some years (Fig. 9).

In terms of the achievement of the annual airborne pollen sum, the dynamics of the Chenopodiaceae/Amaranthaceae pollen seasons in the individual years is presented in Fig. 13. In many cases, these seasons differed with regard to the date when 50% of the annual pollen sum was reached. However, this parameter was similar in 2004, 2007–2013, and 2019, which account for almost half of the seasons studied. In most of the pollen seasons (13), 50% of the annual pollen sum was released into aerosol before the first 10 days of August and before the end of the second 10-day period of this month in the other years (Fig. 12). The stages of the cumulative pollen percentages from 25% to 75%, i.e. the main part of the pollen season, had different durations. This shortest period (17 days) was noted in 2001, which indicates rapid and abrupt release of large amounts of pollen during this time. In turn, we recorded the longest period in this respect in 2019 (36 days), which means that the release of large amounts of pollen lasted almost twice as long as in 2001.

The length of the other stages of airborne pollen grains during the pollen season generally varied (Fig. 12). Nevertheless, in some years, there were similarities in the duration of the stages from 5% to 95% of pollen grains. The seasons with the shortest above-mentioned stages (2001, 2009, 2013) were the most condensed; at the same time, they were the shortest of those recorded in the study period and lasted 85, 87, and 79 days, respectively. 2003, 2007, and 2011 were the years with the longest stages (5%–95%). Simultaneously, these seasons were characterized by a varying length, i.e. 107, 129, and 123 days, respectively.

The Chenopodiaceae pollen seasons varied in terms of the symmetry determined by the position of the cumulative percentage of 50% of pollen grains. Ten of the 19 seasons analyzed were right skewed due to their longer period after reaching the cumulative percentage of 50% of pollen grains. In the other years, the seasons were left skewed.

Application of cluster analysis to compare the pollen seasons with the determination of periods of the cumulative percentages of pollen grains allowed identification of similarities between some seasons in terms of their dynamics (Fig. 13). The last years (2012–2019) were included in the more numerous group in the dendrogram, comprising 12 years, which implies that they had many common characteristic features as regards the successive stages of the pollen season.

3.2.4. Effect of meteorological factors on pollen seasons

The statistical analysis indicated that the onset of the

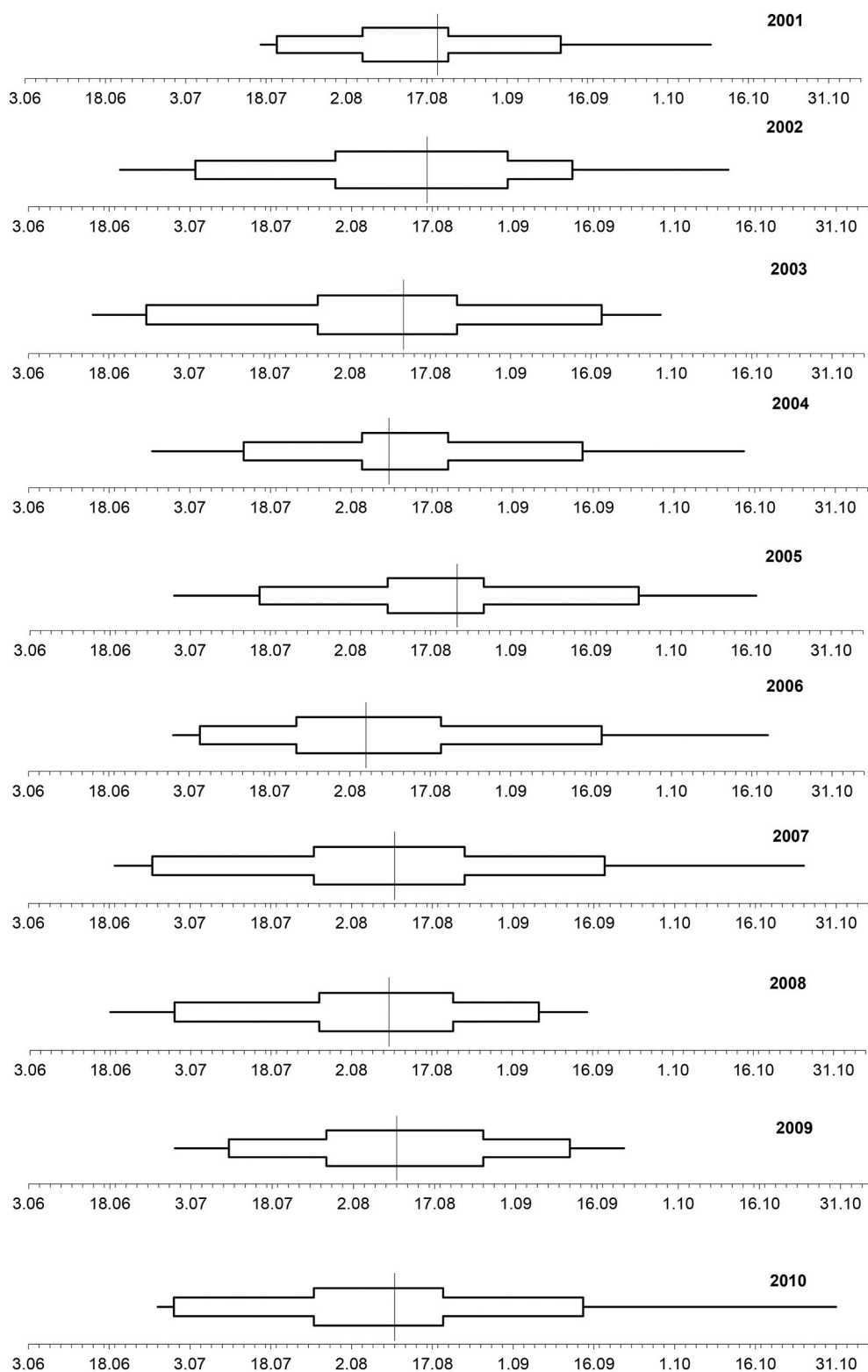


Fig. 12. Dynamics of the Amaranthaceae pollen seasons in 2001–2019.

Chenopodiaceae/Amaranthaceae pollen season was predominantly dependent on temperature in May and June. An increase in temperature in these months promoted an earlier occurrence of airborne pollen grains. The highest significant correlation was found between the season onset and the mean maximum temperature in June. High cloud cover in June delayed the onset of the pollen season (Table 3). The season end

date was positively correlated with relative air humidity and cloud cover in August. The study demonstrated a statistically significant correlation of the season duration with precipitation and humidity in March (negative correlation) and with precipitation in August (positive correlation). The pollen season lasted longer when the total cloud cover in August increased. A correlation was found between the peak value and

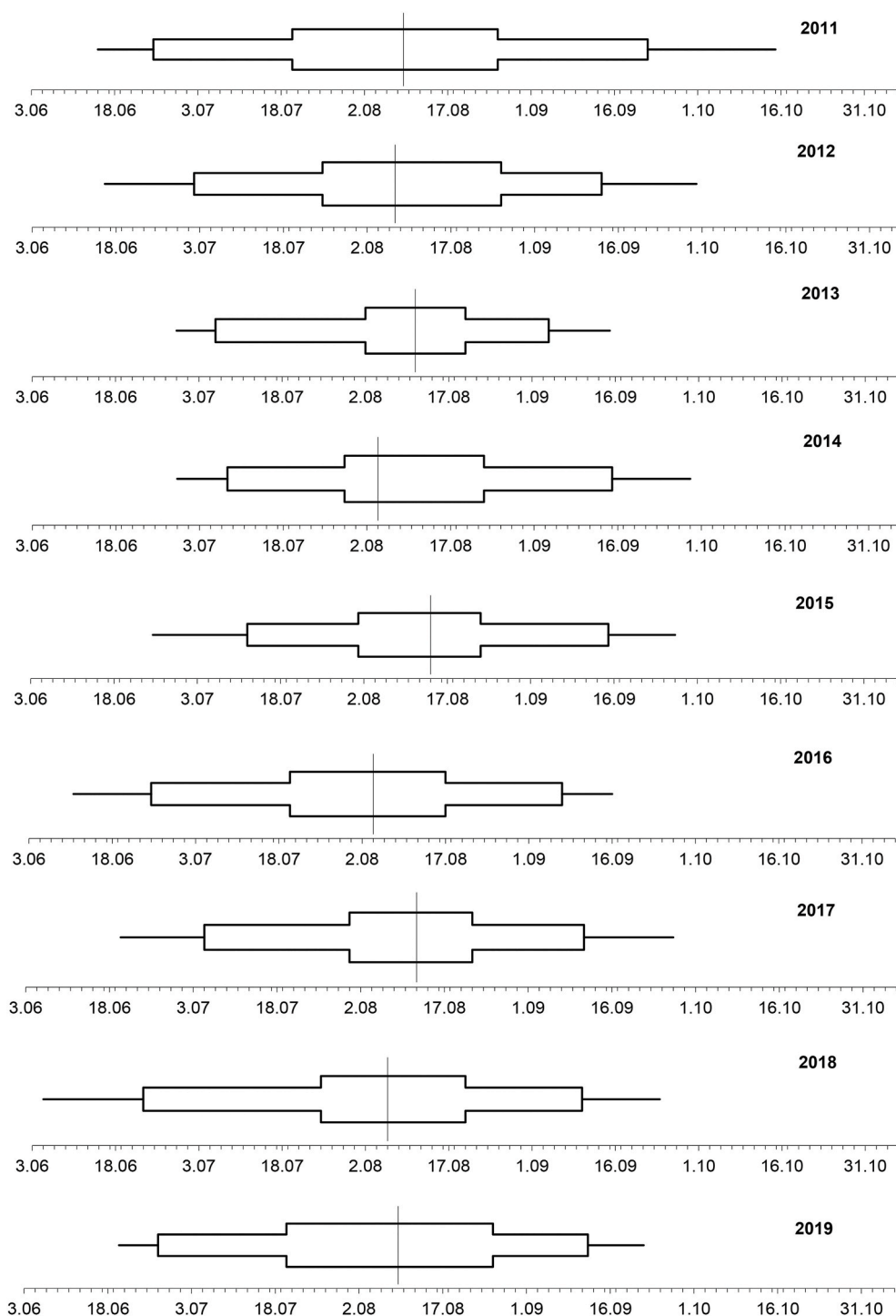


Fig. 12. (continued).

the cloud cover in July (positive correlation), whereas the peak date was correlated with humidity in March (negative correlation). The statistical analysis revealed a significant negative correlation between the annual total and relative air humidity in May (Table 3).

The statistical analysis indicated that the temperature did not have a significant effect on the annual pollen sum. Nevertheless, we found that the average of maximum temperature in May and July in the years characterized by abundant pollen production (2002, 2003, 2006, 2018) was by 1.4 °C and 1.6 °C higher, respectively, than in the years with the lowest annual pollen sum (2008, 2009, 2010, 2013). The lower relative humidity in May in the years with the highest amount of pollen grains

was accompanied by substantially lower daily sums of atmospheric precipitation than in the years with the low pollen count, i.e. on average by 45% over four years.

4. Discussion

4.1. Size of *Spinacia oleracea* pollen grains and presence of pollenkitt in *amaranthaceae*

The diameter of the pollen grains of *Spinacia oleracea* cv. 'Winter Giant' was in the range of 13.2–38.1 µm, with an average of 31.3 µm. As

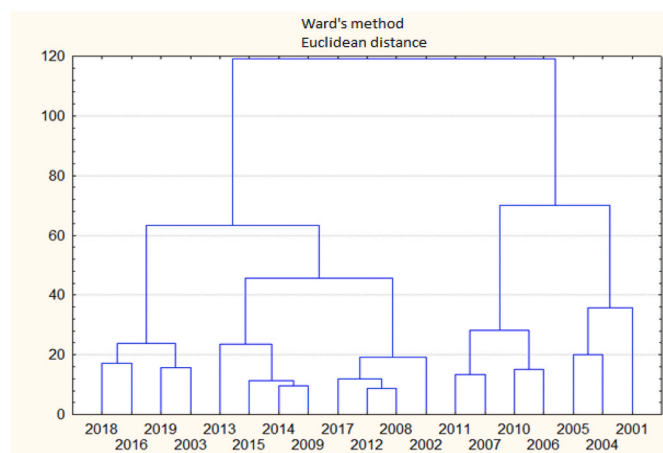


Fig. 13. Cluster analysis of the 2001–2019 pollen seasons.

Table 3

Significant Spearman's correlations between pollen season parameters and meteorological factors in Lublin (2001–2019).

Season parameter vs meteorological factor	Spearman coefficient
Dependent variable: season start	
Mean daily average temperature in May	−0.4594*
Mean daily average temperature in June	−0.6940**
Mean June daily minimum temperature	−0.6332**
Mean May daily maximum temperature	−0.5044*
Mean June daily maximum temperature	−0.7690**
Mean daily cloud cover in June	0.6006*
Dependent variable: season end	
Mean daily relative humidity in August	0.5037*
Mean daily cloud cover in August	0.6868**
Dependent variable: duration	
Total rainfall in March	−0.5015*
Total rainfall in August	0.4923*
Mean daily relative humidity in March	−0.4848*
Mean daily relative humidity in April	−0.5198*
Mean daily cloud cover in August	0.5637*
Dependent variable: peak value	
Mean daily cloud cover in July	0.6691**
Dependent variable: peak date	
Mean daily relative humidity in March	−0.4706*
Dependent variable: annual total	
Mean daily relative humidity in May	−0.6175**

Level of significance * 0.05, ** 0.01.

shown by Wodehouse (1965), the size of pollen grains in this taxon is 30–35 μm , whereas Beug (2004) reported a size range of 27.5–37.5 μm , on average 32.2 μm . The size of the dwarf grains observed in the present study was in the range of 13.2–17.2 μm , while the values for these grains reported by Wodehouse (1965) ranged from 12 to 16 μm . The mean and maximum values of the size of pollen grains in the species analyzed in the present study are comparable to those presented by Beug (2004).

The results of our study indicated that most *Spinacia oleracea* pollen grains at the anther dehiscence stage were coated by pollenkitt. The pollenkitt covered either the entire exine surface or only its parts. The pollen grain surface in many plants is covered with pollenkitt, which is also called the pollen coat or tryphine. This layer is formed by a viscous matrix derived from remnants of disintegrated tapetal cells (Pacini and Hesse, 2005). The pollen coat is a mixture of hydrophobic materials composed of lipids, carotenoids, flavonoids, proteins, and carbohydrates. The pollen coat composition also includes alkanes, waxes, and lipidic volatiles (Hesse 1981; Pacini and Hesse 2005). This extra-exinous layer can also contain allergenic proteins, which are synthesized in the interior of pollen grains and then transmitted to the pollen surface (Gong et al., 2015).

Literature data show an ambiguous position of the family

Chenopodiaceae, currently the subfamily Chenopoideae, in terms of pollination ecology (Wodehouse 1965; Pacini and Hesse 2005). As reported by Wodehouse (1965), plants from this family originate from an entomophilous ancestor which is evidenced by e.g. the presence of numerous pores in pollen grains, and now represent secondary anemophily. Pacini and Hesse (2005), on the other hand, included Chenopodiaceae in families in which primary anemophily occurs. As shown by these authors, in the case of primary anemophily, pollenkitt disappears and, consequently, the pollen surface is devoid of the extra-exinous layer. The results of our study showing the presence of pollenkitt on the surface of *S. oleracea* pollen allow a conclusion that Chenopodiaceae represent plants exhibiting secondary anemophily, which is in agreement with the findings reported by Wodehouse (1965). This is also confirmed by our aerobiological analysis, as we repeatedly found that the Chenopodiaceae/Amaranthaceae pollen grains in the slides were covered with pollenkitt masking the sculpture of the exine surface and had very poorly visible pores.

All *S. oleracea* pollen grains located in the marginal part of the anther locus were covered with pollenkitt. In turn, pollen grains without pollenkitt were also observed in the central part of the anthers. This may have been related to the uneven distribution of the pollenkitt within the locus, which was also observed by Hesse (1979) in other anemophilous plants. This author found that a major part of pollenkitt in *Fraxinus excelsior* was located in the peripheral part of the locus, while considerable amounts of this viscous substance were observed in pollen grains located in the central locus part.

Another interpretation of the presence of small amounts of pollenkitt on the surface of some *S. oleracea* pollen grains is the possibility that this viscous fluid penetrates through the micropores into the exine cavities; hence, there is no visible extra-exinous layer on the grain surface (Hesse 1979, 1980; Hesse 1979; Hesse 1980; Pacini and Hesse 2005).

Particularly interesting is the fact that pollenkitt persist longer in the region of protruded pores on the surface of the *S. oleracea* pollen grain. Thanikaimoni (1986) noted the presence of common internal lamellas in the apertural region; hence, this region often contains more surface coating with the pollen coat than the non-apertural regions.

Most frequently, the pollenkitt layer covered the pollen of *S. oleracea* evenly. A similar distribution of pollenkitt was observed in the entomophilous pollen grains of *Cucurbita pepo* (Nepi and Pacini 1993), *Crocus vernus*, and *Narcissus poeticus* (Chichiricò et al., 2019). We observed the occurrence of conspicuous bridges among the *S. oleracea* pollen grains. Such bridges formed by pollenkitt between pollen grains and between pollen grains and the locus wall were also observed in *Plantago media* by Hesse (1979). Similar bridges located at a distance of a few micrometers from each other were also found in *Crocus vernus* and *Narcissus poeticus* pollen grains by Chichiricò et al. (2019).

Our SEM examination facilitated determination of the stage of the *S. oleracea* pollen grains and visualization of the pollenkitt layer. This study demonstrated that it is possible to use *in vivo* material for scanning microscopy observations. We used this technique for the first time to show the pollenkitt layer on the surface of fresh pollen grains.

4.2. Aerobiological studies

Amaranthaceae pollen belongs to the airborne herb pollen types, which are abundant in the air in Europe (Alcázar et al., 2009). Weeds and ruderal plants growing in uncultivated lands with frequent nitrophilous communities rich in plants of this family are most often the source of this pollen (Giner et al., 2002). The Amaranthaceae pollen seasons are extended because numerous species in this family differ in their flowering season. Therefore, their allergenic pollen is active in the air for a long time (Waisel et al., 2004).

Our research demonstrated that, over the last two decades, the Amaranthaceae pollen season in Lublin lasted on average from the third 10 days of June to the first 10 days of October. The date of the onset of the Amaranthaceae pollen season is largely dependent on latitude. In

Spain, the season began much earlier than in Poland. It was shown that it started two months earlier in Cordoba than in Poznań (Poland), as it began in late June and lasted until September (Alcázar et al., 2009). In Murcia (Spain), the season of the analyzed taxon lasted from the third 10-day period of March until the third 10-day period of August (Giner et al., 2002). The pollen season started much later in Münster (Germany) where airborne pollen of this taxon was recorded in July and August (Melgar et al., 2012). In Skien (Norway), Chenopodiaceae/Amaranthaceae pollen was observed in aerosol from July to October (Piotrowska 2010). In Israel, on the other hand, the pollen of plants from this family was present in the air throughout the year (Waisel et al., 2004), while in Kuwait it was predominant in aeroplankton in spring and autumn (Al-Dousari et al., 2018).

Our long-term study demonstrated that currently the Amaranthaceae pollen season starts 14 days earlier than at the beginning of the study period. Similarly, the end of this season was accelerated by about 24 days. The changes in the onset and end of the analyzed pollen seasons probably result from global warming. Earlier flowering of many herbaceous plant species has been recorded by Fitter and Fitter (2002), Primack et al. (2004), and Mercuri et al. (2016). Many researchers have also found a clear trend towards earlier flowering and pollen release in different tree species (Rasmussen 2002; Clot 2003; Puc 2011; Smith et al., 2014; Malkiewicz 2014; Weryszko-Chmielewska et al., 2019). A shift in pollen seasons of anemophilous allergenic plants may have adverse effects on pollen allergy sufferers. The present study demonstrated that, in our aerobiological monitoring site located in the central-eastern part of Europe, the Amaranthaceae pollen seasons started much earlier during the last two decades. Hence, the pollen season of representatives of the analyzed plant family may coincide with intense pollen release in other plants, for example grasses, which may cause more severe pollen allergy symptoms in sensitive people. Due to the acceleration in the pollen seasons of allergenic plants, the seasons are also harder to predict and deal with efficiently (Damialis et al., 2019).

In 2018, the Amaranthaceae pollen season began exceptionally early, i.e. on June 5 in Lublin and between May 30 and June 5 in 12 other cities of Poland (Malkiewicz et al., 2018). This significant acceleration in the flowering of plants from this family was probably caused by the increase in temperature in April, May, and June 2018, which rose in Lublin by 4.5 °C, 3.3 °C, and 1.8 °C, respectively, relative to the average temperature in the previous 17 years. This was confirmed by the results of the statistical calculations, which revealed a significant negative correlation of the minimum, maximum, and average daily temperature in May and June with the onset of the season. This means that the acceleration in the onset of Amaranthaceae pollen seasons was associated with the increase in temperature in May and June.

The results obtained in the present study, in particular those from 2018, clearly prove that a rise in temperature accelerates plant flowering and pollen release. This is consistent with the results of investigations on the effect of temperature on the acceleration of the onset of pollen seasons in many other plant taxa (Frei and Gassner 2008; Zhang et al., 2014; Puc et al., 2015; Weryszko-Chmielewska et al., 2019; Ziska et al. 2019).

Different results were obtained in Denver (Colorado, USA) by Gross et al. (2019), who analyzed the impact of meteorological variables on pollen seasons in many plant taxa in a 10-year study. An earlier onset of the pollen season with a rise in temperature was observed only in some trees (linden, oak). Chenopodiaceae/Amaranthaceae also exhibited a slightly accelerated onset of the season, although the trend was statistically insignificant. The pollen concentration during the study years was at a similar level in many taxa, e.g. in Chenopodiaceae/Amaranthaceae. An increase in the pollen count was noted in some taxa, e.g. in linden. The data on the earlier onset of the Amaranthaceae pollen season obtained in the present study correspond with the results reported by these authors. The differences in the changes in the pollen count of this taxon in the atmosphere of Lublin and Denver may be associated with the difference in the duration of the investigations, i.e.

we conducted a 9-year longer study.

The average annual total of Amaranthaceae pollen calculated based on the 19-year study carried out in Lublin was 352. A several-year-long study (2001–2005) conducted in 6 cities of Poland showed that the highest values of the annual Amaranthaceae pollen sums were recorded for Lublin. In Cracow, Poznań, Rzeszów, Sosnowiec, and Szczecin, the average annual total for these years ranged from 116 to 359, whereas the value of this parameter in Lublin reached 462 (Weryszko-Chmielewska 2006). In 2018, the annual Chenopodiaceae/Amaranthaceae pollen sum (478) recorded in Lublin substantially exceeded the values (149–282) determined for 12 other Polish cities (Malkiewicz et al., 2018).

Aerobiological studies conducted in various European countries indicate that the annual totals of Chenopodiaceae/Amaranthaceae pollen reach very different values. In Spain, the annual total of this type of pollen was 2820 in Murcia (Giner et al., 2002), from 200 to 1600 in Cordoba (Alcázar et al., 2009), and only 197 in Salamanca (Rodriguez de la Cruz et al., 2012). The mean annual total pollen sum of this taxon (453) recorded in Bratislava (Slovakia) (Ščevková et al., 2010) is comparable to the values recorded in Lublin. On the other hand, much lower annual pollen concentrations were found in Porto (Portugal), where on average only 51 pollen grains were recorded (Ribeiro and Abreu 2012), and in Münster (Germany), where the average value of this parameter was 36 (Melgar et al., 2012).

Our study conducted in Lublin revealed a downward trend in the annual total and maximum concentration of Amaranthaceae pollen during the study period. The annual total decreased by about 35%, while the latter parameter was reduced by even 60%. A similar downward trend in the annual pollen sum of Chenopodiaceae/Amaranthaceae was found in Poznań (Poland), Cordoba (Spain) (Alcázar et al., 2009), and Szczecin (Puc 2009). The annual total value of *Artemisia* pollen was also found to exhibit a similar downward trend in Poznań (Stach et al., 2007). An opposite trend in the airborne Chenopodiaceae/Amaranthaceae pollen count in the atmospheric air of Salamanca (Spain) was noted by Rodriguez de la Cruz et al. (2012). The authors reported a significant increase in the annual pollen index in a 7-year study period. A negative impact of climate warming on birch pollen production has been found in some European countries (Ziello et al., 2012; Newhman et al., 2013). Currently, an increase in the amount of airborne allergenic pollen in response to a temperature rise is recorded for many species (Ziello et al., 2012; Beck et al., 2013; Puc et al., 2015; Mercuri et al., 2016; Ziska et al., 2019). Studies of various authors have proved that an increase in pollen concentrations is related to specific bioclimatic regions and specific pollen types (Ziello et al., 2012).

In the years characterized by the abundant production of Amaranthaceae pollen in Lublin (2002, 2003, 2006, 2018), the mean values of the maximum annual temperature in the four years were by 0.8 °C higher than in the years with the lowest pollen production. However, the statistical analyses did not reveal any significant effect of temperature on the magnitude of the annual pollen sum in this taxon.

The long-term investigations of thermal conditions conducted in Lublin showed that the mean annual temperature in 1951–2000 was 8.1 °C (Piotrowska and Kaszewski 2011) and increased by 1.3 °C in 2001–2018. Laboratory experiments have revealed that increased temperature has an adverse impact on the metabolism of flowers at the time of flowering in some plant species (Borghi et al., 2019). The authors showed a reduction in the number of pollen grains and pollen viability in heat-induced stress conditions (32 °C: 26 °C, day: night) in *Solanum lycopersicum*. As suggested by these authors, these changes result from the reduced content of starch and soluble sugars in the cells of the tapetum and pollen grains accompanied by a higher level of soluble sugars in the locular fluid.

The statistical analysis revealed a significant correlation between the cloud cover in July and the maximum daily Amaranthaceae pollen concentration. The significant negative correlation between the relative air humidity in May and the annual total indicates that these plants

prefer moderate air humidity at this stage of their growth (before flowering). The highest annual totals were recorded in the years when the relative humidity in May reached much lower values than in the years with low annual pollen sums. We also noted that the daily rainfall totals in the years with high annual total values (2002, 2003, 2006, 2018) were much lower in the months preceding the flowering of *Amaranthaceae* (III–V) than in the years with the lowest annual pollen sums (2008–2010, 2013). This may indicate moderate requirements of plants from this family with regard to irrigation conditions. The negative impact of relative air humidity on the pollen concentration has also been noted in the case of other taxa of herbaceous plants: *Artemisia* (Puc 2009), *Poaceae* (Puc et al., 2015), and *Urtica* (Kluska et al., 2020).

Many phenological studies have demonstrated specific trends, frequently divergent, in responses of various plant species to climate change, but only 40% of them have been confirmed statistically (Schleip et al., 2010). Therefore, it is important to continue research in this area. The results presented in our study not only contribute to expanding the knowledge of flowering and pollen production of herbaceous plants in environmental change conditions, but also have a practical aspect, as they can be used in allergology.

As demonstrated in the present study, climate changes contribute to an earlier onset of pollen seasons in *Amaranthaceae* and many other plants reported in the literature, (Zhang et al., 2014; Myszkowska et al., 2015). In contrast to *Chenopodiaceae*/*Amaranthaceae* plants, the concentration of airborne pollen increases in many taxa (D'Amato and Akdis, 2020; Prescott 2020). Climate change thus increases the risk of an earlier occurrence of allergies with a more severe course and creates a need for stronger medication (Cecchi et al., 2010; Prescott 2020). Allergic symptoms caused by the presence of *Chenopodiaceae*/*Amaranthaceae* pollen include rhinitis, allergic conjunctivitis, and sometimes asthma (Stemeseder et al., 2014). The allergenic potential of *Chenopodiaceae*/*Amaranthaceae* pollen has recently been evaluated as high (Ruiz-Valenzuela and Aguilera 2018). A considerable threat may be posed by very high airborne content of pollen of this taxon, as demonstrated in some countries, e.g. Algeria (Kiared et al., 2017), Egypt (Taia et al., 2019), Israel (Helfman-Hertzog et al., 2018), Qatar (Al-Nesf et al., 2020), and India (Singh et al., 2017). In Europe, high concentrations of *Chenopodiaceae*/*Amaranthaceae* pollen are recorded in certain regions of Spain (Majeed et al., 2018), Hungary, and Slovakia (Puc et al., 2020). A very large proportion of the population is affected by allergy symptoms induced by *Chenopodiaceae*/*Amaranthaceae* pollen in some countries, e.g. in Spain (Giner et al., 2002) and Qatar (Al-Nesf et al., 2020).

The impact of environmental changes, including pollution, is reflected not only in respiratory illnesses but also in other aspects of public health, as such changes have been shown to cause cardiovascular, brain and mind, gastrointestinal, skin, immunological, and metabolic disorders (D'Amato and Akdis, 2020; Fairweather et al., 2020).

The accelerated appearance of *Chenopodiaceae*/*Amaranthaceae* pollen in the air of Europe associated with global warming may constitute an increasing problem. Therefore, it is important to monitor the course of pollen seasons, including pollen concentrations. It is essential to conduct large-scale aerobiological research in consecutive years, since such results will provide researchers and allergists with important information about environmental changes. The research presented in this paper addresses a common problem, as *Amaranthaceae* pollen causes allergies on different continents and its allergenicity is classified as “high” by many researchers. It is worth emphasizing that few studies so far have focused on the characteristics of *Amaranthaceae* pollen seasons in Europe, and the present study fills the gap in this regard. Since the results were collected in the multiyear study and were analyzed statistically, they may be especially important for assessment of environmental changes occurring in Central Europe. We have demonstrated that the response to climate change is evident not only in plants flowering in early spring, as shown in many literature reports, but also in species flowering in summer, e.g. *Amaranthaceae*. For the first

time, we have shown the presence of pollenkitt on the surface of fresh *Amaranthaceae* pollen grains using a very simplified method facilitating observation of fresh intact plant material in a scanning electron microscope. The data obtained in this study expand the knowledge of changes in the course of the *Amaranthaceae* pollen seasons and describe the characteristic traits of pollen in representatives of this plant family. Given its important environmental and health aspects, the research will be continued.

5. Conclusions

- * Currently, the *Amaranthaceae* pollen season in Poland lasts approx. 3.5 months, and the highest pollen concentrations are recorded in the second and third tens of days of August and at the beginning of September. In this period, sensitive subjects are at high risk of development of a pollen allergy.
- * The results of our multiyear research (19 years) show a clear response of plants from the *Amaranthaceae* family to climate changes. It is reflected in the accelerated onset (by 14 days) and end (by 24 days) of pollen seasons, a decrease in the annual pollen sums (on average by 35%), and reduced maximum pollen concentrations (on average by 60%) during the study years.
- * Some climatic factors exert a significant impact on the course of the *Amaranthaceae* pollen season. We have shown a positive effect of temperature prevailing in months preceding the pollen season (May and June) on the onset of the pollen season and a negative effect of relative humidity in May on the annual pollen sum, which results in acceleration of the onset of pollen seasons and production of larger amounts of pollen.
- * Pollenkitt covers *Amaranthaceae* pollen grains to varying degrees. The layer protects pollen grains from water loss, which may prolong viability of airborne pollen and play a significant role during global warming.
- * Due to the high allergenic potential of *Amaranthaceae* pollen grains, further studies of their concentration in the air of various regions of the world should be conducted.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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