



Atmospheric exposure to the major *Artemisia* pollen allergen (Art v 1): Seasonality, impact of weather, and clinical implications

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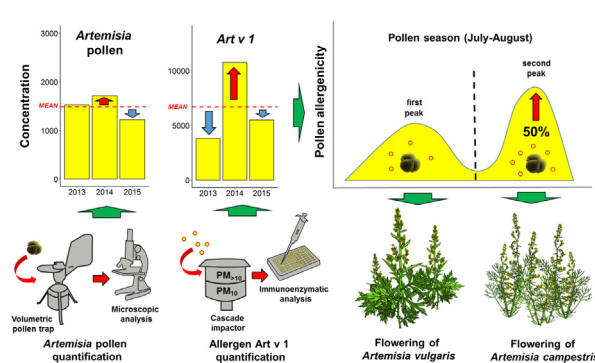
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HIGHLIGHTS

- Art v 1 in the PM₁₀ air fraction accounted for 86% of the total Art v 1 level.
- The daily Art v 1 and *Artemisia* pollen concentration were significantly correlated.
- Seasonal *Artemisia* pollen allergenicity varied from 2.5 to 4.7 pg Art v 1/pollen.
- High seasonal pollen allergenicity was observed during the wet and warm seasons.
- Pollen allergenicity increased by 63% during the *A. campestris* flowering period.

GRAPHICAL ABSTRACT



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ABSTRACT

Artemisia pollen grains are important aeroallergens worldwide. The amount of allergenic proteins produced by pollen, or pollen allergenicity, is regulated by both genes and the environment. As a result, even closely related plant taxa may release pollen with distinctly different allergen contents. Here, we determined the variability in atmospheric exposure to the major *Artemisia* pollen allergen, Art v 1, during the pollination seasons of two common species, i.e., *A. vulgaris* (early flowering species) and *A. campestris* (late flowering species), in Poznań, Poland (2013–2015). *Artemisia* pollen grains were collected using Hirst-type volumetric trap, while Art v 1 was collected by a two-stage cascade impactor (PM₁₀ and PM_{10-2.5} air fractions) and quantified by immunochemical analysis. The results showed that daily Art v 1 levels correlated significantly with mean daily concentrations of *Artemisia* pollen (from $r = 0.426$ to $r = 0.949$, depending on air fraction and peak of the season). Significant differences were observed between 1) the median pollen allergenicity in different seasons (from 2.5 to 4.7 pg Art v 1/pollen) and 2) the median pollen allergenicity in different peak periods of the season (from 1.8 to 6.7 pg Art v 1/pollen). During the late peak (flowering of *A. campestris*), the median pollen allergenicity was significantly higher (on average by 63%, $p < 0.05$) than that during *A. vulgaris* flowering. The highest mean seasonal pollen allergenicity was observed during the wettest season, while the lowest was observed during the driest season (from July–August). In summary, our study showed distinct differences in *Artemisia* pollen allergenicity, that were not only related to daily and seasonal variability, which may exceed 800% and 80%, respectively but also noticeable when two common *Artemisia* species were compared. Therefore, we argue that variability in pollen allergenicity (both seasonal and species-specific) should be considered in future studies assessing pollen exposure.

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1. Introduction

In the Northern Hemisphere, pollen produced by *Artemisia* species (Asteraceae) is an important cause of respiratory allergy (Katial et al., 1997; Burbach et al., 2009; Tang et al., 2015). The highest levels of *Artemisia* pollen are often recorded during midday hours between July and October (Laursen et al., 2007; Grewling et al., 2012). The daily atmospheric concentrations of *Artemisia* pollen commonly reach several hundreds of pollen grains per cubic meter of air (pollen grains/m³), while the threshold for allergic reactions in *Artemisia* pollen-sensitive subjects is estimated to be only 5–15 pollen grains/m³ (de Weger et al., 2013). Due to the relatively low transport ability of *Artemisia* pollen compared to other wind-pollinated plants, the highest pollen exposures are recorded in area in close proximity to *Artemisia* populations (Spieksma et al., 2000; Grewling et al., 2016b). Many *Artemisia* species prefer certain habitats, often dry and sunny, such as sea shores, dunes, fallow lands and xeric steppe (Li et al., 2008; Grewling et al., 2012). Others, such as the common mugwort (*A. vulgaris*), are eurytopic plants found in a wide range of environments (Bogawski et al., 2016). Several *Artemisia* species are cultivated as culinary herbs (Wightman and Whitford, 1984) or medicinal plants (Kumar et al., 2004) and are considered a noxious invasive species that infest field-grown horticultural crops (Barney et al., 2005; Barney, 2006).

Pollen grains produced by different *Artemisia* species are very similar in size and morphology: small (equatorial length: 13–18 µm, polar length: 22–34 µm), elliptical and tricolporate with thick (1–6 µm) echinate pollen walls (Lin et al., 2005; Ghahreman et al., 2007). Therefore, during microscopic analysis of aerobiological samples, *Artemisia* pollen grains are routinely identified at the botanical genus level. Consequently, in allergy forecasts, no differentiation is applied between the pollen seasons of different *Artemisia* species, such as *A. vulgaris* and *A. campestris* (field wormwood), which often co-occur (Bogawski et al., 2016). Due to high structural similarity and strong in vitro cross-reactivity among pollen allergens of different *Artemisia* species (Brandys et al., 1993; Katial et al., 1997; Pablos et al., 2019), it has been suggested that such an approach would be sufficient for clinical and epidemiological purposes. However, genetic and environmental factors affect the expression level of allergens in pollen grains. As a result, pollen grains produced by different species may differ in allergen content (so-called pollen allergenicity). For instance, the amount of released Ole e 1 (major olive pollen allergen) showed strong geographical variations (higher in Spain than in Portugal) (Galán et al., 2013). Similarly, the differences in the exposure to Phl p 5 (major grass pollen allergen) in Europe were associated with both variations in grass species composition and species-specific phenological patterns (Buters et al., 2015).

In addition, in many plant species, allergenic proteins have also been localized in parts other than pollen of plants, e.g., in inflorescences (Vinckier et al., 2005), leaves (Agarwal et al., 1984), orbicules (Ubisch bodies) (Suarez-Cervera et al., 2003) and nanosize pollensomes (Prado et al., 2015). When airborne, these particles may serve as additional sources of allergenic proteins. For instance, it was suggested that allergenic reactions in ragweed-sensitized subjects observed after the pollen season were caused by the allergens originating from leaves (Agarwal et al., 1984). Moreover, under specific atmospheric conditions (mainly increased humidity), pollen grains of certain species, e.g. birch and grasses, can burst through osmotic shock and expel their cytoplasmic content with hundreds of micronic (<3 µm in diameter) immunoreactive starch granules (Suphioglu et al., 1992; Schäppi et al., 1999; Grote et al., 2000; Grote et al., 2001; Taylor et al., 2002). Consequently, it has been suggested that exposure to pollen grains is not equal to allergen exposure (Jato et al., 2010; Cecchi, 2013; Galán et al., 2013). Despite the clinical importance of *Artemisia* pollen in allergic respiratory diseases, the data concerning the release process and atmospheric behavior of *Artemisia* allergens are scarce (Hoidn et al., 2005).

Thus, in this study, we described the intra- and interseasonal (2013–2015) variations in atmospheric content of Art v 1 sampled in two air fractions: PM₁₀ (related to pollen) and PM₁₀ (related to the subpollen fraction). In addition, as the study site was located in an area where the *Artemisia* pollen season has a distinct bimodal pattern (Grewling et al., 2015; Bogawski et al., 2016), the difference in *Artemisia* pollen allergenicity during the flowering periods of two common *Artemisia* species, i.e., *A. vulgaris* and *A. campestris*, was determined.

2. Materials and methods

2.1. Data collection

Aerobiological monitoring was conducted during three consecutive *Artemisia* pollen seasons (2013–2015) in Poznań, Poland (52.47°N, 16.92°E). Poland is located in a temperate climate zone with a mixture of continental and Atlantic climates. The lowest mean monthly temperature (1996–2015 average) occurs in February (0.5 °C), while July is characterized by the highest mean monthly temperature (19.5 °C) (based on data supplied by the Polish Institute of Meteorology and Water Management, www.imgw.pl). July is also the wettest month, with the average total rain reaching 93.6 mm. The monitoring station was located at the roof level (16 m a.g.l.) in the outskirts of Poznań (university campus located approximately 7.5 km north of the city center). The station was surrounded by small pine and alder forests, meadows, lawns and agricultural fields (Fig. 1). Two main *Artemisia* species occur in the study area, i.e., *A. vulgaris* and *A. campestris* (Bogawski et al., 2016). A field survey was performed to assess the distribution of *Artemisia* species in the proximity (a radius of 1 km) of the pollen sampler. First, the potential locations were indicated based on aerial, high-resolution images (raster cell = 25 cm) obtained from the Main Office for Cartography and Land Surveying in Poland (GUGIK, 2012). Second, the locations were visited in the field, and the surfaces covered by *Artemisia* species were mapped using Google Earth software (Google Ireland Ltd.), and the final formatting of the map was performed using ArcGIS 10.3 (ESRI) (See Fig. 1).

Artemisia pollen grains were collected by a seven-day Hirst type volumetric trap (Hirst, 1952). The air with pollen grains was pulled into the trap at a rate of 10 l/min and flowed over a rotating drum covered with transparent adhesive tape. The tape was divided into segments corresponding to 24-h periods to provide a time-related sample (12:00–12:00). Each segment was mounted between a glass slide and cover slip using a mixture of gelatin, distilled water and basic fuchsin. The samples were examined by light microscopy (at 400× magnification). *Artemisia* pollen grains were counted along four longitudinal transects according to the recommendation of the European Aerobiology Society (Galán et al., 2014). Pollen concentration was expressed as a number of pollen grains per cubic meter of air (pollen grains/m³). Daily pollen and Art v 1 concentrations were recorded between 15 July and 27 August ($n = 44$ days). During these periods, most of the *Artemisia* pollen grains (observed throughout the year) were recorded (95.6%, 95.1% and 95.3% of the total pollen recorded in 2013, 2014, and 2015, respectively) (Table S1, Supplementary Materials).

A two-stage, high-volume (800 l/min) ChemVol cascade impactor (Butrac Inc., Son, Netherlands) was used to collect and separate airborne particles (1st air fraction: PM > 10 µm and 2nd air fraction: PM: 2.5–10 µm). The particles were collected on polyurethane filters that were stored separately in polypropylene tubes until extraction (−80 °C). Filters were extracted in the dark over a 4-h period using 0.1 M ammonium bicarbonate buffer (NH₄) HCO₃, pH 8.1, and lyophilized. Lyophilized material was dissolved in 1/10 of the original volume in phosphate-buffered saline (PBS). The major *Artemisia* pollen allergen, Art v 1, was quantified by a “sandwich type” enzyme-linked immunosorbent assay (ELISA) using anti-Art v 1 monoclonal antibodies and biotinylated monoclonal antibodies supplied by Bial-Aristegui (Spain). Streptavidin-peroxidase (Sigma-Aldrich S5512) was used as an

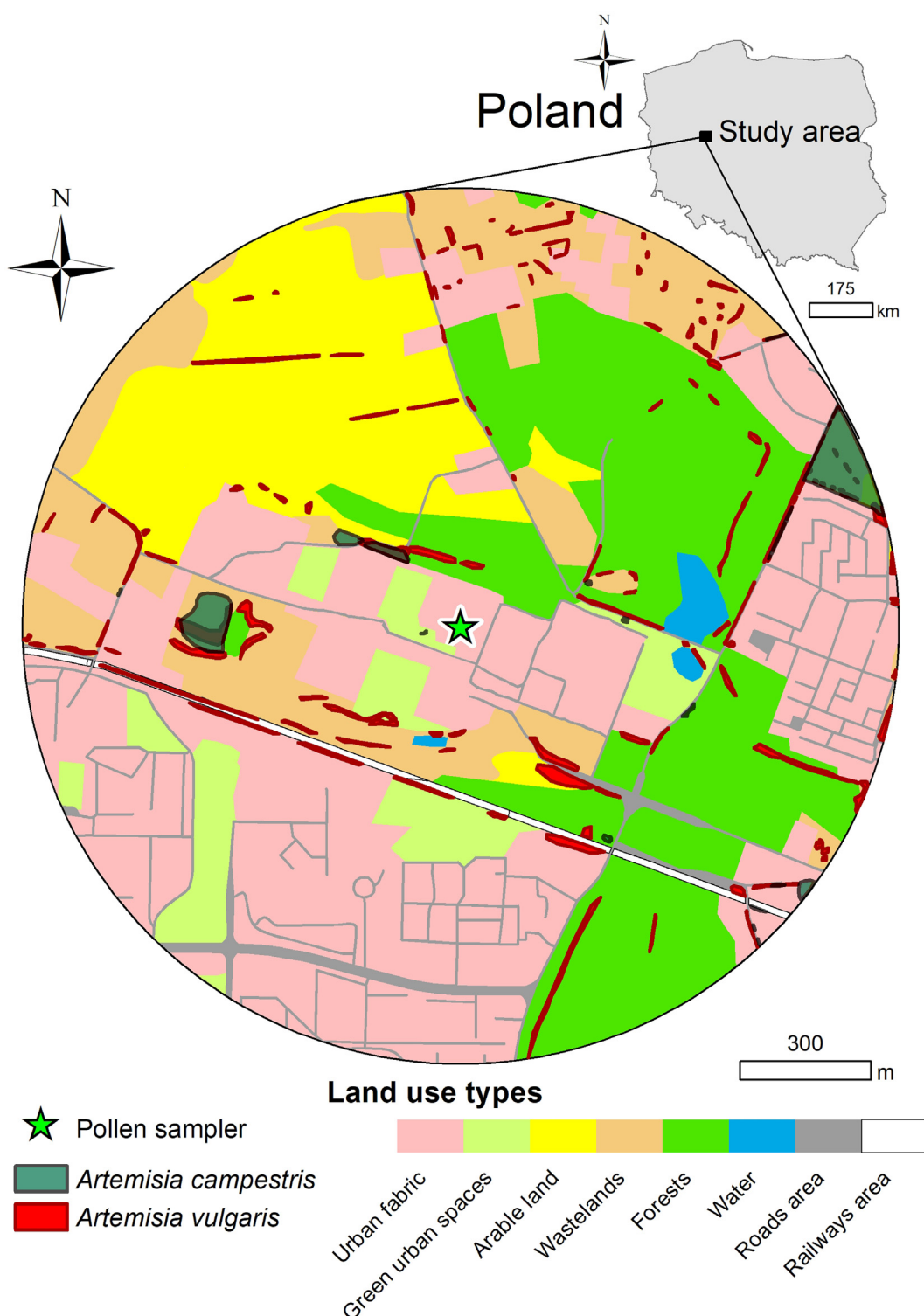


Fig. 1. Study area with both the pollen monitoring station and locations of the main stands of *A. vulgaris* and *A. campestris* marked.

enzyme, and 3,3',5,5'-tetramethylbenzidine (Sigma-Aldrich T0440) was used as a substrate. The reaction was stopped by adding 2.5 M H_2SO_4 (5 N). The concentration of Art v 1 in the sample was determined by reading the absorbance at 450 nm.

Meteorological data, i.e., daily mean, maximum and minimum temperature ($^{\circ}C$), wind speed (m/s), wind direction ($^{\circ}$), total precipitation (mm), and relative humidity (%) were collected from stations belonging to the Polish Institute of Meteorology and Water

Management located at the Ławica airport (approximately 7 km southwest of the pollen monitoring trap). Hourly values of meteorological variables were averaged according to 24-hour periods established for allergen measurements except for wind speed and direction used in polar plots. In this case, we used a frequency approach that relies on attributing all 24 values of wind speed and direction to one daily value of a dependent variable (e.g. allergen content).

2.2. Division of pollen season

The *Artemisia* pollen seasons were divided into two parts: 1) the first peak related to flowering of *A. vulgaris* and 2) the second peak related to flowering of *A. campestris*. Pollen grains of *A. vulgaris* and *A. campestris* are practically indistinguishable under light microscopy (see Supplementary Materials, Fig. S1). Therefore, the division was based on phenological observations of flowering and pollen production analyses of these two *Artemisia* species carried out in the study area (Bogawski et al., 2016). In addition, for a more accurate division, the *Artemisia* pollen seasons in the study area were compared with the pollen seasons recorded in the city center, where *A. campestris* is very rare. This was because, in the city center, the *Artemisia* pollen season had only one main peak in August in the first decade, and the second peak was very low (Grewling et al., 2015). Additionally, to limit the mixing of the pollen grains of the two *Artemisia* species, a 5-day “buffer period” was applied between the two pollen peaks (data from this buffer period were excluded from statistical analysis) (see Supplementary Materials, Fig. S2).

2.3. Data analysis

Due to uncertainties in pollen counts and allergen determination (Buters et al., 2012) and to ensure reliability of the study results, only days with *Artemisia* pollen concentrations >15 pollen grains/m³ and Art v 1 > 10 pg/m³ were included into statistical analysis. No days with *Artemisia* pollen levels below the adopted threshold were recorded outside the investigated collection periods. To calculate the daily allergenicity of airborne *Artemisia* pollen, the mean daily amount of released Art v 1 (collected in PM_{>10} air fraction) was divided by the mean daily *Artemisia* pollen concentration and expressed as pg Art v 1/pollen. Two different aerosol collection traps with different suction rates were used, i.e., ChemVol (for Art v 1) and Hirst traps (for *Artemisia* pollen). It should be stressed, however, that both impactors express the airborne particle concentration with the same units, i.e., particles/m³ of air (regardless of the suction rate). This method is accepted and widely used for the calculation of pollen allergenicity (Buters et al., 2012; Galán et al., 2013; Celenk, 2019). In addition, both traps were located at the same platform, only 2.0 m from each other.

The median daily concentrations of *Artemisia* pollen, Art v 1 and pollen allergenicity during three seasons (2013–2015) were compared using a Kruskal-Wallis test and Dunn’s procedure for multiple pairwise comparisons. *P*-values were adjusted using the Benjamini-Hochberg correction. A Mann-Whitney test was used to compare the median daily levels of Art v 1, *Artemisia* pollen grains and pollen allergenicity during the two peaks of the pollen season (Dunn, 1964; McDonald, 2014). The ratio between average (for 2013–2015) median pollen allergenicity during two peaks was calculated and expressed as an allergenicity “correction factor” (*f_c*). Next, calculated factor was multiplied by daily pollen concentrations during *A. campestris* pollen season (*f_c* for *A. vulgaris* was fixed to 1).

The effect of weather conditions on the daily particle concentrations (pollen and Art v 1 in both air fractions), Art v 1 ratio (PM₁₀ to PM_{>10}) and pollen allergenicity was calculated by the Spearman correlation coefficient (McDonald, 2014). The statistical significance of all results was determined by comparing the *p*-value with the significance level ($\alpha = 0.05$). The impact of wind direction and speed on the daily Art v 1 and pollen levels as well as pollen allergenicity and Art v 1 ratio (PM₁₀ to PM_{>10}) was graphically expressed by polar plots using the openair package (Carslaw and Ropkins, 2012). Statistical analysis was performed using R statistical software version 3.6.1 (R Core Team, 2018).

3. Results

3.1. Weather background

The July–August period in 2013 was characterized by a very low amount of rain, i.e. 82.6 mm (87% lower than the 10-year average from 2003 to 2012). In contrast, the highest rainfall values were observed in 2014 (146.6 mm). In 2015, very low precipitation was recorded during August (25.5 mm, 155% lower than the 10-year average). The July–August average temperatures in 2013 and 2014 were 0.6 °C higher than the 10-year averages. In 2015, this difference was higher, reaching 1.7 °C. The July mean temperatures varied from 19.7 °C (in 2015) to 22.1 °C (in 2014), while those in August varied from 18.0 °C to 22.7 °C (in 2014 and 2015, respectively). In August 2015, the average maximum temperature reached 29.4 °C and was 5.0 °C higher than the 10-year average. The monthly mean wind speed values varied from 2.7 to 3.5 m/s (in August 2013 and July 2015, respectively) and did not differ markedly from the 10-year averages (see Supplementary Materials, Table S2).

3.2. Inter- and intraseasonal variations in Art v 1

Art v 1 was recorded almost every day during the investigated seasons, i.e., 42 days in 2013 (95% of all days) and 44 days in 2014 and 2015 (100%). The highest seasonal sum of daily Art v 1 levels (10,755.5 pg Art v 1/m³ including both air fractions) was observed in 2014, while the lowest was observed in 2013 (3827.7 pg Art v 1/m³, *p* < 0.05) (Table 1, Fig. 2). Relatively little variation was observed with respect to the seasonal sum of *Artemisia* pollen grains (from 1205 pollen grains in 2015 to 1713 pollen grains in 2014) (Table 1). The maximum daily Art v 1 level (summing over both air fractions) varied from 319.9 pg Art v 1/m³ in 2013 to 791.7 pg Art v 1/m³ in 2014. In 2013, and 2014, the highest Art v 1 level was recorded in the third week of August, while in 2015, the peak occurred at the beginning of August (Table 1, Fig. 3). Generally, a high level of Art v 1 coincided with a high level of *Artemisia* pollen (and vice versa), which was expressed as statistically significant correlations (*p* < 0.05) during all three years studied. These correlations were the most distinct during the second peak (related to flowering of *A. campestris*) and varied from *r* = 0.668 to *r* = 0.949 (PM₁₀ in 2014 and PM₁₀ in 2015, respectively). In comparison, during the first peak, when *A. vulgaris* was the main pollen contributor, the correlations between Art v 1 and *Artemisia* pollen varied from *r* = 0.426 to *r* = 0.833 (Fig. 4).

In 2013, the median seasonal pollen allergenicity reached 2.5 pg Art v 1/pollen. In two subsequent seasons, the median pollen allergenicity

Table 1
Main characteristics of *Artemisia* allergen season (15 July–27 August).

Characteristics	Season 2013	Season 2014	Season 2015
Aerobiological data			
Sum of Art v 1 in PM ₁₀ (pg/m ³)	352.1	925.5	938.0
Sum of Art v 1 in PM _{>10} (pg/m ³)	3474.6	9830.0	4565.4
Sum of Art v 1 in both air fractions (pg/m ³)	3827.7	10,755.5	5503.4
Ratio Art v 1 in PM ₁₀ to PM _{>10}	10.2%	9.4%	20.5%
Maximum daily Art v 1 (both fraction) (pg/m ³)	319.9	791.7	446.4
Day with maximum Art v 1 level (both fraction)	18 August	20 August	4 August
Sum of <i>Artemisia</i> pollen (pollen/m ³)	1526	1713	1205
Maximum daily <i>Artemisia</i> pollen level (pollen/m ³)	114	157	121
Day with maximum <i>Artemisia</i> pollen level	17 August	18 August	4 August
Median allergenicity of pollen (pg Art v 1/pollen)	2.52	4.71	3.66
Daily variation in pollen allergenicity (pg Art v 1/pollen)	1.02–4.52	1.45–14.13	1.39–7.17

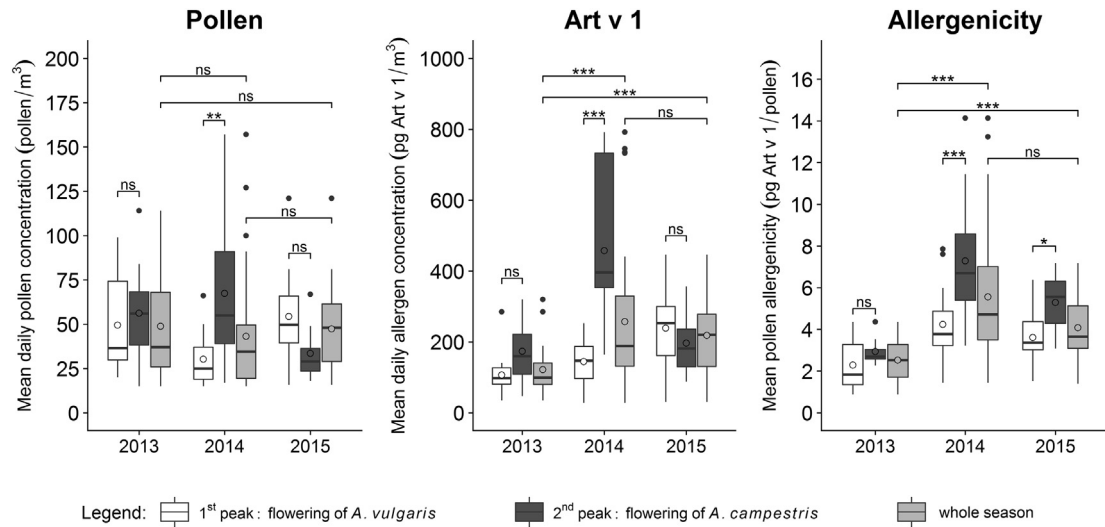


Fig. 2. Inter- and intraseasonal comparison (by Kruskal-Wallis and Mann-Whitney test, respectively) of the median daily *Artemisia* pollen level, Art v 1 and *Artemisia* pollen allergenicity recorded in Poznań, Poland (2013–2015) (ns- not significant, * $p < .05$, ** $p < .01$, *** $p < .001$). Boxes indicate the interquartile ranges, the median is shown as a solid line, the mean is shown as an open circle, and whiskers represent the 5th and 95th percentiles.

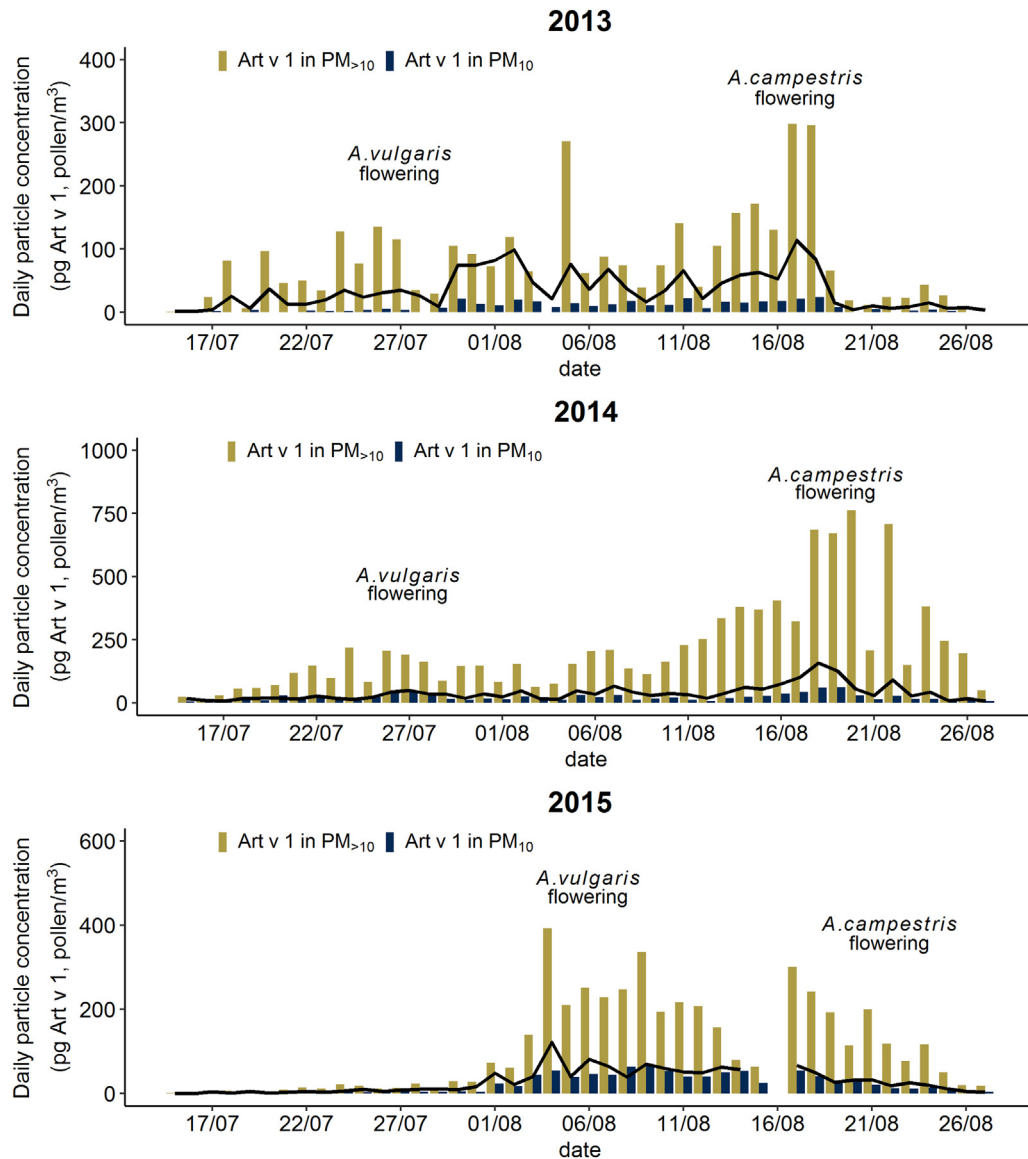


Fig. 3. Temporal variations in the Art v 1 level in two air fractions and *Artemisia* pollen grains (solid black line) recorded in Poznań, Poland (2013–2015).

was significantly higher than that in 2013, i.e., 3.7 ($p = 0.0006$) and 4.7 pg Art v 1/pollen ($p = 0.0001$) (in 2015 and 2014, respectively) (Fig. 2). When all investigated days were considered, the daily allergenicity of *Artemisia* pollen grains varied from 0.9 to 14.1 pg Art v 1/pollen (Table 1). The median allergenicity of *Artemisia* pollen during all three years was higher during the second part of the pollen season than during the first part. The most distinct differences were observed in 2014, when the median daily level of Art v 1 released per pollen grain reached 3.8 pg during the first peak and 6.7 pg during the second peak ($p = 0.0007$). In the other two seasons, the pollen allergenicity during the second peak was 65.3% ($p = 0.031$, year 2015) and 46.8% ($p = 0.192$, year 2013) higher than that in the first part of the seasons (Fig. 2). Overall, the allergenicity of *Artemisia* pollen during the second peak was 63% higher than during the first peak. Therefore, the species-specific allergenicity “correction factor” (f_c) for *A. campestris* was 1.63 (Fig. 5).

3.3. Impact of weather conditions on daily pollen and Art v 1 levels

The strength and direction of the correlations between the selected weather conditions and daily pollen and Art v 1 levels varied between seasons and peaks. Some statistically significant ($p < 0.05$) correlations were observed, e.g., positive relations between Art v 1 in both air fractions and daily temperatures (during the first peak in 2015) and a positive correlation between pollen and Art v 1 and wind speed (during the second peak in 2014). In contrast, wind speed was negatively correlated ($r = -0.567$) with Art v 1 (in $PM_{>10}$) during the first peak in 2013

(Fig. 6). Polar plots showed that the highest concentrations of Art v 1 during the second peak were recorded when winds were coming from the southwest direction (in 2013 and 2014). In 2015, the Art v 1 levels were the highest when air masses were arriving from the east. With respect to the allergen level during the first peak, no clear relationship between the Art v 1 level and wind direction was observed (Fig. 7).

Similarly, no clear tendencies were noted between *Artemisia* pollen allergenicity, the Art v 1 ratio (Art v 1 in PM_{10} to $PM_{>10}$ air fractions) and meteorological parameters. The only statistically significant correlation was recorded between pollen allergenicity during the first peak and daily mean temperature ($r = 0.881$) (Fig. 6). The highest pollen allergenicity was recorded when air masses came from the southeast (during the first peak) and west (during the second peak). In both peaks, the highest Art v 1 ratio (PM_{10} to $PM_{>10}$ air fractions) was observed when the wind was blowing from the west (Fig. 7).

4. Discussion

4.1. *Artemisia* pollen allergenicity and Art v 1 distribution

During the studied period (2013–2015), the median seasonal allergenicity of *Artemisia* pollen varied from 2.5 to 4.7 pg Art v 1/pollen (in 2013 and 2014, respectively). In addition, five- to ten-fold differences in the daily pollen allergenicity were observed, which concurred with previous studies in which pollen allergens of birch (Buters et al., 2010; Buters et al., 2012), grass (Buters et al., 2015), ragweed (Grewling

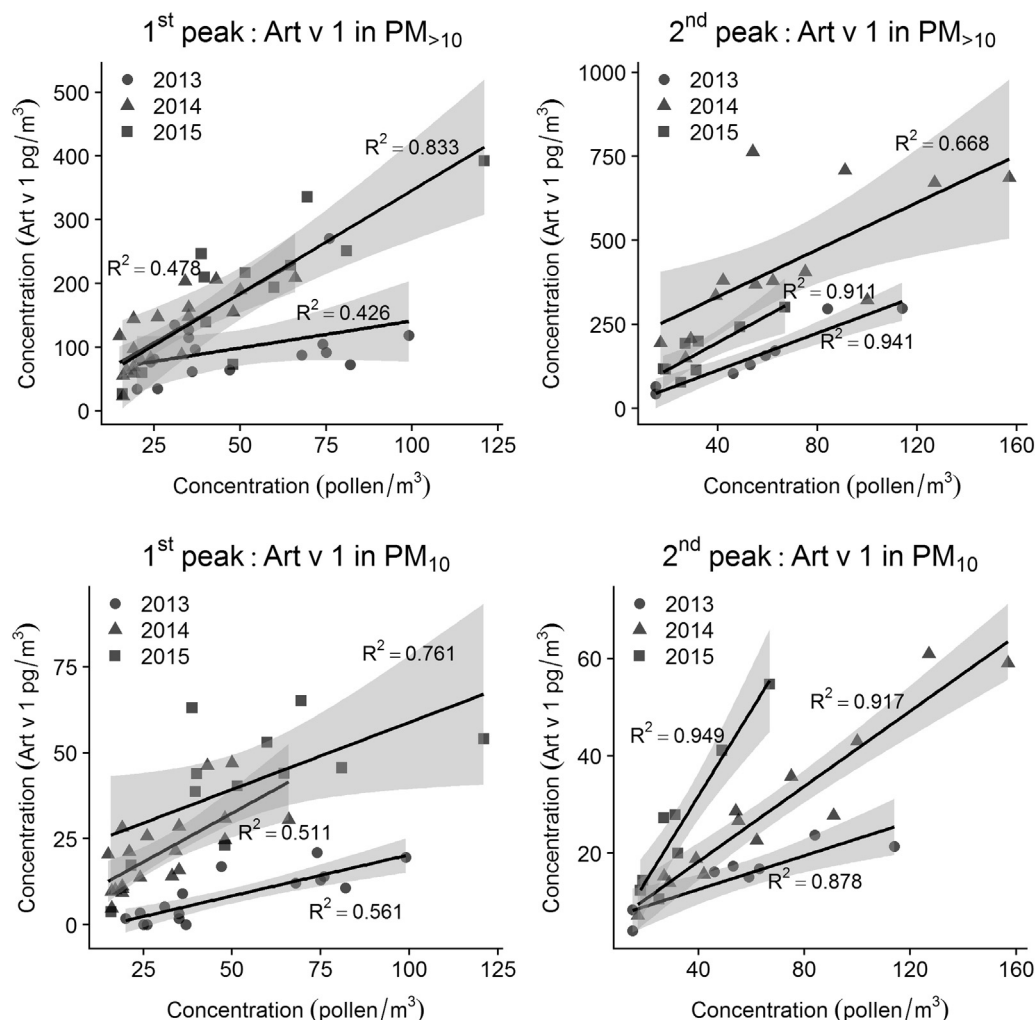


Fig. 4. Correlations between daily concentrations of Art v 1 and *Artemisia* pollen recorded during the investigated seasons (gray area represents the 95% confidence intervals).

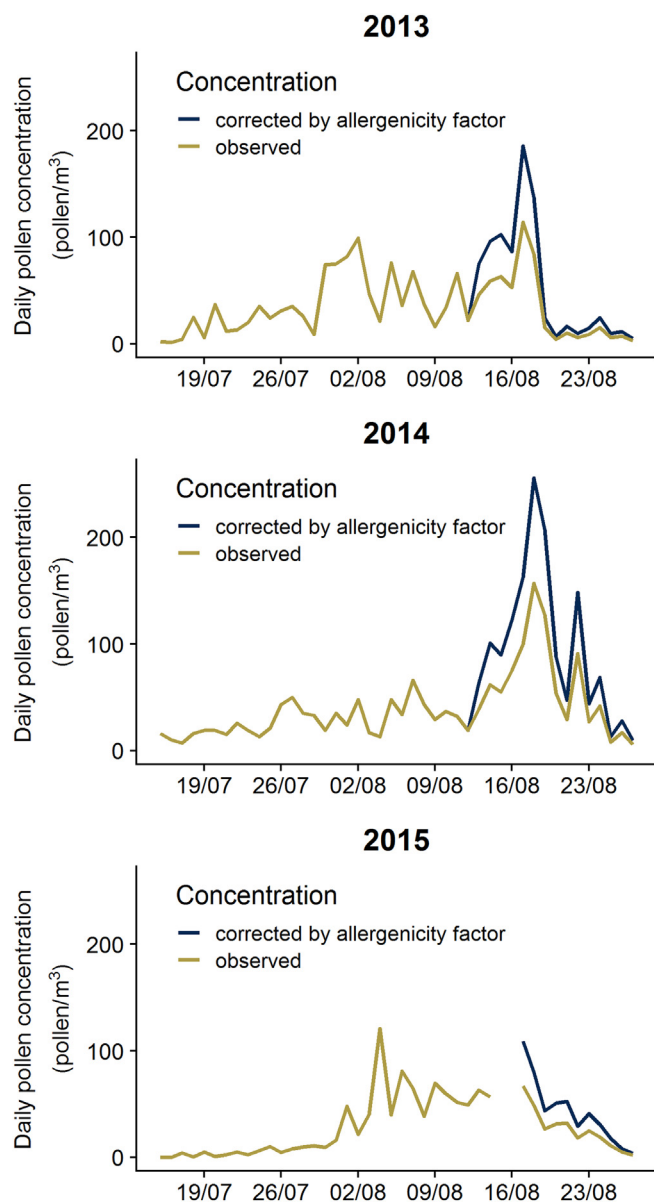


Fig. 5. Mean daily *Artemisia* pollen levels (observed value) and corrected by allergenicity factor ($f_c = 1.63$). Note: due to pollen trap failure, no pollen data are available for 15 and 16 August 2016.

et al., 2016a) and olive (Galán et al., 2013) were investigated. Interestingly, in our study, distinct increases (from 46.8 to 77.7%) in the Art v 1 amount per pollen grain were observed during the second part of the *Artemisia* pollen season (second fortnight of August). In Poznań, *Artemisia campestris* is predominantly flowering during this period and is the major contributor of pollen (Grewling et al., 2015; Bogawski et al., 2016). In contrast, flowering of *A. vulgaris* (a common *Artemisia* species in the studied area) is limited to the end of July and beginning of August, i.e., period with distinctly low pollen allergenicity. The cross-reactivity experiments conducted by Katial et al. (1997) on nine *Artemisia* species revealed that, although most of the species shared similar pollen allergens, their relative concentration in pollen differed. For instance, *A. tridentata* (species native to western North America) was one of the strongest inhibitors (suggesting high allergen concentrations), while *A. ludoviciana*, which is widespread across North America, showed relatively poor inhibitory activity (presumably due to the low concentrations of relevant allergens). In a recent study (Pablos et al., 2019), *Artemisia ludoviciana* (as well as *A. californica*) presented significantly lower IgE reactivity than five other *Artemisia* species (including

A. vulgaris). In addition, in another *Artemisia* allergen study, Brandys et al. (1993) revealed that *A. campestris* showed a larger number of more intensively stained IgE-binding bands than *A. vulgaris*, and the pollen grains of these two species differed in relative contents of total protein and carbohydrate. Moreover, different *Artemisia* species may differ not only in allergen content but also in the location of the major allergen in pollen, what, in turn, may impact on the release mechanism of allergens from pollen grains (Hoidn et al., 2005; Gao et al., 2019). For instance, the location of the Art v 2 homologous protein varied across *Artemisia* species, being intracellular around nuclei for *A. annua* and *A. argyi*, and in both the pollen wall and around nuclei for *A. capilaris* and *A. sieversiana* (Gao et al., 2019).

Considering the Art v 1 size distribution, the highest Art v 1 level was detected in the PM₁₀ air fraction. The seasonal amount of Art v 1 in the subpollen (2.5–10 µm) air fraction accounted for 9.4, 10.2, and 20.5% of the total Art v 1 (in 2013, 2014 and 2014, respectively). This tendency concurs with that revealed in other studies in which the ChemVol cascade impactor was used to separate airborne pollen allergens (Buters et al., 2010; Buters et al., 2012; Galán et al., 2013; Grewling et al., 2016a). The daily amount of Art v 1 in the PM₁₀ and PM₁₀ air fractions followed the concentration of *Artemisia* pollen in the air. During days with high pollen levels, high concentrations of allergens were observed, and vice versa. This phenomenon was commonly described for many other allergenic plant taxa, such as birch (Spieksma and Nikkels, 1999; Buters et al., 2012), grasses (De Linares et al., 2010), pellitory (Rodríguez-Rajo et al., 2011), plantain (Alcazar et al., 2015), and ragweed (Grewling et al., 2016a; Celenk, 2019), indicating that the main source of allergens are intact pollen grains. Intriguingly, the correlations between *Artemisia* pollen and Art v 1 were weaker during the flowering of *A. vulgaris* ($r = -0.600$) than *A. campestris* ($r = -0.850$). We suspect that this difference could be related to the higher ecological diversity of eurytopic *A. vulgaris* than stenotopic *A. campestris*, which predominantly occupied only one type of habitat, i.e., dry sandy soils. In the studied area, the distribution of *A. vulgaris* is much more heterogeneous than that of *A. campestris*, which grows in only a few stands (Fig. 1). Presumably, the higher ecological diversity of *A. vulgaris* populations (occupying various habitats) results in the production of pollen with a more differentiated content of allergens. This hypothesis could be tested experimentally by comparing the pollen allergen content between *Artemisia* populations cultivated under controlled growing conditions (e.g. in plant growth chamber) with different environmental treatment conditions, with respect to such parameters as air temperature, humidity, soil type etc. Such experimental analysis was however outside the scope of this study.

4.2. Impact of weather conditions on Art v 1 content

The highest *Artemisia* pollen allergenicity was observed during the season with the highest daily sum of rain (146.6 mm in the July–August period), while the lowest pollen allergenicity was recorded during the driest season (82.6 mm). It has been previously shown (Stach et al., 2007) that a high amount of rain positively affects the number of *Artemisia* pollen grains that are produced, while high temperatures limit the production of *Artemisia* pollen (Bogawski et al., 2014). We suspect that environmental factors, such as water availability, may affect not only the production of *Artemisia* pollen but also its allergenicity. The role of environmental conditions (e.g., weather and pollution) in the production of pollen allergens has been documented for many plant species (Ahlholm et al., 1998; Aina et al., 2010; Gnani et al., 2012; Beck et al., 2013; Garcia-Gallardo et al., 2013). For instance, Ghiani et al. (2016) suggested that the allergenicity of *Ambrosia* pollen (an allergenic plant belonging to the same family as *Artemisia*) depends on the environmental conditions during plant growth and flowering, which regulate the total protein content. Recently, it has been found that pollen grains released by *Ambrosia artemisiifolia* showed increased allergenicity when the plants were cultivated at high air temperatures



Fig. 6. Spearman correlation coefficients between the daily concentrations of Art v 1 (in PM_{10} , PM_{10} and total) and *Artemisia* pollen, daily pollen allergenicity level, Art v 1 ratio (PM_{10} to PM_{10}) and selected weather conditions. Statistically significant correlations are bold.

(Gentili et al., 2019) and low soil pH (Gentili et al., 2018). There is also evidence that air pollution may impact *Artemisia* pollen by alternating the structures and concentrations of proteins (Depciuch et al., 2016). Art v 1 is a glycoprotein that displays a high homology to plant defensins (Himly et al., 2003). This protein family shows strong antifungal and antibacterial activity (Stotz et al., 2009). Interestingly, Oteros et al. (2019) revealed that *Artemisia* pollen grains are the main vector for bacterial endotoxins. It is therefore tempting to hypothesize that increased Art v 1 production could also be caused by both bacterial occurrence as well as weather conditions favoring bacterial reproduction and later colonization of *Artemisia* pollen grains.

In contrast to studies by Miguel et al. (2006) and Buters et al. (2015), no direct effect of increased relative humidity on increased levels of Art v 1 in the subpollen fraction (2.5–10 μm) was observed in our study. In fact, the highest level of Art v 1 in the 2.5–10 μm air fraction (relative to PM_{10} air fraction) was detected during the period with the highest daily air temperature and the lowest relative humidity, i.e., August 2015. This observation suggests that a high ratio of Art v 1 (PM_{10} to PM_{10}) cannot be linked to pollen grains bursting due to high humidity (Taylor et al., 2004). In this context, it is worth adding that *Artemisia* pollen has a thick wall (up to 5 μm) (Rowley et al., 1981), and the bursting phenomenon of *Artemisia* pollen has not been documented thus far. In addition, orbicules that are considered to be a source of subpollen allergens in some allergenic species (El-Ghazaly, 1999; Vinckier et al., 2005; Ruggiero and Bedini, 2018) do not, however, occur in *Artemisia* (Vinckier and Smets, 2001). We therefore suspect that the high level of Art v 1 in the 2.5–10 μm air fraction was related to the increased deposition of “shrunk” pollen grains on the lower filter due to the hot and dry weather. It has been shown that pollen grains very easily lose water and become dry and desiccate when they become airborne (Gilissen, 1977). The desiccation of airborne pollen grains is a function of air temperature, relative humidity and time (Gilissen, 1977; Fonseca and Westgate, 2005). Due to this process, the shapes of pollen grains change from circular to oval, and the presence of axially elongated apertures (such as in *Artemisia* pollen) is critical for achieving this folding pattern (Katifori et al., 2010). During folding of tricolpate pollen the polar length slightly increase, but the main changes concern

equatorial diameter, that may be additionally reduced by 25–30%. As a result, at high temperatures and low humidity, the diameter of airborne pollen grains decreases (Pacini and Dolferus, 2016), e.g., as shown for *Juniperus ashei*, *Lilium longiflorum* and *Euphorbia milii* pollen grains (Katifori et al., 2010; Bunderson and Levett, 2015). Desiccated smaller *Artemisia* pollen grains (presumably, with reduced equatorial diameter to around 12–13 μm) may therefore pass more easily through the upper PM_{10} filter and be deposited on the lower filter. It should be stressed that aerodynamic diameter of pollen (that determines the deposition on filters) is often lower (by 5–10%) than physical dimensions of pollen grains (Hirose and Osada, 2016). Indeed, during our analysis, we observed *Artemisia* pollen grains deposited on the PM_{10} filter (Supplementary Materials, Fig. S3). The incomplete separation of particles by a cascade impactor that resulted in pollen deposition on a PM_{10} air fraction filter was also claimed by Buters et al. (2010).

Our study reveals a distinct relationship between wind direction and the relative level of subpollen Art v 1. The highest amount of Art v 1 in the subpollen fraction was recorded when winds were blowing from the east, i.e., area dominated by forests and university buildings. These structures may potentially serve as physical obstacles that cause damage to transported pollen grains. Such a phenomenon was also observed by Visez et al. (2015), who noted that due to mechanical shock (e.g., contact with obstacles), pollen grains become damaged and release subpollen particles (1–2.5 μm). The impact of wind direction on the total Art v 1 level was also clearly observed. For instance, in 2015, very low concentrations of allergens (and pollen) were recorded during the second peak. In this period, winds were predominantly coming from the northeast, i.e., areas where populations of *A. campestris* do not occur at high densities. In previous seasons (2013 and 2014), characterized by intensive second peaks of pollen, the air masses were mainly from the southeast, where the main stands of *A. campestris* are located. It should be however stressed that this analysis were conducted with relatively low number of cases (see Fig. S2) therefore the results could be biased. As previous studies showed (Spijksma et al., 2000; Grewling et al., 2015) the local sources have the highest impact on *Artemisia* pollen exposure. For instance, Spijksma et al. (2000) reported that cutting dense *Artemisia* stands located within 100 m from pollen trap markedly

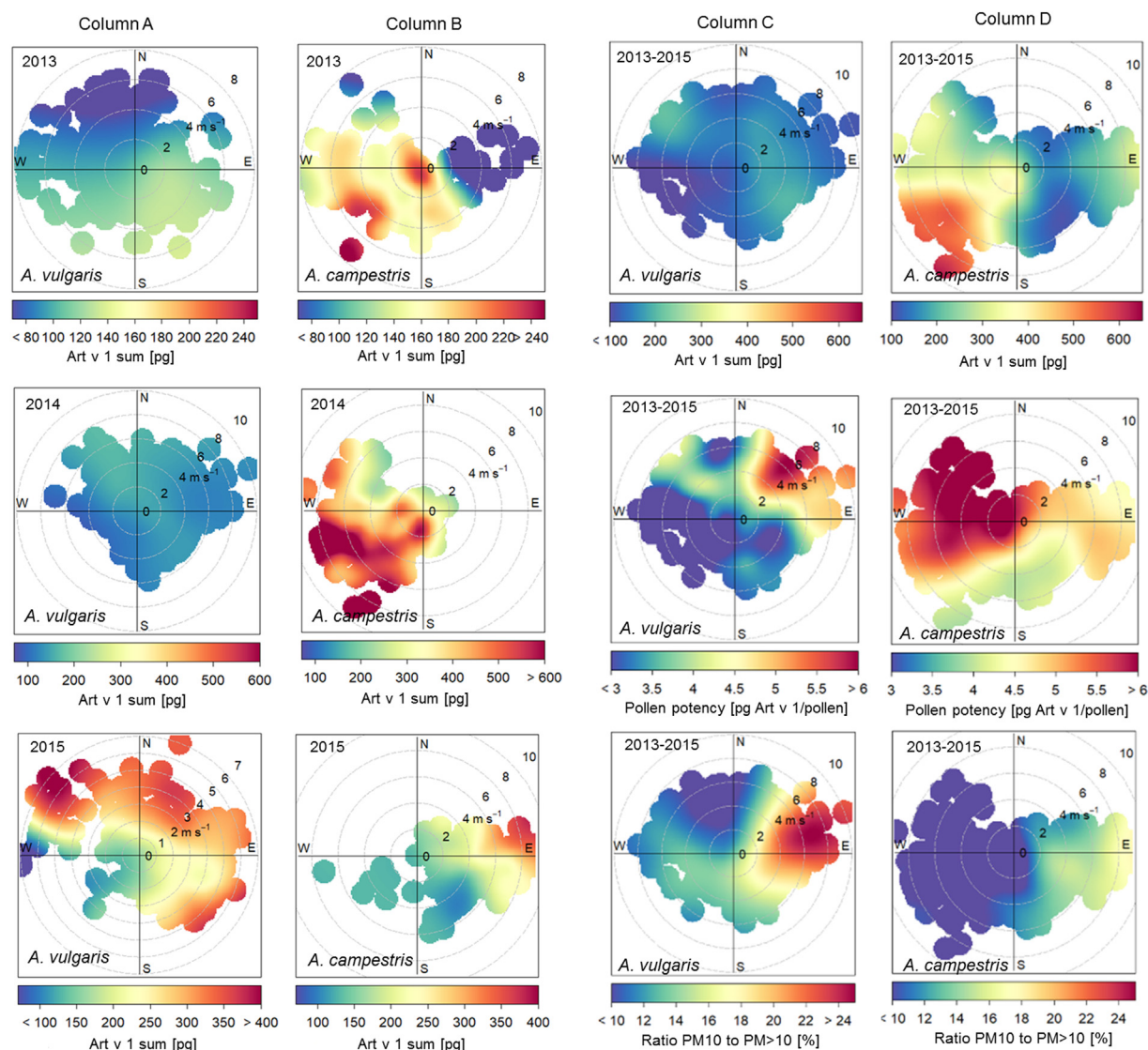


Fig. 7. Polar plots showing the relations between wind speed and direction and: Column A) daily Art v 1 levels in every *A. vulgaris* pollen season (2013, 2014, and 2015), Column B) daily Art v 1 levels in every *A. campestris* pollen season (2013, 2014, and 2015), Column C) mean (2013–2015) daily Art v 1 levels, pollen potency and ratio (PM₁₀ to PM_{>10}) during *A. vulgaris* pollen season, Column D) mean (2013–2015) daily Art v 1 levels, pollen potency and ratio (PM₁₀ to PM_{>10}) during *A. campestris* pollen season.

decreased the pollen concentration in the air. Also, Skjøth et al. (2013) estimated that the majority of pollen released by anemophilous weeds are deposited within 500 m from the source plant. However, in certain atmospheric situation *Artemisia* pollen could also be transported long distances (Qin et al., 2017).

4.3. Clinical implications

Our findings suggest that pollen grains released by two common *Artemisia* species that are phenologically distinct also differ in allergen content. Thus, although pollen grains of different *Artemisia* species showed high cross-reactivity (Brandys et al., 1993; Katial et al., 1997), pollen grains of *A. vulgaris* and *A. campestris* are not equal from an allergic point of view. This difference may partially explain why it is difficult to determine the exact concentration of pollen lead to allergic symptoms in individuals, which is the so-called threshold value (Bergmann et al., 2012; Pfaar et al., 2017). Currently, the only available method to relate symptoms with exposure to pollen allergens remains a comparison with airborne pollen grains/m³ (airborne allergen monitoring is still too expensive and time consuming and not adjusted for real-time measurements) (Pfaar et al., 2017). However, as Bergmann et al. (2012)

stressed, the threshold values can be calculated only by combining symptom severity with the actual exposure to the individual pollen type. Our study, as well as several others, where taxon-specific pollen allergenicity was determined (Alche et al., 2007; Buters et al., 2015; Jung et al., 2018), may help to achieve this important goal. Introduction of pollen allergenicity data into pollen-based exposure models certainly improves model accuracy. For instance, in Fig. 5, we showed the daily *Artemisia* pollen levels in Poznań with a “correction factor” (here, $f = 1.63$) that considers the average differences in median pollen allergenicity between *A. vulgaris* and *A. campestris* (obtained in this study from 2013 to 2015). Although this is a very simple approach (e.g., it does not consider the diurnal variation in pollen allergenicity), we believe it better expresses the real risk to *Artemisia* allergens than models solely based on pollen grains. Importantly, this approach can be easily included in standard forecasting models.

The further refinement of statistical models based on pollen data will, however, require additional information, e.g., species abundance and distribution, flowering periodicity and pollen productivity (Bogawski et al., 2016; McInnes et al., 2017). With respect to *Artemisia*, such information is still very scarce, but fortunately some progress has been observed in recent years. For instance, a detailed map of the

distribution ranges of twelve *Artemisia* species has been prepared for the Iberian Peninsula and Canary Islands (Sainz et al., 2017). In Great Britain, the abundance of *Artemisia* sp., although without species recognition, has been presented on a high-resolution spatial scale (1 km × 1 km grids) (McInnes et al., 2017). Additionally, Bogawski et al. (2016) determined the abundance, pollen production and flowering variations of three *Artemisia* species in Poznań (Poland). Finally, pollen DNA metabarcoding data could potentially be used to precisely determine the taxonomic composition of pollen exposure (Bell et al., 2019; Brennan et al., 2019).

5. Conclusions

Our study showed distinct variations in *Artemisia* pollen allergenicity, i.e., the amount of the major allergen, Art v 1, per pollen grain. These differences were not only related to daily and seasonal variability, which may exceed 800% and 80%, respectively but also noticeable when two common *Artemisia* species were compared. We calculated that during the second peak, which was related to the flowering of *A. campestris*, pollen allergenicity was on average 63% higher than during the beginning of the pollen season when *A. vulgaris* pollinates. These two species differ not only in phenology but also in distribution, abundance and pollen production; therefore, they may affect sensitive subjects with different intensities. We suspect that in other parts of the world that are dominated by various *Artemisia* species, these differences could be even higher. Thus, information about species-specific pollen allergenicity could be highly useful in allergy prophylaxis and treatment. It would be wise to include such data into standard forecasting models (e.g., by introduction of “allergenicity factor”) to more correctly express the real allergen exposure in certain areas.

Declaration of competing interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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Appendix A. Supplementary data

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Supplementary Materials

Atmospheric exposure to the major Artemisia pollen allergen (Art v 1): seasonality, impact of weather, and clinical implications.

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Figure S1. Pollen grains of three common *Artemisia* species under light microscope (scale bar: 20µm).

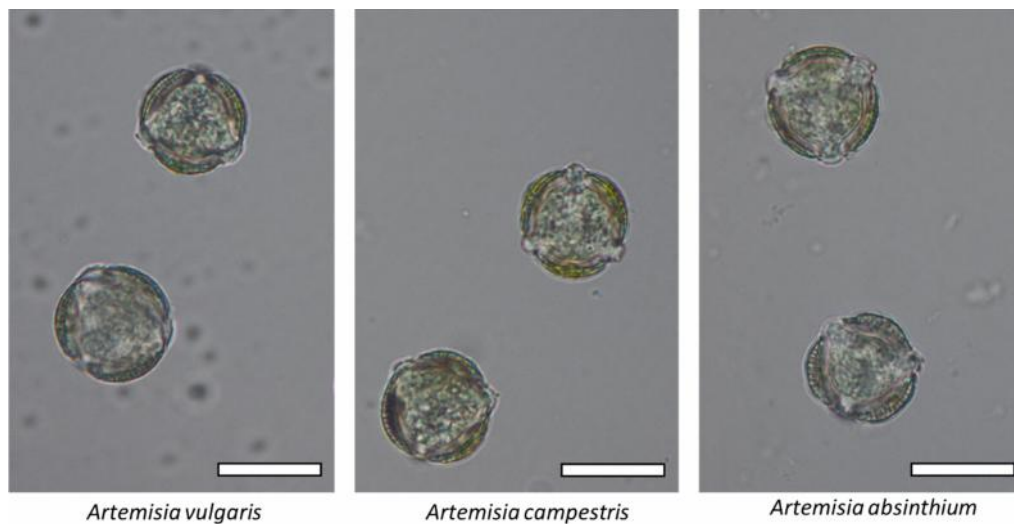


Figure S2. Division of *Artemisia* pollen season into two peaks. Shaded area showed “5-days buffer period”, that were excluded from statistical analysis. Note lower pollen concentration during second peak in city center.

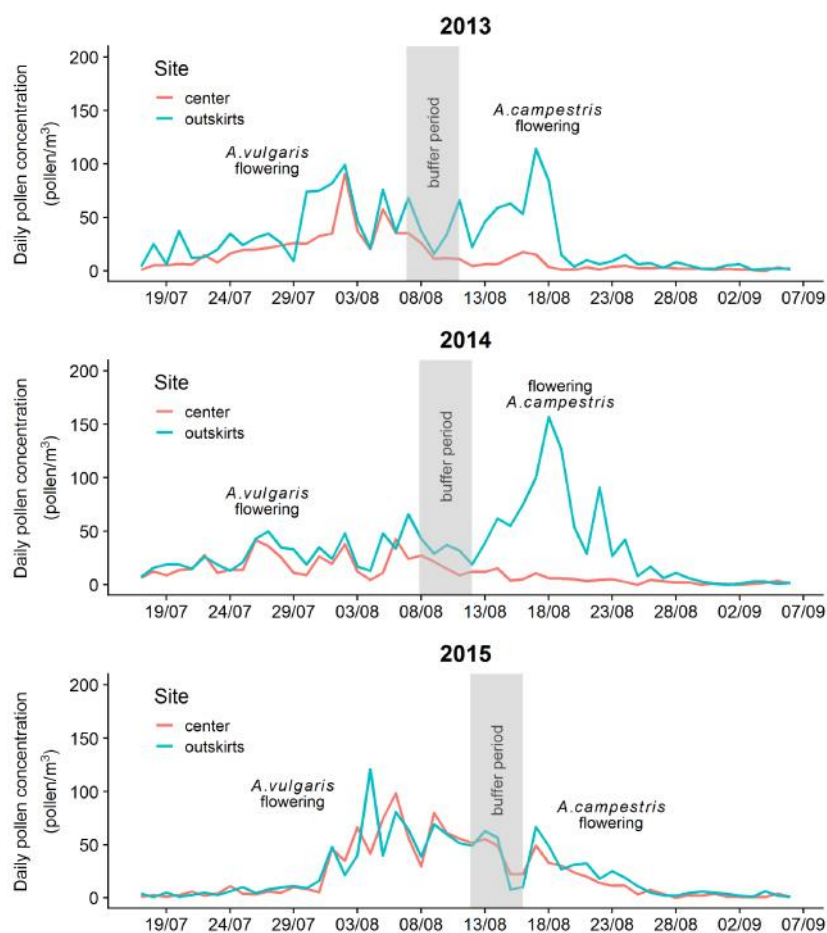


Figure S3. *Artemisia* pollen grain deposited and extracted from lower filter (2nd stage, 2.5-10µm).

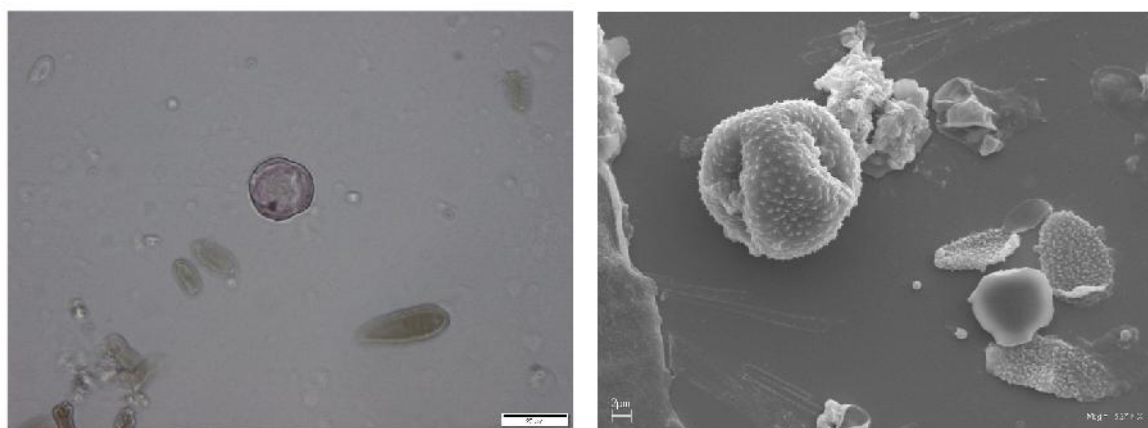


Table S1. Contribution of *Artemisia* pollen collected during selected periods, i.e. 15 July and 27 August, to the total *Artemisia* pollen sum recorded during the whole year.

Parameter	2013	2014	2015
Pollen collected during selected period	1526	1713	1205
Pollen collected during the whole year	1597	1802	1264
Percentage (%)	95.6%	95.1%	95.3%

Table S2. Weather conditions between July-August during studied years in comparison to 10-year average (2003-2012).

Season	Month	Temp mean [°C]	Temp max [°C]	Temp min [°C]	Humidity [%]	Wind speed [mm]	Rain [mm]
2013	July	20.6	26.2	14.7	66.6	3.0	44.9
	August	19.6	25.3	14.2	67.6	2.7	37.7
2014	July	22.1	27.9	16.4	64.6	3.2	89.8
	August	18.0	23.3	13.1	70.2	2.8	56.8
2015	July	19.7	25.6	14.5	61.1	3.5	88.8
	August	22.7	29.4	16.1	52.3	3.4	25.5
2003- 2012	July	20.0	25.6	14.3	68.2	3.3	89.3
	August	18.9	24.4	13.2	70.7	3.2	65.1