



Comparison of *Ambrosia* L. Pollen Seasons in Lublin (Poland) and Ivano-Frankivsk (Ukraine) and Presentation of the Morphotypes of Trichomes on *A. artemisiifolia* L. Shoots in Terms of the Allergenic Properties of the Plant

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Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). **Abstract:** *Ambrosia* pollen contains strong allergens. Allergic reactions can also be caused by direct contact with the plant. The investigations of the dynamics of *Ambrosia* pollen seasons were conducted in Lublin (Poland) and Ivano-Frankivsk (Ukraine) in 2013–2015. The onset and end of the seasons, maximum concentrations, annual sums, and the number of days with an allergy risk were determined. Additionally, the types of trichomes present on different parts of *Ambrosia artemisiifolia* L. shoots were determined using light microscopy. Morphometric studies were carried out on trichomes sampled from staminate inflorescences. The maximum concentrations and annual sums of *Ambrosia* pollen were shown to be substantially higher in Ivano-Frankivsk than in Lublin. Similarly, the risk of allergies is higher in the study site in Ukraine. The study results indicate that the presence of *Ambrosia* pollen grains in Lublin may be associated with long-distance transport. The presence of non-glandular and glandular trichomes was found on the examined organs. The staminate inflorescences were covered by two types of non-glandular trichomes (short and long) and two types of glandular trichomes (linear and biseriate), whose secretory product can cause dermatitis in sensitive subjects upon contact with the plant.

Keywords: ragweed; pollen grains; concentrations; trichomes; allergy; Poland; Ukraine

1. Introduction

Ambrosia artemisiifolia L. (ragweed), i.e., a native North American species, was introduced in many European countries with cereal grains in the 19th century [1,2]. In Poland, it grows in dispersed localities, with the highest concentration in the south of the country [3–5]. *Ambrosia* is an anemophilous species, and its pollen grains are present in the air from August to October [6,7]. *Ambrosia* pollen present in the air of Poland originates from local plants or from long-distance transport, mainly from the south [8,9] and the east [10,11].

Symptoms of allergy caused by pollen of such plants as *A. artemisiifolia* include rhinitis, watery eyes, allergic conjunctivitis, cough, asthma, and rash [12,13]. Pollen concentration, location, date in the season, and environmental pollutants are factors that increase the risk of allergy, i.e., exposure of hypersensitive subjects to pollen allergy [14].

The pollen of *Ambrosia* contains strong allergens, and even its small concentration in the air may trigger allergic reactions. In studies conducted in France, Thibaudon [15] concluded that even one pollen grain/m³/24 h is a threshold value for the occurrence of allergies triggered by *Ambrosia* pollen. As shown by Dechamp et al. [16], allergy symptoms



can be induced by *Ambrosia* pollen in the amount of 5 grains per 1 m³ of air. Various investigations have revealed an upward trend in the sensitivity to *Ambrosia* pollen and in the occurrence of allergies in recent years in Europe [17]. According to IgE (immunoglobulin E) reactivity, among the 11 allergens that have been described in *A. artemisiifolia*, *Amb a* 1, and *Amb a* 11 seem to be major allergens [18].

The harmful impact of *Ambrosia* species on the human organism is exerted not only by their allergenic pollen but also by direct contact with the plants, which may cause atopic and contact dermatitis in susceptible subjects [19].

In many European countries, *Ambrosia artemisiifolia* is an invasive plant [20–27]. In Poland, *A. artemisiifolia* has been classified into category W4 as a highly expansive high-risk species, which is widely distributed in the natural environment. The degree of invasiveness of the plant was estimated as very high [3]. In France [28], Austria [29], Hungary [30], Romania [31], and other European countries, *A. artemisiifolia* is mainly a nuisance weed in crop fields, meadows, pastures, orchards, and vineyards. However, it is the commonest along waterways, roads, railways, and wasteland. Its presence significantly reduces yields and impedes the harvest of seeds, mainly cereals [32], bean [33], soya [34], and sunflower [30]. This species produces a large number of long-lived seeds [35]. One plant can produce 30,000–40,000 seeds (up to 100,000), which are viable for 5–14 years [36]. *Ambrosia* has been classified as one of the most dangerous invasive alien species in Europe, as it spreads quickly, exhibits resistance to certain herbicides, and does not have fully effective natural enemies [37].

The most effective method of controlling the spread of roadside populations of *A. artemisiifolia* is to mow the plants shortly before the development of male flowers in order to limit the amount of released pollen, followed by subsequent mowing before the appearance of new flowers on the developing side shoots [27]. In turn, in agricultural habitats, the species can be managed by shallow tillage and subsequent management of emerging seedlings to reduce the soil seed bank [38]. High hopes are raised by the biological control agent *Ophraella communa*, LeSage which can reduce *A. artemisiifolia* pollen production by 82% [39,40].

Ambrosia artemisiifolia is an annual 60 cm high plant. It has double- or triple-pinnate leaves and a hairy stem. Staminate and pistillate flowers are gathered in capitula [2,41]. The staminate capitulum comprises on average 25 flowers, whereas the pistillate capitulum has only one flower. The staminate capitula form spikes at the apical part of the shoots [42]. The staminate capitulum involucre is completely fused and sub-glabrous or hairy [2]. One staminate flower produces on average 16,875 pollen grains [42].

Species of the genus *Ambrosia* contain biologically active substances, which have been used in medicine. Triterpenoids present in *Ambrosia* species have been shown to be active against *Trypanosoma* [43]. In Eastern Europe, *A. artemisiifolia* has been applied in folk medicine as a medicinal plant with anti-inflammatory, antibacterial, and antiviral properties [3]. Herbicidal effects of sesquiterpene derivatives from *A. artemisiifolia* have been demonstrated as well [44].

The induction of contact allergies may be associated with the presence of trichomes on the plant, as glandular trichomes are the site of the production of bioactive secondary metabolites [45–47]. Therefore, in addition to the analysis of the airborne concentrations of *A. artemisiifolia* pollen, which is responsible for inhalation allergies, the types of trichomes present on the aboveground *A. artemisiifolia* organs were investigated.

The aim of the study was to compare the course of the *Ambrosia* pollen seasons in Lublin (Poland) and Ivano-Frankivsk (Ukraine), i.e., their onset and end dates, duration, pollen concentration in the air, and annual sums. Due to the allergenic properties of *A. artemisiifolia* aboveground organs, microscopic analyses were performed to determine the types of trichomes present on these organs. The detailed studies of the trichome morphotypes were carried out in the staminate flower perianth and in capitulum involucres present in these flowers, as staminate inflorescences constitute an important well-exposed part of plants with a characteristic appearance encouraging contact with their shoots.

2. Materials and Methods

2.1. Aerobiological Analyses

The research was conducted in Lublin and Ivano-Frankivsk cities from 2013 to 2015. Lublin is located in Lublin Upland in central-eastern Poland. The climate of this region is influenced by continental air masses. The average annual air temperature in Lublin (1951–2007) is 8.2 °C. January is the coldest month of the year (average temperature -2.8 °C), and July is the warmest month (average temperature 17.8 °C). The mean annual precipitation sum is 546 mm [48,49]. Currently, no representatives of the genus *Ambrosia* can be found in the city.

Ivano-Frankivsk ($24^{\circ}42'$ E and $48^{\circ}55'$ N, 260 m a.s.l.) is a city located on the Pokut plain in the southwest of Ukraine between the Bystritsa Nadvirnyanska and Bystritsa Solotvynska Rivers. The climate of Ivano-Frankivsk is temperate continental. The average temperature is 17–19 °C in July and -5 °C in January. The average humidity is 68–70%, and the average annual rainfall is 600–700 mm. From a landscape perspective, the city is considered an urbanized territorial complex. It is part of the natural landscape heavily transformed by buildings, communication infrastructures, and other urban elements.

Since it is characterized by rapid spread and is regularly found on contaminated seed lots, forage, and fodder, A. artemisiifolia is a quarantine species in Ukraine. This taxon requires special attention and control as well as the activation of an early warning system, as it may pose a threat in other European countries and other parts of the world. In 2000, A. artemisiifolia was added to the EPPO (European and Mediterranean Plant Protection Organization) Alert List covering all quarantine plant species [50]. In Ivano-Frankivsk, the highest density of the plants (50–100 per 1 m²) is recorded in the northern and western part of the city near railway stations and roadside lanes. It is rare in the central part of the city. The distance between the pollen monitoring station and city areas with a high accumulation of A. artemisiifolia plants is 4-6 km. In 2017, the species was present in an area of 0.52 ha in Ivano-Frankivsk and 10.8 ha in the nearest region. In Lublin, pollen concentrations were measured with the volumetric method using a Hirst-type sampler (Lanzoni VPPS 2000, LANZONI srl. Bologna, Italy). The pollen monitoring station was located near the city center (22°32′25″ E and 51°14′37″ N; 197 m a.s.l.). The analyses were carried out in accordance with the International Association for Aerobiology (IAA) and the European Aerobiology Society (EAS) standards [51–53]. As specified by the requirements, the sampler must be placed on a flat horizontal surface, preferably on the roof of a building, away from its edge, in order to reduce the effect of turbulence. It is recommended to locate the sampler at the height of 15 to 30 m. The pollen sampler was installed in Lublin on a flat roof of a building at the height of 18 m above ground level. The device simulates the work of the human respiratory system. The air and bioaerosol were sucked onto a transparent tape wound on a drum moved by a clock mechanism. The constant airflow was 10 L/min. Biological and inorganic particles contained in the air settled on the tape covered with a sticky adhesive. After one week exposure, microscope slides were prepared. The tape was cut into lengths corresponding to 24 h, which were placed on slides coated with glycerogelatin and then covered with glycerogelatin-coated coverslips. Pollen grains were counted with the method of 4 horizontal stripes using a light microscope Nikon YS 100 (Nikon, Tokyo, Japan) (×400 magnification).

In Ivano-Frankivsk, the gravimetric method was employed for pollen collection using a Durham trap that was installed at a height of 24 m above the ground. Glycerin-lubricated slides were replaced daily from early February to mid-October. The pollen grains were counted using an Olympus CX-300 (Olympus, Tokyo, Japan) light microscope (×400 magnification) by continuous vertical transects (the analyzed area of the glass was 11.52 cm²). To facilitate the comparison of the data obtained with the different methods, the results of the gravimetric method were recalculated using the standard factor for *Ambrosia* as recommended by Basett et al. [54].

The pollen concentration in both sites was expressed as the daily average number of pollen grains in 1 m³ of air per 24 h (P/m^3). The length of the pollen seasons was

calculated with the 98% method with days on which 1 and 99% of the annual pollen sum were recorded and assumed as the onset and end of the season, respectively [55].

The study was focused on the analysis of the dynamics of the occurrence of *A. artemisiifolia* pollen in the air. The pollen seasons were divided into stages corresponding to the cumulative percentages of the annual pollen sum, i.e., 1, 5, 25, 50 (vertical line), 75, 95, and 99% (Figure 1). The graphs depict 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 [56].



Figure 1. 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%, and (7) 95–99%.

The correlation between the pollen concentration and selected meteorological factors was investigated to determine the impact of abiotic factors on the presence of *Ambrosia* pollen in the air. In Lublin, the following meteorological factors were analyzed: average air temperature, relative air humidity, precipitation, cloud cover, and wind speed. In turn, the average air temperature and relative air humidity were taken into account in Ivano-Frankivsk. Spearman correlation coefficients were calculated to show the dependence of the pollen concentration on the weather. The correlation was checked for each year separately and for the three years of the study.

2.2. Analysis of Trichome Morphotypes

Trichomes on the *Ambrosia artemisiifolia* L. shoots were analyzed in 2019 and 2020. Plants growing along railway tracks on the outskirts of Lviv (Ukraine) were collected during the flowering period in the second half of August 2019. Herbarium specimens of these plants were deposited at the Department of Botany and Plant Physiology, University of Life Sciences in Lublin.

Stem and leaf fragments were collected from the middle and upper parts of the plant (n = 5). Capitula with pistillate and staminate flowers were observed under a stereoscopic microscope SMT 800 (Nikon, Tokyo, Japan) coupled with a Nikon Coolpix 4500 camera (Nikon, Tokyo, Japan). The lower and middle parts of staminate inflorescences with trichomes were selected for detailed examination in a light microscope. For this analysis, fresh and fixed (in 70% ethanol) samples of male capitula were used for the preparation of semi-permanent microscopic slides. Cross and tangential sections of the capitulum bracts (n = 10) and perianth (n = 10) of staminate flowers were cut by hand with a razor blade and viewed in water with glycerol (1:1). Trichomes were subjected to morphometric analyses. In total, 30 measurements were made for each type of trichomes. A Nikon Eclipse E-400 (Nikon, Tokyo, Japan) light microscope was used for the observations, measurements, and photographic documentation.

The photographs were taken by Galyna Melnychenko (Figure 8A,B), Agata Konarska (Figure 8C–M), Krystyna Piotrowska-Weryszko (Figure 9), and Elżbieta Weryszko-Chmielewska (Figure 10).

3. Results

3.1. Aerobiological Analyses

The three-year research conducted in Lublin and Ivano-Frankivsk showed a similar date of the onset of the *Ambrosia* pollen season in 2013–2014 in both cities. In turn, the beginning of the pollen season was recorded 17 days earlier in Ivano-Frankivsk in 2015 (Table 1).

	Lublin			Ivano-Frankivsk		
Pollen Season Parameters –	2013	2014	2015	2013	2014	2015
Pollen season period by the 98% method	24.07–29.10	29.07-28.09	05.08–16.10	24.07-26.09	26.07-23.09	19.07–01.10
Season duration/number of days with concentration above zero	98/18	62/45	73/47	65/65	60/59	75/63
Peak value (P/m ³) peak date	5	57	25	25	184	52
	20.08	12.09	16.09	20.08	2.09	28.08
Annual pollen sum	27	450	182	332	783	396
Number of days with concentration \geq threshold 5 P/m^3	1	21	9	23	35	28

Table 1. Characteristics of Ambrosia pollen season in Lublin (Poland) and Ivano-Frankivsk (Ukraine).

The onset of the A. artemisiifolia pollen season was recorded between 24 July (2013) and 5 August (2015) in Lublin and between 19 July (2015) and 26 July (2014) in Ivano-Frankivsk. The pollen seasons in Ivano-Frankivsk were characterized by a compact course, as airborne Ambrosia pollen grains were recorded each or almost every day of the season. In contrast, the pollen seasons in Lublin were discontinuous, i.e., days with the presence or absence of airborne A. artemisiifolia pollen were recorded alternately. In 2013, low concentrations of A. artemisiifolia pollen grains were detected in Lublin, but they were highly dispersed over time, which significantly prolonged the pollen season (Figure 2A). The pollen season calculated using the 98% method was particularly long in Lublin in 2013 (98 days). The beginning of the pollen season was determined when the sum of daily mean concentrations reached 1% of the total sum, while the end of the season was determined by the date when 99% of the total sum was recorded. The first Ambrosia pollen grains appeared relatively early (24 July); however, after that time, there were days when no pollen of this plant was observed, then small amounts of pollen were recorded for 2 or 3 days, and again there was no pollen. The pollen season was discontinuous; as a result, there were only 18 days with the presence of A. artemisiifolia pollen grains. The end of the Ambrosia pollen season in Lublin in 2013 was registered extremely late, i.e., on 29 October. In 2014 and 2015, the A. artemisiifolia pollen seasons in Lublin were similarly discontinuous, but there were substantially higher numbers of days with the presence of pollen of this taxon (Figure 2B,C).

The *A. artemisiifolia* pollen seasons in Ivano-Frankivsk were characterized by substantially greater pollen abundance than in Lublin (Figure 2A–C). The maximum daily pollen concentrations (peak value) were in the range of 25–184 P/m³ in Ivano-Frankivsk and ranged from 5 P/m³ to 57 P/m³ in Lublin. These values in Ivano-Frankivsk were 5-fold higher in 2013, over 3-fold higher in 2014, and over 2-fold higher in 2015 than in Lublin. Moreover, the annual *Ambrosia* pollen sums were over 12-fold higher in 2013 and approximately 2-fold higher in 2014–2015 (Table 1).

The peak date was recorded on the same day (20 August) in both cities only in 2013. In the other years, the maximum daily concentration of *A. artemisiifolia* pollen was noted 10 (2014) or 19 days (2015) later in Lublin than in Ivano-Frankivsk. The seasonal maximum was recorded in the second ten days of September in Lublin and at the end of August or at the beginning of September in Ivano-Frankivsk. The intensity of pollen seasons is also evidenced by the number of days with pollen concentrations exceeding certain threshold values specified by Dechamp et al. (1997). A concentration equal to or exceeding 5 P/m³ was recorded in Lublin on 1–21 days, whereas 23–35 such days were reported from Ivano-Frankivsk. In both cities, the lowest and highest numbers of days with a threshold pollen concentration were recorded in 2013 and 2014, respectively (Table 1). The curves presenting the course of the pollen seasons were multimodal; nevertheless, one clear peak was noted in Ukraine in 2014 and 2015 (Figure 2). In both sites, *Ambrosia* pollen was recorded mainly



in August and September, and only small amounts of pollen were recorded in July and October (Figure 3).

Figure 2. Comparison of *Ambrosia* pollen seasons in Lublin and Ivano-Frankivsk in (**A**) 2013; (**B**) 2014; (**C**) 2015.



Figure 3. Frequency of *Ambrosia* pollen in the air of Lublin and Ivano-Frankivsk in particular months of the year; means for 2013–2015.

The analysis of the data on the annual sums of pollen grains shows a similar trend in the changes in the pollen concentration in both cities (Figure 4). In both study sites, the highest sums of pollen grains were found in 2014, and the lowest amounts were reported in 2013.



Figure 4. Comparison of annual pollen sums for *Ambrosia* in the air of Lublin and Ivano-Frankivsk, 2013–2015.

The dynamics of achievement of the annual pollen sum differed both between the years and between the measurement sites. In 2013, the maximum concentrations were recorded even before the 50% phase: three days earlier in Lublin and one day earlier in Ivano-Frankivsk. In 2014 and 2015, the maximum pollen concentrations and the 50% phase were recorded simultaneously in Ivano-Frankivsk, whereas the seasonal maximum in Lublin was recorded 2–3 days after reaching 50% of the cumulative pollen grain sum. In both localities, the pollen seasons were right-skewed in 2013 and left-skewed in 2014 and 2015. This was particularly evident in Lublin, where the concentration increase was relatively slow and the 50% phase and maximum concentrations were recorded later than in Ivano-Frankivsk (Figure 5).



Figure 5. Dynamics of Ambrosia pollen seasons in 2013-2015 (A) in Lublin; (B) in Ivano-Frankivsk.

In 2013, the stages of *Ambrosia* cumulative pollen grains with percentages from 25% to 75%, constituting the main part of the pollen season, had a similar duration in both cities. This period lasted 23 days and 24 days in Lublin and Ivano-Frankivsk, respectively. Similarly, the main part of the pollen season in 2015 had a similar duration in these cities (22 and 20 days). This indicates that the phase of release of large amounts of *Ambrosia* pollen grains in both years was relatively long and slow. In turn, the duration of stages with percentages from 25 to 75% in 2014 differed significantly in both cities (Figure 5). In Ivano-Frankivsk, this phase lasted only 11 days, which suggests a short and rapid release of large amounts of pollen grains. In Lublin, this stage of the pollen season in 2014 was twice as long as in Ivano-Frankivsk. This present analysis of the dynamics of the *Ambrosia* pollen seasons revealed a significant similarity in the course of their main part in the analyzed cities in 2013 and 2015.

The analysis of the Spearman test shows that, in Lublin, the greatest impact on the concentration of *Ambrosia* pollen over the three years was exerted by the temperature (positive correlation) and relative air humidity (negative correlation) (Table 2). The highest values of the coefficients were obtained in relation to the temperature in 2015 and humidity in 2014 in Lublin. In Ivano-Frankivsk, the greatest impact of the above-mentioned meteorological factors was noted in 2015. The highest concentrations of *Ambrosia* pollen were recorded on days with high air temperature, which is illustrated in Figure 6 for Lublin. High pollen content accompanied low air humidity, which was clearly visible in Ivano-Frankivsk in 2015 (Figure 7).

City	Season	Average Temperature (°C)	Relative Humidity (%)
Lublin	2013	0.28806 **	-0.2225
	2014	0.23593	-0.35029 **
	2015	0.36367 **	-0.18414
	2013–2015	0.32517 **	-0.22546 **
Ivano-Frankivsk	2013	0.10482	-0.21311
	2014	-0.25033	-0.11309
	2015	0.23635 *	-0.29116 *
	2013–2015	0.03794	-0.15981 *

Table 2. Spearman's correlation coefficients between *Ambrosia* pollen concentration and meteorological factors in Lublin (Poland) and Ivano-Frankivsk (Ukraine).

Level of significance * 0.05, ** 0.01.



Figure 6. Course of the Ambrosia pollen season in Lublin in 2015 versus the mean air temperature.

The influence of other weather parameters on the pollen concentration was investigated in Lublin. The statistical analysis showed that the cloud cover and wind speed had no significant effect on the concentration of *Ambrosia* pollen in the air, whereas a relationship with precipitation was found (at the level of 0.05) only in 2013 (R = 0.20965) and 2014 (R = -0.31345).



Figure 7. Course of the *Ambrosia* pollen season in Ivano-Frankivsk in 2015 versus the relative air humidity.

3.2. Morphological Analyses

3.2.1. Distribution of Trichomes on Ambrosia artemisiifolia Shoots

The plant specimens were observed before flowering (Figure 8A) and in the anthesis phase (Figure 8B). All aboveground organs were covered by trichomes. Non-glandular trichomes with varied lengths and two types of glandular trichomes were present on the stems, leaves, and capitulum involucres. In turn, the perianths of the staminate flowers were covered by only one type of glandular trichomes.



Figure 8. Stems, leaves, inflorescences, and pollen grains of *Artemisia artemisiifolia*: (**A**) shoot with inflorescence buds (arrows) in top view; (**B**) flowering plant; (**C**–**F**) leaves on the top part of the shoot; (**C**,**D**) adaxial surface of the leaf blade; (**E**,**F**) fragments of the abaxial leaf surface with numerous non-glandular trichomes; (**G**) fragment of an inflorescence with male capitula; (**H**,**I**) male capitula with an involucre bearing trichomes; (**J**) cluster of capitula with pistillate flowers; (**K**) capitulum with one pistillate flower equipped with two long pistil stigmata; (**L**,**M**) pollen grains with a visible layer of pollenkitt (asterisk) on the surface; Scale bars: **G**–3 mm, **H**–**J**–2 mm, **K**–1 mm, **L**,**M**–10 μ m.

The longest trichomes were found on the top leaves (Figure 8C). The area near the midrib on the adaxial surface of the leaf blade exhibited very long trichomes, whereas substantially shorter trichomes were visible at the lamina margins (Figure 8D). The longest trichomes on the abaxial surface of the leaf blade were observed along the midrib and at the margins of its basal part (Figure 8E). Higher magnifications showed the presence of dense shorter trichomes along the leaf veins (Figure 8F). The largest non-glandular trichomes on the inflorescences were located at the margins of the bracts in the staminate capitula (Figure 8G–I) and in the pistillate inflorescences (Figure 8J,K). Pollen grains were often attached to the surface of the bracts forming white deposits (Figure 8I). The adherence of pollen to the surface is facilitated by a layer of pollenkitt covering the exine (Figure 8L,M).

3.2.2. Diversity of Trichomes in Staminate Inflorescences

Two types of non-glandular trichomes and two types of glandular trichomes were distinguished in the capitulum of the staminate flowers. The images of these trichomes are presented in Figure 9 and their sizes are shown in Table 3.



Figure 9. Different types of trichomes from *Artemisia artemisiifolia* male inflorescences: (**A**–**E**) short non-glandular trichomes; (**A**–**C**) trichomes with yellow–green cell content; (**D**,**E**) trichomes with transparent content and visible cell wall ornamentation; (**F**–**H**) long non-glandular trichomes; (**I**,**J**) linear glandular trichomes; (**K**,**L**) biseriate glandular trichomes with secretory product in subcuticular space (arrow); Scale bars: **F**–200 µm, **G**,**H**–100 µm, **A**,**D**,**I**,**J**–50 µm, **K**,**L**–30 µm, **B**,**C**,**E**–20 µm.

Type of Trichomes	The Number of Trichome Cells	Trichome Length min-max (μm)	Trichome Diameter * min-max (μm)
Short non-glandular	3–5	207–330	42–52
Long non-glandular	5–8	620–1260	62–83
Linear glandular	7–8	120–131	21–26
Short glandular	10	47–63	39–60

Table 3. Characteristics of *Ambrosia artemisiifolia* trichomes present on the perianth and bracts of staminate flowers.

* The diameter was measured at the widest area of the trichome.

Non-glandular trichomes were present on the fused bracts (involucre) and pedicels of the capitulum. All trichomes of this type were viable, uniseriate, and subulate with a strongly elongated apical cell. They differed in the length, diameter, number of their cells, and the content of protoplasts.

The length of the short non-glandular trichomes was in the range of 207–330 μ m, and their diameter at the widest point was 42–45 μ m. The trichomes were composed of 3–5 cells (Table 3).

The basal trichome cells had a substantially larger diameter than the apical ones (Figure 9A,D). Chloroplasts and green-yellow or brown secretion were contained in the protoplasts of these cells (Figure 9A–C). No secretion was observed in older trichomes, but the granular or ridged wall ornamentation was visible more clearly (Figure 9D,E).

The long non-glandular trichomes had a length in the range of 620-1260 μ m and a maximum diameter of 62–83 μ m. They were composed of 5–8 cells (Table 3). Geniculate thickenings were visible at the junction of the adjacent cells of these trichomes (Figure 9F,G). Very poor ornamentation was visible in the cell walls (Figure 9H). The cytoplasm in the cells of these trichomes was located parietally.

The linear glandular trichomes were observed on the surface of the capitulum involucre. They were 120–131 μ m long and had a diameter of 21–26 μ m. The trichomes were composed of 7–8 uniseriate cells (Figure 9I,J). The cells contained chloroplasts and greenish content. Some trichomes had orange–brown secretion in the apical cells (not shown).

The short glandular trichomes covered the perianth surface and the involucre abundantly (Figure 8H,I). The biseriate trichomes were the shortest (Table 3) and consisted of two columns of five cells each; two basal cells formed the stalk. The apical cells were surrounded by the subcuticular space, which was often filled with secretion. It gradually enlarged during floral development and surrounded four or six apical cells (Figure 9K,L). Next, the cuticle surrounding the subcuticular space ruptured and the accumulated secretion was released.

3.2.3. Stomatal Complexes

The stomata were located in the abaxial epidermis of the leaves and involucre (Figure 10A–C). The guard cells were surrounded by four subsidiary cells (Figure 10A,B). The guard cells were 18–24 μ m long. The surface of the abaxial epidermis exhibited distinct cuticle striation (Figure 10A–D), whereas the wall of the guard cells was smooth (Figure 10C). The outer walls of the epidermal cells were relatively thick. The stomata were irregularly distributed in the epidermis at different distances from each other.



Figure 10. Characteristics of the abaxial epidermis of bracts in *Artemisia artemisiifolia* male capitula: (**A**,**B**) Stomatal complexes composed of two guard cells, four subsidiary cells, and epidermis cells with striated cuticle; (**C**) Dense arrangement of stomata; (**D**,**E**) Cross-section of epidermis cells (Ep); (**D**) Outer cell walls covered by cuticle with distinct striation; (**E**) Thick outer wall in epidermis cells; Scale bars: **A**,**D**,**E**—10 μm, **B**,**C**—20 μm.

4. Discussion

4.1. Ambrosia Pollen Seasons

In 2013 and 2014, the *Ambrosia* pollen seasons in Lublin started relatively early, i.e., in the third ten days of July, whereas the onset of the pollen season of this taxon in 2000–2004 was mainly recorded in the second ten days of August [6]. In Ivano-Frankivsk, the pollen seasons of the taxon were recorded earlier than in Lublin in 2014 and 2015, which may be related to the shorter distance between the monitoring station in Ivano-Frankivsk and the pollen sources. During the three-year comparative studies, the *Ambrosia* pollen seasons observed in Ivano-Frankivsk in Ukraine were characterized by at least two-fold higher annual sums and maximum daily concentrations of pollen grains than the seasons in Lublin. Nevertheless, a similar trend in the abundance of pollen seasons was observed in both cities. The lowest pollen concentrations were recorded in 2013, whereas the highest pollen concentrations were noted in both sites in 2014. A similar trend in the changes in the amounts of pollen in both sites may indicate long-distance transport of *Ambrosia* pollen, e.g., from Ukraine to Poland. This phenomenon was evidenced in previous studies based on the analysis of retrograde trajectories of air masses [9].

Based on studies conducted in several European countries, Šikoparija et al. [57] found that the number of days with the presence of *Ambrosia* pollen grains in the air decreased with the increasing distance from the plant's localities. This is reflected in the intermittent nature of atmospheric transport episodes from areas where *Ambrosia* is abundant to areas where this plant is less common or absent (such as Lublin). Therefore, we suppose that the discontinuity of the pollen seasons observed in Lublin may have been associated with the transport of pollen from longer distances. In turn, in Ivano-Frankivsk, where there are many *Ambrosia* localities, pollen of this taxon was present in the air almost every day of the pollen season.

The findings of the more abundant and more intense pollen seasons in Ivano-Frankivsk are confirmed by the substantially higher number of days with concentrations exceeding the threshold value (5 P/m^3) than in Lublin. This is associated with the presence of numerous localities of *A. artemisiifolia*, most often growing along the tracks and on roadsides as a ruderal plant in the territory of Ukraine, and also in many cities. Many localities situated at a certain distance from the pollen monitoring station have been found in Ivano-Frankivsk. The very light *A. artemisiifolia* pollen is transported easily over long distances [9]. In turn, no plants of this taxon have recently been found in Lublin, and its closest localities are relatively remote from the monitoring station [4].

The moderately intensive *Ambrosia* pollen seasons in Lublin with lower annual sums and the absence of pollen on many days of the season indicate that the pollen of this taxon originates largely from long-distance transport. Therefore, the results of the present study support earlier findings reported by other authors, who described the transfer of *Ambrosia* pollen to Poland from southern or eastern European areas [8,9,11].

The high concentrations of *Ambrosia* pollen in Ivano-Frankivsk are in agreement with earlier data from aerobiological studies conducted in the neighboring area of Ukraine [58–60]. The southeastern areas of Ukraine are characterized by very high pollen concentrations. Data reported by Kaidashev et al. [61] support the concentration of more than 10 pollen grains as clinically significant for Ukrainian patients due to the *Ambrosia* allergenicity. As demonstrated by the authors, the highest number of allergy cases were diagnosed in 2013, which was shown by studies conducted from 2013 to 2015. The Carpathian Basin and the Rhone valleys in France and Northern Italy are other areas with the occurrence of naturalized *Ambrosia* species and the highest pollen concentrations. In these regions, *Ambrosia* pollen is often the main source of allergens [60,62,63] and pollen concentrations provoking symptoms in allergic patients range from 1 grain/m³ to 30 grains/m³ [15,16,22].

There are several causes of the spread of *Ambrosia* species in Europe: changes in agricultural land use, an increase in suburban areas due to the reduction of the surface area of arable land, and climate change [64].

In the present study, we investigated the influence of various meteorological factors on the concentration of *Ambrosia* pollen in the air of Lublin and the impact of temperature and humidity on the concentration of pollen of representatives of this genus in Ivano-Frankivsk. We found a significant positive interaction between the concentration of pollen grains and average temperature. We also showed a significant negative correlation between the pollen concentration and relative humidity in Lublin and in Ivano-Frankivsk.

Investigations conducted by other authors also show that the analyzed meteorological variables have a significant effect on the content of *Ambrosia* pollen in the air. Stępalska et al. [65] reported the highest *Ambrosia* pollen concentrations on days with high values of maximum temperatures. In turn, the research carried out by Rodinkova et al. [59] indicates that an elevated mean daily temperature accelerates the start of the *Ambrosia* pollen season. Ianovici and Birsan [66] reported a significant negative correlation of *Ambrosia* pollen concentrations with relative humidity. The results presented by Šaulienė and Veriankaitė [67] indicate that the presence of airborne *Ambrosia* pollen in Lithuania was accompanied by approximately 70% relative air humidity and the minimum air temperature not less than 12 °C. In turn, it has been found that droughts may also significantly decrease *Ambrosia* pollen season, as reported from Ukraine by Rodinkova et al. [59].

4.2. Ambrosia Trichome Morphotypes

A characteristic trait of Asteraceae is the simultaneous presence of different types of glandular and non-glandular trichomes. Nine types of non-glandular trichomes have been distinguished [68]. In the present study, we observed two types of non-glandular trichomes in the inflorescences of *A. artemisiifolia*. Both types of the trichomes are uniseriate and have a long terminal cell. They differ in the number of cells, length, diameter, and the presence of secretion in their cells. Several types of non-glandular trichomes have been observed in *Centaurea cyanus* by Chiru et al. [69] and Haratym et al. [70].

The analyzed *A. artemisiifolia* organs exhibited the presence of two types of glandular trichomes: longer linear uniseriate and short biseriate trichomes. Linear uniseriate trichomes were observed previously in *Helianthus annuus* and many other species from the genera *Aldama, Heliomeris, Rudbeckia,* and *Tithonia* [71,72]. As reported by Spring et al. [73], the linear glandular trichomes of *Helianthus annuus* contain sesquiterpenes.

All *A. artemisiifolia* organs examined in this study had biseriate glandular trichomes composed of 10 cells arranged in five tiers. The same trichome structure, typical for Asteraceae, has been found in, e.g., *Artemisia annua* [74], *Chamomilla recutita* [75], and *Chrysanthemum morifolium* [76]. In turn, the presence of biseriate glandular trichomes with

6-7 tiers of cells was reported by Werker [77] in *Artemisia dracunculus* and by Haratym et al. [70] in *Centaurea cyanus*.

Biseriate glandular trichomes were also observed on floret corollas in other Asteraceae representatives, e.g., *Helichrysum* [78], *Artemisia* [74], *Chamomilla*, and *Inula* [75,79]. In contrast, no glandular trichomes were detected on the corolla in *Petasites* [80] and *Centaurea* [70].

It has been reported that biseriate glandular trichomes on the leaves of *Artemisia annua* are the sole sources of artemisinin and artemisitene [46,81]. Artemisinin is an endoperoxide sesquiterpene lactone with effectiveness against some strains of *Plasmodium* responsible for malaria [82].

The types of glandular and non-glandular trichomes have taxonomic value for plant species [68]. The types of trichomes found in the present study on *A. artemisiifolia* shoots have not been described in the literature to date. The secretory products contained in the cells of these trichomes may trigger contact dermatitis in sensitive subjects.

5. Conclusions

The concentration of *Ambrosia* pollen during the pollen season in the western part of Ukraine is substantially higher than in central-eastern Poland.

The highest and the lowest concentrations of *Ambrosia* pollen grains in Lublin and Ivano-Frankivsk were noted in the same years. The highest number of pollen grains was recorded in 2014, which was associated with the highest risk of allergy due to the highest number of days with concentrations that could trigger the reaction. In the air of both cities, the pollen grains were most abundant in August and September.

In Lublin and Ivano-Frankivsk, the stage of the pollen season, in which large amounts of *Ambrosia* pollen grains were released, was relatively long and slow. An exception was the 2014 season in Ivano-Frankivsk, as the stage was short and rapid. The lower concentrations of *Ambrosia* pollen, lack of plants of this genus in Lublin (Poland), and the fact that there were many days without airborne pollen may indicate the transport of the pollen of this taxon from distant areas.

Our study shows the greatest impact of average temperature and relative humidity on the concentration of *Ambrosia* pollen in the air. The two types of glandular trichomes and the two types of non-glandular trichomes present on *A. artemisiifolia* shoots have not been described in this species to date. The secretion produced by the trichomes of this species can cause contact dermatitis in sensitive subjects.

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